Electroosmotic flow hysteresis and effect of nanostructure orientation in a microfluidic channel

Lim, An Eng

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**NANYANG
TECHNOLOGICAL
UNIVERSITY**

SINGAPORE

**ELECTROOSMOTIC FLOW HYSTERESIS AND
EFFECT OF NANOSTRUCTURE ORIENTATION IN
A MICROFLUIDIC CHANNEL**

LIM AN ENG

SCHOOL OF MECHANICAL AND AEROSPACE ENGINEERING

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ORIENTATION IN A MICROFLUIDIC CHANNEL

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Abstract

Electroosmotic flow (EOF) has been exploited in a wide range of microfluidic applications. Most of these applications require the driving fluid to be inhomogeneous, i.e. differing in either concentration or conductivity. There is an experimentally observed anomaly in the EOF of two fluids with different concentrations, whereby the flow behavior is found to be direction-dependent. This observation is termed as “EOF hysteresis”. The conventional theoretical models for EOF contain critical limitations for describing the hysteresis phenomenon. Thus, a generalized theoretical model was formulated in this thesis for a proper description of the two-fluid displacement flow process, with appropriate simplifications for specific experimental conditions to ease the demand of computational effort when implemented for numerical simulation.

The abovementioned EOF hysteresis was demonstrated numerically to originate from the accumulation/depletion of minority pH-governing ions, such as the hydronium ion (H_3O^+), as a result of the imbalanced electric-field-induced flux at the two-fluid interface, which subsequently widened and spread during the displacement flow process. The resultant pH changes caused the variations of zeta potential and EOF flow rate that gave rise to the hysteretic behavior. To provide conclusive evidence on pH changes in EOF hysteresis, direct experimental observation of the pH changes and their quantifications for comparison with the numerical simulations were performed. The experimental results showed good quantitative agreements with the simulation results.

Hitherto, no investigation has been performed to examine the flow direction-dependent behavior during electroosmotic displacement flow of solutions with dissimilar ionic species. Hence, EOF hysteresis involving solution pair with dissimilar cation or anion species was investigated in this thesis, both experimentally and numerically. Two different mechanisms had been identified as the causes of EOF hysteresis for dissimilar cationic solutions: (a) widening/sharpening effect of the interfacial region generated by conductivity difference between the two solutions, and (b) difference in average zeta potentials caused by the concentration adjustments in different flow directions.

For the investigation of EOF hysteresis involving dissimilar anionic solutions, the displacement flow was discovered to exhibit an unusual behavior, for which the equilibrium concentration in the microchannel deviated from the initial displacing electrolyte. This was found to be caused by the ion concentration adjustment when the displacing anions migrated in opposition to EOF, and a second ion concentration adjustment due to the diffusive interface at the junction between the anode reservoir and microchannel which was convected throughout the entire channel by EOF. The resultant ion distributions (thus difference in average zeta potentials) led to the direction-dependency of the EOF flow rate.

This investigation reveals that an in-depth understanding of EOF hysteresis for solutions with different concentrations and ionic species is important to the precise manipulation of fluids and analyte transports in microfluidic applications, where the fluids involved are typically inhomogeneous.

Nanoscale structures are usually incorporated within a microchannel for various applications. Even though there are numerous investigations on EOF reduction due to the presence of nanostructures in microchannels, a proper study on the orientation effect of nanostructures on EOF has never been conducted. This thesis presents a novel fabrication method for microchannels with nanostructure designs that have significant orientation difference, i.e. parallel versus perpendicular indented nanolines. The fabrication process consisted of four phases: fabrication of silicon master, creation of mold insert via electroplating, injection molding with cyclic olefin copolymer (COC), and thermal bonding and integration of practical inlet/outlet ports.

The effect of nanostructure orientation on EOF in a microfluidic channel was examined both experimentally and numerically. The experimental results showed that the perpendicular nanolines significantly reduced the EOF velocity (by approximately 20%). The numerical simulation revealed that the flow velocity reduction was due to the local electric field distortion at the nanostructured surface. In contrast, the parallel nanolines had no effect on the EOF, as it was deduced that the parallel nanolines did not result in distortion of the electric field. The outcomes of this investigation enhance the fundamental understanding of the effect of nanostructures on EOF behavior. These have implications on the precise EOF control in devices utilizing nanostructured surfaces for chemical and biological analyses.

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List of symbols

<u>Symbol</u>	<u>Unit</u>	<u>Description</u>
a	m	Radius of charged particle
a_i	-	Weight coefficient for Shapiro-Wilk test
c_i	mol.m ⁻³ (mM)	Concentration of ionic species
$c_{o(i)}$	mol.m ⁻³	Bulk concentration of ionic species
c_L	mol.m ⁻³	Equilibrium solution concentration in microchannel
D_i	m ² .s ⁻¹	Diffusion coefficient of ionic species
$\mathbf{E}$	V.m ⁻¹	Electric field vector
E	V.m ⁻¹	Electric field
E_a	V.m ⁻¹	Electric field at anode reservoir junction
E_L	V.m ⁻¹	Electric field at other locations in the microchannel during steady-state
E_{str}	V.m ⁻¹	Streaming electric field
E_{sed}	V.m ⁻¹	Sedimentation electric field
E_g	J	Energy generation in fluid
ΔE_{st}	J	Energy storage in fluid
F	C.mol ⁻¹	Faraday constant
g	m.s ⁻²	Acceleration due to gravity
H	m	Suspension height
H_o	-	Null hypothesis
H_l	-	Alternative hypothesis
I	A	Time-dependent current
I^o	A	Initial current
$I_{Steady-state}$	A	Steady-state current
I^*	-	Normalized current

I^{-1}	A^{-1}	Inverse of experimental current
I_s	mol.m^{-3}	Ionic strength of solution
I_{str}	A	Streaming current
I_c	A	Conduction current
$\mathbf{J}$	$A.m^{-2}$	Current density vector
J	$A.m^{-2}$	Axial current density
k	m^{-1}	Debye-Hückel parameter
k_b	$J.K^{-1}$	Boltzmann constant
K_A	-	Equilibrium constant of silanol groups (SiOH) deprotonation reaction
K_H	$L.atm.mol^{-1}$	Henry's law constant
K_{fw}	$\text{mol.dm}^{-3}.s^{-1}$	Apparent specific forward rate constant of water
K_{rw}	$\text{mol}^{-1}.\text{dm}^3.s^{-1}$	Apparent specific reverse rate constant of water
K_w	$\text{mol}^2.\text{dm}^{-6}$	Equilibrium constant of water auto-ionization
K_{fC1}	s^{-1}	Apparent specific forward rate constant of carbonate system 1
K_{rC1}	$\text{mol}^{-1}.\text{dm}^3.s^{-1}$	Apparent specific reverse rate constant of carbonate system 1
K_{C1}	mol.dm^{-3}	Equilibrium constant of carbonate system 1
K_{fC2}	s^{-1}	Apparent specific forward rate constant of carbonate system 2
K_{rC2}	$\text{mol}^{-1}.\text{dm}^3.s^{-1}$	Apparent specific reverse rate constant of carbonate system 2
K_{C2}	mol.dm^{-3}	Equilibrium constant of carbonate system 2
K_{fP1}	s^{-1}	Apparent specific forward rate constant of phosphate system 1
K_{rP1}	$\text{mol}^{-1}.\text{dm}^3.s^{-1}$	Apparent specific reverse rate constant of phosphate system 1
K_{P1}	mol.dm^{-3}	Equilibrium constant of phosphate system 1
K_{fP2}	s^{-1}	Apparent specific forward rate constant of phosphate system 2
K_{rP2}	$\text{mol}^{-1}.\text{dm}^3.s^{-1}$	Apparent specific reverse rate

		constant of phosphate system 2
K_{P2}	mol.dm^{-3}	Equilibrium constant of phosphate system 2
KRF	mol.V.s.m^{-5}	Kohlrausch regulating function
L	m	Length of microcapillary
$\mathbf{n}$	-	Unit vector normal to boundary
n	-	Number of samples
n_i	-	Molecular formula of ionic species
N_a	mol^{-1}	Avogadro constant
N_{Total}	m^{-2}	Total number site density of SiOH
p	Pa	Pressure
Δp	Pa	Pressure difference
P	-	Dimensionless interface position of two fluids
PCO_2	atm	Partial pressure of carbon dioxide
Q	$\text{m}^3.\text{s}^{-1}$	Flow rate of fluid
R_i	$\text{mol.m}^{-3}.\text{s}^{-1}$	Total reaction rate of ionic species
r	$\text{mol.m}^{-3}.\text{s}^{-1}$	Reaction rate of reversible acid-base chemical reaction
r_f	$\text{mol.m}^{-3}.\text{s}^{-1}$	Forward reaction rate
r_r	$\text{mol.m}^{-3}.\text{s}^{-1}$	Reverse reaction rate
r_c	m	Radius of microcapillary
R	$\text{J.mol}^{-1}.\text{K}^{-1}$	Gas constant
$R_{I/2}$	Ω	Instantaneous resistances of two fluids during displacement flow
R_T	Ω	Total instantaneous resistance of system
R_c	m^2	Capillary cross-sectional area
Ra	m	Surface roughness
R^2	-	Coefficient of determination
s_{HL}	s	Sample standard deviation of T_{HL}

S_{LH}	s	Sample standard deviation of T_{LH}
$S_{HL(Turn)}$	s	Sample standard deviation of $T_{HL(Turn)}$
$S_{LH(Turn)}$	s	Sample standard deviation of $T_{LH(Turn)}$
S	$C.m^{-3}$	Surface charge density
t	s	Time
t^*	-	Normalized time
t_d	s	Displacement time
t_s	-	t -score for Student's t -test
T	K	Temperature
T_{HL}	s	Displacement time for a high conductivity solution displacing a low conductivity solution
T_{LH}	s	Displacement time for a low conductivity solution displacing a high conductivity solution
$T_{HL(Turn)}$	s	Turning time for a high conductivity solution displacing a low conductivity solution
$T_{LH(Turn)}$	s	Turning time for a low conductivity solution displacing a high conductivity solution
$\bar{T}_{HL}$	s	Sample mean of T_{HL}
$\bar{T}_{LH}$	s	Sample mean of T_{LH}
$\bar{T}_{HL(Turn)}$	s	Sample mean of $T_{HL(Turn)}$
$\bar{T}_{LH(Turn)}$	s	Sample mean of $T_{LH(Turn)}$
$\mathbf{u}$	$m.s^{-1}$	Fluid velocity vector
u	$m.s^{-1}$	Axial fluid velocity
u_{eo}	$m^2.V^{-1}.s^{-1}$	Electroosmotic flow (EOF) mobility
$u_{m(i)}$	$m^2.V^{-1}.s^{-1}$	Ionic mobility of ionic species
U_{avg}	$m.s^{-1}$	Average EOF velocity (numerical)

v_{eo}	m.s^{-1}	EOF velocity
v_{avg}	m.s^{-1}	Average EOF velocity (experimental)
v_{ep}	m.s^{-1}	Electrophoretic velocity
ΔV	V	Electric potential difference
w	-	Test statistic for Shapiro-Wilk
x^*	-	Dimensionless x (axial coordinate)
X	m	Interface position of two fluids
z_i	-	Ionic charge number of ionic species
α	-	Volume fraction of particles
ε_o	$\text{C.V}^{-1}.\text{m}^{-1}$	Permittivity of free space
ε_r	-	Relative permittivity
ρ	kg.m^{-3}	Density of fluid
ρ_p	kg.m^{-3}	Density of particle
ρ_e	C.m^{-3}	Net charge density
μ	$\text{kg.m}^{-1}.\text{s}^{-1}$	Viscosity of fluid
λ_D	m	Debye length
ψ	V	Electrostatic wall potential
ζ_0	V	Electrostatic surface potential
ζ	V	Zeta potential
ζ_{avg}	V	Average zeta potential
ζ_{eff}	V	Effective zeta potential
$\bar{\zeta}$	V	Length-average zeta potential
σ	S.m^{-1}	Conductivity of fluid
$\sigma_{1/2}$	S.m^{-1}	Conductivities of two fluids during displacement flow
σ_b	S.m^{-1}	Bulk fluid conductivity

σ_a	S.m^{-1}	Conductivity at anode reservoir junction
σ_L	S.m^{-1}	Conductivity at other locations of microchannel during steady-state
ϕ	V	Applied electric potential
$\Delta\phi_{str}$	V	Streaming potential
$\Delta\phi_{sed}$	V	Sedimentation potential
∇	-	Del operator

Chapter 1 Introduction

1.1 Background

Micro-electro-mechanical systems (MEMS) refer to a class of miniature integrated devices, which consists of the mechanical and electrical components. The functions of these devices are generally to sense, control and actuate on the micro-level. The fabrication of MEMS devices evolved from the fabrication techniques used to create integrated circuits (ICs), i.e. lithography, etching and deposition. Over the past decades, elements in these devices are no longer restricted to purely electrical and mechanical disciplines, but also encompass the chemical, optical and/or microfluidic components.

Microfluidics manipulates volumes of fluids that vary from microliters to femtoliters in networks of channels with dimensions typically ranging from a few micrometers to millimeters. Biological micro-electro-mechanical systems (BioMEMS), lab-on-a-chip (LOC) and micro total analytical systems (μ TAS) are the terms given to MEMS devices which incorporate microfluidic components in a miniaturized format for chemical or biological analyses.

Such devices have found emerging opportunities in engineering, medicine, biology and chemistry (Amin et al. 2013, Pang et al. 2013, Mohammadi et al. 2015). The advantages that attract users in these fields are small volumes of samples and reagents consumption, little waste production, short reaction times, low costs and small footprints as compared with other analytical devices (Weibel and Whitesides 2006).

The application of microfluidic technology has proven to be more efficient and less complex in detecting lethal viruses during major disease outbreaks, such as severe acute respiratory syndrome (SARS) (Long et al. 2004, Zhou et al. 2004, Qi et al. 2006), swine flu (H1N1 flu) (Lee et al. 2012, Tseng et al. 2016, Qiu et al. 2017), Eobola epidemic (Cai et al. 2015, Piraino et al. 2016, Du et al. 2017) and Zika virus (Lee et al. 2016, Song et al. 2016).

There are several methods available for the transportation of fluids in a microfluidic channel. The two most commonly used techniques are pressure driven flow and electrokinetically driven flow. Electrokinetically driven flow, also known as electroosmotic flow (EOF), involves the motion of fluid driven by an applied electric field that acts on the net electric charge produced due to the spontaneous formation of surface charges at the fluid-solid interface upon contact with an aqueous solution. EOF has several advantages over pressure driven flow as it requires no external mechanical moving parts and provides an easy mean of transporting fluids through the application of an electric field. The velocity profile of EOF is typically uniform across a microchannel and independent of the cross-sectional area (Cummings et al. 2000, Ghosal 2004, Chang and Yang 2007).

1.2 Research motivation

EOF has been exploited in numerous microfluidic applications including manipulation of biomolecules (Bown and Meinhart 2006, Andreev 2013, Di Fiori et al.

2013, Asandei et al. 2016), drug delivery (Pikal 2001, Abla et al. 2005, Prausnitz and Langer 2008), fuel cell (Karimi and Li 2005, Buie et al. 2006, Litster et al. 2009), sludge treatment (Yuan and Weng 2003, Glendinning et al. 2007, Zhan et al. 2016), soil remediation (Schultz 1997, Gill et al. 2014, Mena et al. 2016), chemical species/particles separation (Li et al. 2000, Yan et al. 2003, Kawamata et al. 2008), pumping (Wang et al. 2009, He et al. 2011, Gao and Gui 2014) and mixing of fluids (Chang and Yang 2007, Lim et al. 2010, Nayak 2014).

In many of these applications, the fluid driven by EOF is inhomogeneous, i.e. differing in conductivity or concentration. There is an experimentally observed anomaly in the displacement of two fluids with *different concentrations* (Gan et al. 2006, Lim and Lam 2012). Contrary to the prediction of prevailing EOF theory, the flow behavior is found to be different, and dependent on the flow direction. For example, the flow of a lower concentration potassium chloride (KCl) solution displacing a higher concentration KCl solution is slower than the flow in the reverse direction. This observation is termed as “EOF hysteresis”. Theoretical/numerical studies had been attempted to explain this hysteretic behavior (Gan et al. 2006, Lim and Lam 2012). However, there were significant discrepancies between the theoretical/numerical predictions and experimental observations. Therefore, the aim of this investigation is to uncover the mechanics of this hysteresis effect experimentally in a systematic manner, and to provide theoretical/numerical explanations and quantifications.

Although there are many investigations on electroosmotic displacement flow with different types of fluids (Ren et al. 2001, Ren et al. 2002, Ren et al. 2003), no investigation has hitherto been conducted to investigate the difference in flow behaviors between opposing flow directions. In ion pre-concentration techniques such as field amplified sample stacking (FASS) (Giordano et al. 2012) and isotachopheresis (ITP) (Smejkal et al. 2013), the electric field is typically applied in one direction throughout the whole experiment, which favors the accumulation of the desired species at the two-fluid interface. Hence, this investigation also specifically examines the EOF hysteresis involving solution pair with *dissimilar ionic species*, i.e. dissimilar cation or anion species, through both experimental and numerical approaches. A proper understanding of EOF hysteresis for solutions with different concentrations and ionic species is critical to the accurate manipulation of fluids and solutes in practical applications, where the fluids involved are typically inhomogeneous.

Even though EOF has been extremely useful for various applications, it can have very dramatic consequences on the performance of certain applications. For instance, EOF generates a counterflow opposite to the electrophoresis migration of negatively charged samples, e.g. DNA and sodium dodecyl sulfate (SDS) denatured proteins, which degrades the resolution of electrophoretic separation (Viovy 2000). The suppression of EOF is conventionally accomplished through the coating of water-soluble polymers (Horvath and Dolnik 2001), e.g. acidified poly(ethylene oxide) (PEO) (Iki and Yeung 1996, Preisler and Yeung 1996).

Nanoscale networks/structures are often integrated within a microchannel for a broad range of applications, such as electrophoretic separation of biomolecules (Li et al. 2003, Kaji et al. 2004), high reaction efficiency catalytic microreactors (Kusakabe et al. 2001, Miyazaki et al. 2004), and enhancement of heat transfer (Nagayama et al. 2007, Li et al. 2012) and sensing (Li et al. 2013, Ng et al. 2016). Even though there are numerous investigations on EOF reduction due to the presence of nanostructures in microchannels (Kang and Suh 2009, Messinger and Squires 2010, Yasui et al. 2011, Koga et al. 2013), the effect of nanostructure orientation on EOF has yet to be examined. This investigation studies the effect of nanostructure orientation on EOF in a microfluidic channel, both experimentally and numerically. The outcomes of this investigation enhance the fundamental understanding of EOF behavior, with implications on the precise EOF control in devices utilizing nanostructured surfaces for chemical and biological analyses.

1.3 Organization of thesis

Chapter 1 discusses the background, research motivation, and organization of the research thesis.

Chapter 2 reviews the fundamental theories on EOF, literature related to electroosmotic-driven flow involving two or more fluids, and the different hysteresis phenomena. Literature on the micro-/nanofabrication techniques and the effects of micro-/nanostructures in a micro-/nanochannel on EOF will also be discussed.

Chapter 3 formulates a generalized theoretical model to describe the two-fluid electroosmotic displacement flow. To ease the demand on computational resources for implementation in numerical simulation, appropriate simplifications of the generalized model are performed for specific experimental conditions.

Chapter 4 presents the origin of EOF hysteresis for fluids with different concentrations, through both experimental and numerical approaches. As elucidated by the numerical simulations, the flow direction-dependent behavior is attributed to the pH changes caused by the accumulation or depletion of pH-governing minority ions, such as hydronium (H_3O^+) and bicarbonate (HCO_3^-) ions.

Chapter 5 focuses on the direct experimental demonstration of pH changes in microchannel during the displacement flow of fluids with different concentrations, and their quantifications for comparison with the numerical simulations.

Chapters 6 and 7 report the experimental and numerical investigations of EOF hysteresis involving solution pair with dissimilar ionic species, i.e. dissimilar cation or anion species respectively.

Chapter 8 documents the fabrication method for microfluidic channels with nanostructure designs that possess maximum orientation difference, i.e. parallel versus perpendicular indented nanolines. It also details the experimental and numerical investigations performed to study the orientation effect of nanostructures on EOF.

Chapter 9 provides the conclusions of this investigation, with recommendations for future work.

Chapter 2 Literature review

2.1 Introduction

This chapter introduces the fundamental theories on the formation of electrical double layer (EDL), and the electrokinetic phenomena with an emphasis on electroosmotic flow (EOF). EOF has been utilized for a wide range of microfluidic applications that usually involve two or more types of fluids. However, there is an experimentally observed anomaly in the two-fluid electroosmotic displacement flow process, i.e. the flow behavior is dependent on the displacement flow direction. The hysteresis phenomenon is termed as “EOF hysteresis” in this thesis. Detailed reviews on electroosmotic driven flow involving two or more fluids, and the different hysteresis phenomena will be discussed.

Nanoscale networks/structures are commonly integrated within microchannels for various applications. The introduction of nanostructures in a microfluidic channel has been known to have an intrinsic ability for EOF reduction. However, the orientation effect of nanostructures within a microchannel on EOF has yet to be investigated. Literature related to micro-/nanofabrication techniques, and the effects of micro-/nanostructures within a micro-/nanochannel on EOF will also be reviewed in this chapter.

2.2 Theoretical background

2.2.1 Electrical double layer (EDL)

When in contact with an electrolyte solution, solid surfaces develop net charge density through mechanisms such as dissociation of surface groups and adsorption processes. As shown in Figure 2.1, the charged surface attracts counterions (ions of opposite charge to the surface) to the region near the wall and repels coions (ions of similar charge to the surface) away from it. Hence, as compared to the bulk fluid, the counterions in this region are present at higher concentration, whereas the coions are present at lower concentration. This region is known as the EDL, where the ions move under the influence of electrostatic force and random thermal motion (Hunter 1988, Probstein 1994, Kirby 2010).

Figure 2.1: Electrical double layer (EDL).

In the conventional theory, the EDL is divided into two parts, namely the Stern layer and the Gouy-Chapman diffuse layer (see Figure 2.1). The compact layer directly

next to the wall is called the Stern layer, which consists of the immobile counterions. Further away from the wall, the Gouy-Chapman diffuse layer comprises the mobile counterions; these counterions are distributed in a “diffuse” manner and will travel under an externally applied tangential electric field.

The plane separating the inner Stern layer and the outer diffuse layer is known as the shear plane, where the fluid motion begins. The electrostatic potential in the Stern layer is assumed to drop linearly from the surface value ζ_0 to ζ at the shear plane, which is commonly known as the zeta potential. This potential value decreases exponentially across the diffuse layer into the bulk fluid.

The net charge on the surface is balanced by the volumetric charge density of the diffuse layer (due to excess counterions over coions), which is equal in magnitude and of opposite polarity to the net surface charge density. The characteristic thickness of the EDL (typically in the order of nanometers) is given by the Debye length (Hunter 1988, Probstein 1994, Kirby 2010):

$$\lambda_D = \sqrt{\frac{\epsilon_o \epsilon_r k_b T}{e^2 N_a \sum z_i^2 c_{o(i)}}} = \sqrt{\frac{\epsilon_o \epsilon_r k_b T}{2e^2 N_a I_s}}, \quad (2.1)$$

where ϵ_o is the permittivity of free space, ϵ_r is the dielectric constant of fluid, k_b is the Boltzmann constant, T is the temperature, e is the electron charge, N_a is the Avogadro constant, $I_s = 1/2 \sum z_i^2 c_{o(i)}$ is the ionic strength of solution, z_i is the ion charge number and $c_{o(i)}$ is the bulk concentration of ionic species. Increasing the ionic strength of a

solution will decrease the Debye length because there will be higher valency/concentration of ionic species for the screening of surface charge.

The bulk fluid region which is far away from the wall is electrically neutral. The concentration of ionic species under the influence of charged surface is presumed to follow the Boltzmann distribution (Hunter 1988, Probstein 1994, Kirby 2010):

$$c_i = c_{o(i)} \exp\left(\frac{-z_i e \psi}{k_b T}\right), \quad (2.2)$$

where the concentration of ionic species far from the wall $c_i \rightarrow c_{o(i)}$ as the electrostatic potential $\psi \rightarrow 0$. The electrostatic potential is governed by the Poisson equation (Hunter 1988, Probstein 1994, Kirby 2010):

$$\nabla \cdot \nabla \psi = -\frac{\rho_e}{\epsilon_r \epsilon_o}, \quad (2.3)$$

where the net charge density $\rho_e = F \sum c_i z_i$ and F is the Faraday constant.

For a symmetric electrolyte, incorporating Equation 2.2 into the one-dimensional (1-D) form of Equation 2.3 will result in the Poisson-Boltzmann equation:

$$\frac{d^2 \psi}{dy^2} = \frac{2c_o F |z_i|}{\epsilon_o \epsilon_r} \sinh\left(\frac{|z_i| e \psi}{k_b T}\right). \quad (2.4)$$

If $|z_i| e \psi / k_b T$ is small (due to small electrostatic potentials), the nonlinear term on the right side of Equation 2.4 can be linearized, which allows the Debye-Hückel approximation to be obtained:

$$\frac{d^2\psi}{dy^2} = k^2\psi, \quad (2.5)$$

where k is the Debye-Hückel parameter, for which the inverse k^{-1} is the Debye length as given in Equation 2.1.

Integrating Equation 2.5 subject to the conditions that $\psi = \zeta$ at $y = 0$ (the shear plane), and $d\psi/dy \rightarrow 0$ and $\psi \rightarrow 0$ as $y \rightarrow \infty$, it yields the Debye-Hückel solution:

$$\psi(y) = \zeta e^{-ky}. \quad (2.6)$$

2.2.2 Electrokinetic phenomena

Electrokinetic phenomena refer to a group of phenomena which is associated with the formation of EDL. Relative movement between the mobile portion of the diffuse double layer and the static charges on the wall surface induces distinct effects which include (Hunter 1988, Probstein 1994, Kirby 2010):

- (a) Electroosmosis (synonymous with EOF) – the movement of fluid relative to a stationary charged surface (in the form of a microcapillary or porous plug) under the influence of an applied electric field. EOF occurs when the external tangential electric field acts upon the excess counterions in the diffuse layer of the EDL (see Figure 2.1), exerting an electrical body force that drives its motion in the direction of the applied electric field. The movement of the counterions is transferred to the bulk of the fluid through viscous effect, thereby dragging the bulk fluid to move

along with it. For a sufficiently thin EDL, the formula for the electroosmotic velocity, also known as the Helmholtz-Smoluchowski slip velocity, is given as (Hunter 1988, Probstein 1994, Kirby 2010):

$$v_{eo} = \frac{-\varepsilon_o \varepsilon_r E \zeta}{\mu} = u_{eo} E, \quad (2.7)$$

where E is the applied electric field, μ is the dynamic viscosity of fluid and u_{eo} is the EOF mobility.

- (b) Electrophoresis – the movement of a charged particle relative to the stationary fluid induced by an applied electric field. As shown in Figure 2.2, a negatively charged particle will move in the direction opposite to the applied electric field. Considering the case for large Debye length λ_D compared to the radius a of the charged particle, the particle may be regarded as a point charge in a uniform electric field, and the electrophoretic velocity is (Hunter 1988, Probstein 1994, Kirby 2010):

$$v_{ep} = \frac{2\varepsilon_o \varepsilon_r E \zeta}{3\mu}. \quad (2.8)$$

Figure 2.2: Electrophoresis of a negatively charged particle.

However, the curvature effects of the EDL can be disregarded if λ_D is small compared to a , and the charged particle is deemed as a plane surface. The applied electric field is considered to be parallel to the charged surface whereby the Helmholtz-Smoluchowski equation (see Equation 2.7) can be applied, with the electrophoretic velocity carrying an opposite sign (Hunter 1988, Probstein 1994, Kirby 2010):

$$v_{ep} = -\frac{\epsilon_o \epsilon_r E \zeta}{\mu}. \quad (2.9)$$

Therefore, electrophoresis is a complement of electroosmosis. Equation 2.9 shows that the electrophoretic velocity of a charged particle is independent of its shape and size for a constant zeta potential, when the EDL is thin compared to the characteristic body dimension.

- (c) Streaming potential – the electric field created when fluid is caused to flow along a stationary charged surface (the opposite of electroosmosis). When a fluid is made to flow through a capillary under a pressure gradient (see Figure 2.3), the charges in the mobile part of the EDL are carried towards one end. Consequently, a streaming current is produced (Hunter 1988, Probstein 1994, Kirby 2010):

$$I_{str} = -\frac{\pi r^2 \epsilon_o \epsilon_r \zeta \Delta p}{\mu L}, \quad (2.10)$$

where Δp is the pressure difference, r_c is the radius and L is the length of the capillary. The accumulation of charges sets up an electric field that causes a backward current flow, which is known as the conduction current (Hunter 1988, Probstein 1994, Kirby 2010):

$$I_c = \frac{\pi r^2 \sigma_b \Delta \phi_{str}}{L}, \quad (2.11)$$

where σ_b is the conductivity of the bulk fluid and $\Delta \phi_{str}$ is the potential difference across the capillary (called the streaming potential). When $I_{str} + I_c = 0$, an equilibrium condition is established, and the streaming potential is given as (Hunter 1988, Probstein 1994, Kirby 2010):

$$\Delta \phi_{str} = \frac{\epsilon_o \epsilon_r \zeta \Delta p}{\mu \sigma_b}, \quad (2.12)$$

whereby the thickness of EDL is assumed to be small compared to the size of the capillary.

Figure 2.3: Streaming potential across capillary.

- (d) Sedimentation potential – the production of an electric field when charge particles move relative to a stationary fluid (the opposite of electrophoresis). When a charged particle moves through a liquid under the effect of gravity (see Figure 2.4), the mobile counterions shift from the lower half of the particle's EDL to the upper surface, which disrupts the equilibrium symmetry of the EDL. Thus, the moving particle creates a net dipole moment and the sum of all dipoles generates an electric field. With the assumption that the EDL is small compared to the particle radius, the sedimentation potential across the suspension height H may be written as (Probstein 1994):

$$\Delta\phi_{sed} = \frac{\alpha g (\rho_p - \rho) \epsilon_o \epsilon_r \zeta H}{\mu \sigma_b}, \quad (2.13)$$

where α is the volume fraction of particles, g is the acceleration due to gravity, ρ_p is the density of particle and ρ is the density of fluid.

Figure 2.4: Sedimentation potential.

2.2.3 Electroosmotic flow (EOF) profile

To achieve fluid flow in a microchannel, the two commonly employed methods are pressure driven flow and electrokinetically driven flow. EOF is immensely useful in microfluidic systems because it is often more convenient and efficient to control flow velocity by adjusting the magnitude of electric field without bulky mechanically driven parts (Cummings et al. 2000, Ghosal 2004, Chang and Yang 2007). The velocity of fluid rises from zero at the shear plane (see Figure 2.1) to a maximum electroosmotic velocity at the edge of the EDL, and after which remains constant. This produces a flat or plug-like flow profile (see Figure 2.5b) which is independent of the channel dimension, for the case with non-overlapping EDL.

In contrast, a pressure driven flow shows a parabolic flow profile (see Figure 2.5a) with a maximum velocity at the middle, and the velocity reduces to zero at the wall (i.e. the no slip boundary condition). This type of fluid flow requires high pressure for delivering fluids in channel of small cross-sectional area, making it less advantageous compared to EOF, whereby the transportation of fluids can be easily achieved through the application of electric field in a small channel.

Figure 2.5: Flow profiles of (a) pressure driven flow and (b) electroosmotic flow (EOF).

Pressure driven flow may not be the ideal choice for sample delivery in analytical methods such as flow injection analysis (Kamholz and Yager 2001, Bai et al. 2002, Kutter and Klank 2004) and liquid chromatography (Jacobson et al. 1994, Dutta and Leighton 2003, Dutta and Leighton Jr 2003). This is because band broadening caused by Taylor dispersion, which is the superposition of band broadenings due to

both diffusion and the parabolic flow profile (Probstein 1994, Kirby 2010), might limit the resolution of the aforesaid methods. Whereas EOF provides a more desirable flow profile for the transportation of samples in an analytical tool as band broadening can be expected to be extremely small for such a uniform flow profile (Martin and Guiochon 1984, McEldoon and Datta 1992, Dasgupta and Liu 1994, Culbertson et al. 1998, Griffiths and Nilson 1999).

2.2.4 Helmholtz-Smoluchowski approximation

EOF arises from the Coulomb force experienced by the mobile net charge in the EDL diffuse layer, which is induced by an external tangential electric field (see Section 2.2.2). The fluid flow can be described by performing integration on the Navier-Stokes (momentum) equation (Hunter 1988, Probstein 1994, Kirby 2010):

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u} + \rho_e \mathbf{E}, \quad (2.14)$$

where p is the pressure, $\mathbf{u}$ is the fluid velocity, and the electrical body force term (third term on the right of Equation 2.14) consists of the product of net charge density ρ_e and electric field $\mathbf{E}$.

For a steady inertia-free flow along a wall surface in the x direction without pressure gradient (see Figure 2.5b), the fluid velocity is only a function of the y direction; Equation 2.14 is reduced to:

$$\mu \frac{d^2 u}{dy^2} = -\rho_e E, \quad (2.15)$$

Using the Poisson equation (see Equation 2.3) to substitute ρ_e in terms of the wall electrostatic potential ψ , this yields:

$$\mu \frac{d^2 u}{dy^2} = \varepsilon_o \varepsilon_r \frac{d^2 \psi}{dy^2} E. \quad (2.16)$$

Integrating Equation 2.16 with respect to y twice and applying the conditions that $du/dy = 0$ and $d\psi/dy = 0$ as $y \rightarrow \infty$ (outside the EDL), and $\psi = \zeta$ (zeta potential) when $u = 0$ (no slip boundary condition at the shear plane), it results in:

$$u = \frac{-\varepsilon_o \varepsilon_r E \zeta}{\mu}, \quad (2.17)$$

which is commonly known as the Helmholtz-Smoluchowski equation (see Equation 2.7). This equation explains the plug-like velocity profile of EOF (see Figure 2.5b) for the region outside the EDL, but does not provide information on the flow field within the double layer.

Hence, to capture the influence of EDL on the flow field, the Debye-Hückel solution (see Equation 2.6) can be incorporated for the integration of Equation 2.16. Applying the no slip boundary condition at $y = 0$ and $du/dy \rightarrow 0$ when $y \rightarrow \infty$, it yields:

$$u(y) = \frac{-\varepsilon_o \varepsilon_r E \zeta}{\mu} (1 - e^{-ky}). \quad (2.18)$$

As dictated by Equation 2.18, the velocity rises exponentially from zero at the shear plane to a maximum value at the edge of the EDL. For cases where the Debye-Hückel linearization is not applicable (i.e. high electrostatic potentials), the non-linear Poisson-Boltzmann equation (see Equation 2.4) can be employed for solving the flow field in the EDL, which is typically accomplished through numerical analysis.

2.2.5 Surface charge formation

EOF occurs due to the formation of EDL, which is the consequence of the development of surface charge density on the channel wall (see Section 2.2.1). For glass/fused silica microchannels, the dissociation of silanol (SiOH) groups is the principal mechanism by which the wall surface acquires a surface charge. When an electrolyte solution comes into contact with the channel surface at $\text{pH} > 2$, deprotonation of the SiOH groups takes place, which leads to the development of a negative surface charge density (due to the presence of SiO^-) (Hunter 1988, Behrens and Grier 2001):

where H_2O represents the water molecule and H_3O^+ is the hydronium ion. The protonation of SiOH groups is only expected at extremely acidic conditions (Hiemstra et al. 1989, Hiemstra et al. 1996), and thus it is usually ignored.

Polymers, such as poly(methyl methacrylate) (PMMA), polydimethylsiloxane (PDMS) and cyclic olefin copolymer (COC), have gained popularity over glass/fused silica for microfluidic devices owing to their inexpensive cost, flexibility in fabrication, and desirable optical and chemical properties (Stone et al. 2004, Mela et al. 2005, Hawkins et al. 2007). However, there are challenges on the understanding of the electrokinetic effects when polymer-based devices are employed. This is due to a lack of proper understanding on the polymer surface chemistry on the origin of the surface charges (Tandon et al. 2008). Various investigations discovered that polymers have a lower zeta potential value compared to glass/fused silica (Tandon et al. 2009, Almutairi et al. 2012, Falahati et al. 2014), which results in a slower EOF. This is due to the polymers having less ionizable surface groups than glass/fused silica.

2.3 Conventional theoretical models for electroosmotic flow

The conventional theoretical models for EOF are classified into three main categories: (a) slip-velocity model, (b) Poisson-Boltzmann (PB) model and (c) Poisson-Nernst-Planck (PNP) model.

The simplest model for describing EOF is the slip-velocity model, which omits the variation of flow field within the EDL. The model assumes that the fluid flow is generated by the slip wall with the Helmholtz-Smoluchowski velocity equation (Equation 2.7), and is solved with the removal of electrical body force term (third term on the right of Equation 2.14) from the Navier-Stokes equation (Equation 2.14):

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u}. \quad (2.20)$$

Due to the simplicity and ease of use of the model, it has been adopted in various investigations, such as the time periodic EOF (Yan et al. 2009, Lim and Lam 2012) and EOF near sharp corners (Zimmerman et al. 2006, Craven et al. 2008),

The PB and PNP models are more complicated models for modelling EOF due to the inclusion of flow field variation in the EDL. This inevitably incurs a substantial amount of computational resources when implemented for numerical analysis. The PB model assumes that the distributions of ionic species follow the Boltzmann distributions (Equation 2.2), which are incorporated to the Poisson equation (Equation 2.3). The Navier-Stokes equation (Equation 2.14) can subsequently be solved to acquire the velocity flow field (Keh and Tseng 2001, Tang et al. 2004).

As for the PNP model, the ion distributions are determined by the Nernst-Planck equation (Probstein 1994, Kirby 2010):

$$\frac{\partial c_i}{\partial t} + \nabla \cdot \left[-D_i \nabla c_i - u_{m(i)} c_i \nabla (\phi + \psi) \right] = -\mathbf{u} \cdot \nabla c_i, \quad (2.21)$$

where ϕ is the applied electric potential, D_i is the diffusion coefficient and $u_{m(i)}$ is the ionic mobility of each ionic species. Since the wall potential, ion distributions and fluid velocity are strongly coupled, the Nernst-Planck equation (Equation 2.21), Poisson equation (Equation 2.3) and Navier-Stokes equation (Equation 2.14) have to be solved simultaneously to obtain the fluid flow field. The PNP model has been employed for

numerous investigations, for example, ion transport and EOF around cylindrical electrodes (Fernandes et al. 2010), and EOF entry flow in a microchannel (Yang et al. 2001).

The existing conventional models are mainly developed to describe a single fluid EOF. Therefore, they cannot reveal the critical phenomena of a two-fluid electroosmotic displacement flow process. The slip-velocity and PB models do not account for the electric field variation, which is the consequence of the evolving solution conductivity due to the transportation of ionic species during a two-fluid electroosmotic displacement flow process.

Moreover, the zeta potential at the wall boundary, an important parameter that dictates the direction and flow velocity of EOF, has to be prescribed *a priori* for the three types of theoretical models (slip-velocity, PB and PNP models) (Ren and Li 2001, Fu et al. 2003, Brotherton and Davis 2004). Indeed, zeta potential is changing and evolving according to the flow conditions when one fluid is displaced by another fluid. As such, the conventional theoretical models will not accurately reflect the flow behaviors of a two-fluid electroosmotic displacement flow process.

Hence, a generalized theoretical model is required to be formulated for a better elucidation of the two-fluid electroosmotic displacement flow process, as well as the revelation of the experimentally observed flow hysteresis as discussed in Section 2.5.

2.4 Electroosmotically driven flow involving two or more fluids

EOF has been employed in many microfluidic applications (see Section 1.2 in Chapter 1). In most of these applications, two or more types of fluids are typically involved. The following sub-sections present the literature on electroosmotically driven flow involving two or more fluids.

2.4.1 Electroosmotic displacement flow

Current monitoring method is a well-known technique for measuring the EOF velocity, which involves two-fluid electroosmotic displacement flow of solutions with a small concentration difference (typically 5% difference) (Huang et al. 1988). This method is simpler and convenient compared to the traditional methods such as neutral marker method (Lauer and McManigill 1986, Walbroehl and Jorgenson 1986) and weighing method (mass of electrolyte transferred) (Devasenathipathy and Santiago 2005). Figure 2.6 shows the experimental setup of the current monitoring technique as proposed by Huang et al. (1988).

Figure 2.6: Current monitoring setup for measuring EOF velocity. Reservoir 1 and reservoir 2 contained solutions with 5% concentration difference, which were connected by microcapillary T (Huang et al. 1988).

When a low concentration solution displaces a high concentration solution (or vice versa) in a microcapillary, electrical resistance across the channel changes due to the conductivity difference, which results in the electrical current to vary during the displacement flow as illustrated by Figure 2.7. Upon completion of the displacement process, the current reaches a steady value and the displacement time can be determined (see Figure 2.7). The average EOF velocity can then be calculated by dividing the length of microcapillary L with the displacement time t_d :

$$v_{avg} = \frac{L}{t_d}. \quad (2.22)$$

Figure 2.7: Current-time curve for 19 mM phosphate buffer displaced 20 mM phosphate buffer in the microcapillary (Huang et al. 1988).

Through substituting the average EOF velocity (Equation 2.22) into the Helmholtz-Smoluchowski slip velocity equation (Equation 2.7), the zeta potential can be obtained:

$$\zeta = \frac{-\mu}{\epsilon_r \epsilon_0 E} \cdot \frac{L}{t_d} \quad (2.23)$$

Over the years, the current monitoring technique has been refined and improved by various researchers (Sze et al. 2003, Almutairi et al. 2009, Saucedo-Espinosa and Lapizco-Encinas 2016) to increase the accuracy of EOF velocity/zeta potential measurement.

Several investigations in the literature had reported the application of electroosmotic displacement flow of solutions with large concentration/conductivity difference. Based on the electroosmotic displacement flow of a solution at a given concentration with solutions at different concentrations (see Figure 2.8), Mampallil et

al. (2010) had proposed a analytical model to determine the surface charge on the microchannel walls, as well as the zeta potential at the specific solution concentration. The zeta potentials of solutions and surface charges were obtained by fitting the experimental current-time curves to the theoretically calculated curves.

Figure 2.8: Experimental (full lines) and theoretically calculated (grey-dashed lines) current-time curves for 25 mM KCl displaced by KCl solutions of different concentrations. The insert illustrates the magnitude of zeta potential $|\zeta|$, as well as surface charge $|\sigma|$, as function of solution concentration C (Mampallil et al. 2010).

Theoretical investigation on the electroosmotic displacement flow of two or three solutions with a range of conductivity ratios between the solutions had been conducted by Tang et al. (2011). Based on Ohm's law and conservation of fluid mass, a series of length-averaged equations were derived to describe the variations of electrical current and two-fluid interface with time. It was found out that the induced internal

pressures due to the conductivity mismatch can affect the propagation of two-fluid interface and the transient current behavior. The applicability of the theoretical analysis was verified by the excellent agreement with the experimental results in Huang et al. (1988) (see Figure 2.9).

Figure 2.9: Comparison between the theoretical prediction by Tang et al. (2011) and experimental data by Huang et al. (1988).

Ren et al. (2001) and Ren et al. (2003) had performed both theoretical and experimental studies on two-fluid electroosmotic displacement flow. They adopted Ohm's law with the inclusion of mixing zone between the two different solutions in their theoretical model, which is different from the work by Tang et al. (2011). In their experiments, they employed the deionized ultrafiltered (DIUF) water, 10 mM potassium chloride (KCl), 0.1 mM KCl and 0.1 mM lanthanum chloride (LaCl_3) solutions as the testing fluids. Good agreement between experimental results and the model predictions was observed (see Figure 2.10). However, the ionic mobility of each species was not taken into account in the theoretical model. Hence, the model might not

be sufficient for describing electroosmotic displacement flow of solutions with dissimilar ionic species.

Figure 2.10: Comparison between experimental and theoretically predicted current-time curves for electroosmotic displacement flows of (a) 10 mM KCl and DIUF water, (b) 0.1 mM KCl and 10 mM KCl, and (c) 0.1 mM LaCl_3 and 0.1 mM KCl (Ren et al. 2001).

2.4.2 Ion pre-concentration techniques

Field amplified sample stacking (FASS) is one of the popular pre-concentration techniques in microfluidic devices. The principle behind FASS is that an axial gradient in electric field is achieved by preparing the conductivity of the sample matrix lower than the background electrolyte (BGE) (Bharadwaj and Santiago 2005, Sustarich et al. 2010) (see Figure 2.11). Upon application of a constant voltage, the sample region acts as a high-electrical-resistance zone which results in a locally high electric field. Under an electric field, there will be migration of sample ions from the high to low drift velocity region. Local accumulation/stacking of sample ions will occur near the interface between regions of high and low conductivities (see Figure 2.11). Extensive review on the recent developments of FASS and its applications can be found in the literature (Giordano et al. 2012, Šlampová et al. 2013).

Figure 2.11: Field amplified sample stacking (FASS) of anionic species in the absence of EOF (Bharadwaj and Santiago 2005).

Another well-known ion pre-concentration technique is the isotachopheresis (ITP). In contrast, ITP is typically conducted under constant current which is critical in ensuring dynamic equilibrium; all ions are moving at the same speed (Delmotte 1979, Garcia-Schwarz et al. 2011, Smejkal et al. 2013). ITP requires a leading electrolyte (LE) that has a higher ionic mobility than the sample components, and a terminating electrolyte (TE) that has a lower ionic mobility than the sample components. The sample components form focused/concentrated zones in order of ionic mobility behind the LE and ahead of the TE (see Figure 2.12).

Figure 2.12: Principal of isotachopheresis (ITP).

EOF occurs naturally during FASS and ITP as the electric field applied to stack or separate the ions tend to drive the fluid motion. The non-uniform EOF velocities due to the mismatch in solution conductivities generate internal pressure gradients that cause unwanted sample dispersion, which affects the sensitivity and resolution of such applications. The resultant frustrated flow profile is a superposition of a plug-like profile of EOF and a parabolic profile of pressure driven flow (Devasenathipathy et al. 2007, Chang and Yeo 2010). Sample dispersion can be reduced by suppressing EOF through coating of water-soluble polymers (Horvath and Dolnik 2001, Hou et al. 2010), e.g. acidified poly(ethylene oxide) (PEO) (Preisler and Yeung 1996), or addition of

additives to the BGE (Mallampati et al. 2010). There are also methodologies which exploit EOF to achieve the desired ion pre-concentration and transport effect in a micro/nanofluidic channel (Sustarich et al. 2010, Zhang and Zhang 2011, Hsieh et al. 2012).

2.5 Hysteresis in two-fluid electroosmotic displacement flow

Gan et al. (2006) performed an investigation on two-fluid electroosmotic displacement flow. They developed a theoretical model which took into account the axial step change of velocity flow fields at the two-fluid interface, continuity of flow and induced local pressure gradient. The model captured the non-linear current change during the electroosmotic displacement flow process.

The theoretical model predicted that the displacement time for EOF of 10 mM and 0.5 mM sodium chloride (NaCl) solutions, i.e. the time required for the current-time curve to reach a steady-state value, was the same regardless of the displacement flow direction (see Figure 2.13). However, it was observed that the displacement time for 10 mM NaCl displaced 0.5 mM NaCl was shorter than the displacement time for 0.5 mM NaCl displaced 10 mM NaCl (see Figure 2.13). This experimentally observed flow direction-dependent behavior cannot be predicted by their model.

Figure 2.13: Experimental and theoretical current-time curves for electroosmotic displacement flow of 0.5 mM NaCl and 10 mM NaCl in two different flow directions (Gan et al. 2006).

Lim and Lam (2012) investigated the flow direction-dependent behavior of two-fluid electroosmotic flow with various concentration differences. Their current monitoring experiments verified that the response of the system indeed exhibited a flow direction-dependent behavior, whereby the displacement time for a high concentration KCl solution to displace a low concentration KCl solution was shorter than that in the reverse flow direction. It was found that the percentage displacement time difference increased with increasing concentration difference, and was independent of the applied voltage, diameter and length of the microchannel (see Figures 2.14a, 2.14b and 2.14c).

Figure 2.14: Percentage displacement time difference between both directions for KCl with various percentage concentration differences at different (a) applied voltages, (b) diameters and (c) lengths of microchannels (Lim and Lam 2012).

Subsequently, Lim and Lam (2012) attempted to explain the abovementioned flow direction-dependent behavior, which is termed as “EOF hysteresis” in this thesis, through the development of a numerical model with finite element method (FEM). The mechanism for this hysteresis was believed to be caused by the distortion of ion distributions in the EDL induced by the internal pressure gradient generated due to the mismatch of fluid velocities during the displacement flow process.

However, the simulations were performed only on low concentration solutions, and the simulation results disagreed quantitatively with the experimental results by a large margin (see Figure 2.15). This indicates that this type of EOF hysteresis may not

be caused by the changes of ion distributions in the EDL, and the mechanisms are yet to be properly elucidated.

Figure 2.15: Comparison between experimental and numerical results for displacement flow of KCl solutions with 80% concentration difference in two different flow directions (Lim and Lam 2012).

EOF hysteresis might affect the accuracy of EOF velocity/zeta potential measurements by the current monitoring technique, which involves two-fluid electroosmotic displacement flow. Therefore, an in-depth understanding of EOF hysteresis will provide an accurate interpretation of the results obtained from the current monitoring method.

In applications such as ion pre-concentration techniques, the electric field is typically applied across fluids of different conductivities to achieve accumulation/focusing of the desired species. The effect of EOF hysteresis can also be

potentially exploited to provide alternative solutions to minimize dispersion and increase stacking efficiency for ion pre-concentration techniques.

2.6 Other hysteresis phenomena in electrokinetically-driven applications

Recently, other hysteresis phenomena in electrokinetically-driven applications had been discovered. In the investigation carried out by Rosentsvit et al. (2015), inversion effect of ion current rectification (ICR) was illustrated in a funnel-shaped nanochannel at voltage threshold approximately equivalent to an under-limiting and over-limiting current regime transition, which differs from the previous investigations on ICR at either under-limiting current region (Weit et al. 1997, Siwy 2006, White and Bund 2008) or over-limiting current region (Jung et al. 2009, Yossifon et al. 2009, Yan et al. 2013). The occurrence of the rectification inversion was due to the shifting of system resistance from being manipulated by the intra-channel ion concentration polarization to external concentration polarization.

The important finding revealed by Rosentsvit et al. (2015) was the hysteresis effect observed during the extraction of the rectification factor, which originated from the residual concentration polarisation and was dependent of the voltage scan rates. The hysteretic behavior (difference in measured ICR-V between different scan directions) was prominent with increasing scan rate (see Figure 2.16a). The experimental results agreed qualitatively with the numerical results (see Figure 2.16b). The understanding of

such hysteresis phenomenon is critical for preventing the current rectification studies from being influenced by the dependency of scan rate and field polarity at non steady-state conditions.

Figure 2.16: (a) Experimental ICR-V response for various scan rates and scan directions. (b) COMSOL numerical simulation results (Rosentsvit et al. 2015).

Yan et al. (2016) reported a robust oscillatory ion-mass flux behavior across a porous monolith attached to a capillary (see Figure 2.17a) under a steady electric field. The oscillations (see Figure 2.17b) were driven by the hysteretic wetting-dewetting dynamics of the air-liquid meniscus when EOF and pressure driven backflow (because

of expanding bubble) competed to achieve balanced flow (zero mass flux) in the monolith. The prolonged bubble advancement (dewetting) triggered a delayed film rupture that cut the circuit and suppressed EOF, which caused the bubble to recede until the rupture was restored (see Figure 2.17c), initiating and sustaining oscillations under a range of applied voltages.

This artificial system exploited the hysteresis phenomenon of the meniscus dynamics to sustain a robust ion current oscillation under steady electric field, and is comparable with the ion channel oscillations (Wang et al. 2010, Hsiao et al. 2011). Hence, the proposed system can serve as an excellent biomimetic model for ion current oscillation in an ion channel.

Figure 2.17: (a) Schematic of experimental setup. (b) Time series of current oscillation at different voltages. (c) On-off state switch position for the voltage 2.0 kV oscillation (Yan et al. 2016).

The exploitation of different hysteresis phenomena for electrokinetically-driven applications in the abovementioned investigations have indicated the possibility for EOF hysteresis being utilized in practical applications.

2.7 EOF reduction by micro-/nanostructures within micro-/nanochannels

Several theoretical/numerical studies had been conducted to investigate the effects of micro-/nanostructures on EOF in micro-/nanochannels. Hu et al. (2003), Hu et al. (2004) and Hu et al. (2007) investigated the effect of microscale rectangular prismatic elements on EOF in microchannel (see Figure 2.18a) with the slip-velocity model. In their study, they demonstrated that the electric field was distorted by the rectangular prismatic elements which resulted in non-uniformity of the EOF velocity (see Figure 2.18b). The presence of the rectangular prismatic elements in the flow passage induced a periodic pressure field (see Figure 2.18c) that greatly reduced the EOF flow rate.

Figure 2.18: (a) 3-D computational grid system for symmetrically arranged rectangular prismatic elements. (b) Slip boundary velocity and (c) induced pressure distributions on the bottom plane of rough microchannel (Hu et al. 2003).

Kang and Suh (2009) employed the PNP model to numerically investigate the two-dimensional (2-D) EOF in a microchannel with rectangle-waved surface structures. They found that the fluid flowed along the surface of waved wall without any flow separation (see Figure 2.19a), and the EOF flow rate decreased exponentially with the amplitude of waved wall (see Figure 2.19b) and increased linearly with the periodic length (see Figure 2.19c).

Figure 2.19: (a) EOF streamlines. Variation of flow rate $Q/2$ with the wall-wave (b) amplitude h and (c) periodic length L (Kang and Suh 2009).

Kim and Darve (2006) performed molecular dynamics simulations to study the effect of 2-D subnanoscale grooves and ridges (rectangle-waved surface structures) (see Figure 2.20a) on EOF in a nanochannel. The velocity profiles corresponded to those with overlapping EDL (Rice and Whitehead 1965, Qu and Li 2000, Stein et al. 2004, Pennathur and Santiago 2005), and the EOF flow rate was found to decrease with decreasing period (see Figure 2.20b) and increasing amplitude (see Figure 2.20c) of the surface structures, which coincides with the investigation of Kang and Suh (2009).

Figure 2.20: (a) Model system for 2-D subnanoscale grooves and ridges with spatial period p and amplitude A . EOF flow rate variation with (b) different periods (for both symmetric and asymmetric wall channels) and (c) different amplitudes (Kim and Darve 2006).

In contrast, Messinger and Squires (2010) demonstrated that sinusoidal nanostructures can significantly suppress EOF when excess surface conductivity within the EDL is appreciable. Upon application of an electric field over the sinusoidal rough surface, the tangential component of the electric field was inhomogeneous which yielded an inhomogeneous slip velocity (see Figure 2.21a). Appreciable surface conductivity led to gradients of the excess counterion surface current where conservation required balancing by the normal counterion flux and electric field (see Figure 2.21b), which reduced the average tangential electric field and EOF velocity.

Figure 2.21: (a) Sinusoidal rough surface results in inhomogeneous tangential electric field $E_{||}$ and slip velocity $u_{||}$. (b) Appreciable surface conductivity ($Du \geq 1$) causes gradients in the excess counterion surface current J_s , and conservation requires balancing by the normal counterion flux $J_\perp$ and electric field $E_\perp$ (Messinger and Squires 2010).

Limited experimental studies have been conducted due to the difficulties in the fabrication of micro-/nanochannels with micro-/nanostructures. Koga et al. (2013) investigated the effect of random nanostructures on EOF with PDMS-glass hybrid microchannels, with the glass surfaces treated with dry and wet etchings (see Figure 2.22a). Their investigation revealed the strong correlation between the EOF velocity and surface roughness Ra (in scale of nanometres); the surfaces treated with dry etching (higher Ra) showed a significant reduction in EOF velocity as compared to the surfaces treated with wet etching (lower Ra) (see Figure 2.22b).

Figure 2.22: (a) PDMS-glass hybrid microchannel (glass surface treated with either dry or wet etching). (b) EOF velocity as a function of surface roughness (Koga et al. 2013).

Yasui et al. (2011) fabricated nanopillar array structures in microchannels on quartz chips (see Figure 2.23a) by electron beam lithography (EBL), photolithography and reactive ion etching (RIE). They demonstrated that the nanopillar array structures had an intrinsic ability to suppress EOF (see Figure 2.23b), and the EOF mobility was affected by the total number of nanopillars inside the microchannel (see Figure 2.23c). However, their fabrication method was prohibitively costly and time consuming for large-area structuring, a limiting factor for a detailed investigation.

Indeed, even though Yasui et al. (2011) reported that there was no difference between the square and tilted nanopillar array patterns in EOF suppression (see Figures 2.24a, 2.24b and 2.24c), the orientation difference between these two arrangements might be insignificant for a significant difference in the EOF velocity to be detected.

Figure 2.23: (a) Photo of nanopillar chip with scanning electron microscopy (SEM) image of the nanopillar array channel. (b) Logarithmic plot of EOF mobility in the microchannel, nanochannel and nanopillar chips. (c) Dependence of EOF mobility on the number of nanopillars in the channel at 5X TBE (Yasui et al. 2011).

Figure 2.24: (a) Square and tilted nanopillar array patterns respectively. Logarithmic plot of EOF mobility in nanopillar chips arranged in (b) square and (c) tilted array patterns (Yasui et al. 2011).

Despite the vast investigations on EOF reduction due to micro-/nanostructured surfaces in micro-/nanochannels (both numerically and experimentally), a proper study on the orientation effect of nanostructures on EOF within a microchannel is yet to be conducted.

2.8 Micro- and nanofabrication techniques

For a rigorous investigation on the effect of nanostructure orientation on EOF, nanostructure designs in the microchannels should have a significant orientation difference for experimental studies, which allow further verifications by numerical simulations. The micro- and nanostructures produced should possess good regularity, cover a relatively large area and can be mass-produced. Despite the availability of various micro- and nanofabrication techniques, most of them do not satisfy the aforementioned criteria.

Traditional methods to produce micro-/nanostructured devices include micro-electro-mechanical system (MEMS) fabrication technologies (Li et al. 2003, Fedder et al. 2008) and rapid prototyping techniques, such as micromilling (Friedrich et al. 1997, Kummrow et al. 2009), laser micromachining (Rossier et al. 1999, Waddell et al. 2002) and PDMS soft lithography (Duffy et al. 1998, Abdelgawad et al. 2008). Although these methods are well-established with their own advantages, they suffer from certain drawbacks.

The main benefits of micromilling and laser micromachining are the high repeatability, flexibility and cost efficiency (Tanvir Ahmmed et al. 2014, Guckenberger et al. 2015). However, their resolutions are limited at micrometer level. Although PDMS soft lithography is popular for its low material costs, fast prototyping and optical transparency (McDonald et al. 2000, McDonald and Whitesides 2002), the filling of high viscosity PDMS into nanoscale features on the master mold remains a challenge (Kang et al. 2005, Koo et al. 2007, Con and Cui 2013).

MEMS technologies, such as lithography and wet/dry etching, allow the fabrication of nanoscale patterns along with microscale structures on silicon (Si) or glass platform (Schlautmann et al. 2003, Archer and Ligler 2008), but the complexity of the process lowers the throughput and increases the cost, especially for single-use microfluidic devices.

Polymer-based replication methods, such as hot embossing (subset of nanoimprint lithography (NIL)) (Heyderman et al. 2000, Ting et al. 2008, Jena et al. 2012) and injection molding (Benitez-Rangel et al. 2007, Nguyen et al. 2011, Utko et al. 2011), are compatible with the requirements of low material costs and high replication accuracy. Among these methods, injection molding is more suitable for mass production due to the short cycle times. However, conventional injection molding tools are expensive and time consuming to fabricate. Furthermore, the machining of the molding tools is challenging for nanoscale features. To overcome these shortcomings, MEMS technologies can be employed for the fabrication of masters for injection molding.

Many cleanroom-based techniques have been reported for the creation of mold inserts (Andresen et al. 2010, Tanzi et al. 2012). The most commonly used technique is LIGA (**L**ithographie, **G**alvanoformung, **A**bformung, which means Lithography, Electroplating, Molding in German) (Piotter et al. 1997, Kupka et al. 2000). This technique may be categorized into two main types, namely X-ray LIGA and UV LIGA, that utilize highly collimated synchrotron X-rays and ultraviolet light respectively. Although X-ray LIGA enables the production of high aspect ratio structures, the process is complicated and requires an expensive synchrotron facility. In contrast, UV LIGA is cheaper and more accessible due to the use of inexpensive UV light source and less sophisticated chromium mask, but it creates relatively low aspect ratio structures.

Despite the employment of versatile polymer photoresist, LIGA faces technical difficulties when multilevel structuring and nano-range precision molds are needed. DEEMO (**D**ry **E**tching, **E**lectroplating, **M**olding) (Elders et al. 1995) substitutes the lithography step of LIGA with dry etching, which makes it possible to produce structures of feature sizes from nanometers to micrometers. However, multilevel structuring involving both micro- and nanoscale still remains a challenge for DEEMO, as the dry etching process might destroy the regularity of the nanoscale patterns.

Both the LIGA and DEEMO processes have their own unique strengths and weaknesses for producing regular micro- and nanostructures that cover a large area. Hence, these two fundamentally different methodologies have the potential to be combined to exploit their individual strengths for the fabrication of nanostructure

designs within microchannels that can demonstrate maximum orientation difference for the examination of the orientation effect of nanostructures on EOF in a microfluidic channel.

2.9 Summary

EOF is one of the electrokinetic phenomena that arise due to the formation of EDL when a solid surface is in contact with an aqueous solution. EOF has been employed for various microfluidic applications which normally involve two or more types of fluids. Electroosmotically driven flow involving two or more fluids includes electroosmotic displacement flow and ion pre-concentration techniques.

However, there is an experimentally observed anomaly in two-fluid electroosmotic displacement flow for solutions with *different concentrations*. The displacement time for a high concentration solution to displace a low concentration solution is shorter than that for the reverse direction, demonstrating that the flow behavior is dependent on the flow direction. This hysteresis phenomenon is termed as “EOF hysteresis” in this thesis. Theoretical/numerical studies had been attempted to explain this hysteretic behavior. However, hitherto there were significant discrepancies between the theoretical/numerical predictions and experimental observations.

The conventional theoretical models such as the slip-velocity, PB and PNP models are mainly developed to describe a single fluid EOF. They cannot reveal the critical phenomena of two-fluid electroosmotic displacement flow. For example, for

these models, the zeta potential at the wall boundary has to be prescribed *a priori*, instead of allowing it to vary according to the flow conditions when one fluid is displaced by another fluid. Thus, a generalized theoretical model is required to be formulated for a better elucidation of the two-fluid electroosmotic displacement flow process, as well as the revelation of the experimentally observed EOF hysteresis.

It is essential to uncover the mechanics of this EOF hysteresis, and to investigate, if any, the presence of other hysteresis effects, i.e. EOF hysteresis for solutions with *dissimilar ionic species*. EOF hysteresis might affect the accuracy of EOF velocity/zeta potential measurements through the current monitoring method, which involves two-fluid electroosmotic displacement flow. Therefore, an in-depth understanding of EOF hysteresis will provide an accurate interpretation of the results obtained via the current monitoring method.

In applications such as ion pre-concentration techniques, the electric field is typically applied across fluids of different conductivities to achieve accumulation/focusing of the desired species. The effect of EOF hysteresis can be potentially exploited to provide alternative solutions to minimize dispersion and increase stacking efficiency for ion pre-concentration techniques. Recent investigations on the exploitation of other hysteresis phenomena in electrokinetic driven applications, such as ion current rectification and oscillation, have also indicated the possibility for EOF hysteresis being utilized in practical applications.

Nanoscale networks/structures are often integrated within microchannels for numerous applications. The presence of micro-/nanostructures in micro-/nanochannels had been discovered to have a suppression effect on EOF. Despite the many investigations, the orientation effect of nanostructures within a microchannel on EOF has yet to be fully investigated. For a rigorous investigation, microchannels with nanostructures designs that demonstrate a significant orientation difference should be fabricated to allow for rigorous experimental studies, which will then be verified by numerical simulations. However, the existing micro-/nanofabrication techniques cannot fulfil the requirements of producing micro- and nanostructures of good regularity, cover a relatively large area and suitable for mass production.

Polymer injection molding that makes use of the clean-room based MEMS technologies for the creation of a master mold insert will allow the mass production of micro-/nanoscale patterns or structures. The two commonly employed techniques are LIGA and DEEMO. Both techniques have their own strengths and weaknesses for producing regular micro- and nanostructures that cover a large area. Hence, these two fundamentally different methodologies have the potential to be combined to exploit their strengths for the fabrication of nanostructure designs in microchannels with maximum orientation difference for the examination of the orientation effect of nanostructures on EOF.

Chapter 3 A generalized theoretical model for two-fluid electroosmotic displacement flow

3.1 Introduction

The conventional theoretical models for electroosmotic flow (EOF) contain critical limitations for describing a two-fluid electroosmotic displacement flow. The existing models are mainly developed to describe a single fluid EOF. They cannot reveal the critical phenomena of two-fluid electroosmotic displacement flow. For example, the zeta potential of the channel wall is prescribed *a priori* in the conventional models, instead of allowing it to vary according to the flow conditions. As such, it will not accurately reflect the flow behaviors of two-fluid electroosmotic displacement flow.

Hence, in this chapter, a generalized theoretical model will be formulated for a better elucidation of the two-fluid displacement flow process. The necessity of each equation of the model will be explained. As substantial computation effort is required when the generalized model is fully implemented for numerical simulation, the model will be simplified for specific experimental conditions to ease the demand on computational resources.

3.2 Formulation of a generalized theoretical model for two-fluid electroosmotic displacement flow

3.2.1 Laplace equation

An external electric field is applied across a microchannel to induce EOF, which generates current across the channel. Assuming that there is no source or sink in the medium, charge conservation requires the divergence of current density $\mathbf{J}$ to be equal to zero (Lim and Lam 2012):

$$\nabla \cdot \mathbf{J} = 0. \quad (3.1)$$

The relationship between the current density $\mathbf{J}$ and the transport of ionic species is given as (Hunter 1988, Probstein 1994, Kirby 2010):

$$\nabla \cdot \left[\sigma \nabla \phi + F \sum z_i D_i \nabla c_i - \mathbf{u} F \sum z_i c_i \right] = 0, \quad (3.2)$$

where the solution conductivity $\sigma = F \sum z_i u_{m(i)} c_i$, ϕ is the applied electric potential, F is the Faraday constant, $\mathbf{u}$ is the fluid velocity, D_i is the diffusion coefficient, z_i is the ion charge number, $u_{m(i)}$ is the ionic mobility and c_i is the concentration of each ionic species.

Equation 3.2 consists of the electromigrative, diffusive and convective current densities. The first term describing the electromigrative current (also known as the conduction current) is the major contributor; the second and third terms describing respectively the diffusive and convective currents can be neglected due to their small magnitudes (Arulanandam and Li 2000, Lim and Lam 2012). As such, Equation 3.2

reduces to the Laplace equation which governs the applied electric potential distribution (Lim and Lam 2012):

$$\nabla \cdot (\sigma \nabla \phi) = 0. \quad (3.3)$$

The single fluid EOF described by the existing conventional models, such as the slip-velocity and Poisson-Boltzmann (PB) models, has no variation of electric field (refer to Section 2.3 in Chapter 2). As such, Laplace equation (Equation 3.3) is not necessary. In contrast, the inclusion of the Laplace equation (Equation 3.3) here, in the generalized theoretical model, is a necessity to allow for a proper description of the critical phenomenon of electric field variation during two-fluid electroosmotic displacement flow due to the evolving conductivity of the solution.

3.2.2 Navier-Stokes and continuity equations

EOF occurs when the non-zero net charge in the diffuse layer of electrical double layer (EDL) (refer to Section 2.2.1 in Chapter 2 for details on charge accumulation) experiences Coulomb force caused by the applied electric field. Similar to other conventional models, the fluid flow (for an incompressible Newtonian fluid) in the generalized theoretical model is described by the Navier-Stokes and continuity equations, which are respectively written as (Hunter 1988, Probst 1994, Kirby 2010):

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u} + \rho_e \left[-\nabla (\phi + \psi) \right], \quad (3.4)$$

$$\nabla \cdot \mathbf{u} = 0, \quad (3.5)$$

where p is the pressure, $\rho_e = F \sum c_i z_i$ is the net charge density, ρ is the density, μ is the viscosity of fluid and ψ is the electrostatic wall potential distribution.

In microfluidics, the Reynolds number is typically less than 1 due to the small channel size (Yang et al. 2001). Thus, the inertial term (second term on the left of Equation 3.4) is usually ignored, and Stokes flow is assumed. The wall potential induced body force can also be neglected from the electric body force term (third term on the right of Equation 3.4) as it is presumed to be balanced by the pressure gradient normal to the channel wall (Kim et al. 2011). As such, Equation 3.4 can be simplified to:

$$\rho \frac{\partial \mathbf{u}}{\partial t} = -\nabla p + \mu \nabla^2 \mathbf{u} + \rho_e \left[-\nabla \phi \right]. \quad (3.6)$$

3.2.3 Poisson equation and charge-regulated wall condition

The electrostatic wall potential distribution ψ (due to the presence of charged wall) is given by the Poisson equation (Hunter 1988, Probstein 1994, Kirby 2010):

$$\nabla \cdot \nabla \psi = -\frac{\rho_e}{\epsilon_r \epsilon_o}, \quad (3.7)$$

where ε_o is the permittivity of free space and ε_r is the relative permittivity of fluid. To allow the description for the variation of zeta potential ζ (electrostatic potential at the shear plane of EDL) according to the local net charge density, which varies with the concentrations of ionic species during two-fluid displacement flow, a surface charge density is specified as the wall condition instead of a constant zeta potential.

Without the Poisson equation (Equation 3.7) together with the specified surface charge density as the wall condition, the conventional models such as the slip-velocity, PB and Poisson-Nernst-Planck (PNP) models will not be able to predict any zeta potential variation. As such, the zeta potential of the channel wall has to be prescribed *a priori* (refer to Section 2.3 in Chapter 2). This is not a deficiency for a single fluid EOF as the zeta potential is a constant, and therefore can be prescribed *a priori*. However, for two-fluid displacement flow, the zeta potential of the channel wall will not remain constant, and is evolving when one fluid is displaced by another fluid. It is therefore cannot be prescribed *a priori*, and its relationship with changes of fluid properties during displacement flow has to be established.

An origin of EOF hysteresis (flow direction-dependent behavior during electroosmotic displacement of solutions with different concentrations, refer to Section 2.5 in Chapter 2) is hypothesized in the current investigation to be caused by the pH-governing minority ions, i.e. hydronium (H_3O^+), hydroxide (OH^-), bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions, which is rather counter-intuitive, and is typically neglected in traditional EOF studies. To reveal the validity of the hypothesis, charge regulation (Yeh et al. 2012) due to the deprotonation/protonation surface reactions occurring on

the functional groups of the solid/liquid interface, is necessary to be incorporated to the generalized theoretical model. This will allow the revelation of the interplay between changes of zeta potential and pH changes due to the pH-governing minority ions.

The deprotonation reaction of silanol (SiOH) groups (see Equation 2.19 in Chapter 2) is assumed to occur when the glass/silica surface is in contact with the electrolyte solution. The relation between the specified surface charge density and the charge-regulated nature of the wall surface may be written as (Yeh et al. 2012):

$$S_{(Charge-regulated)} = \frac{-eN_{Total}K_A}{K_A + c_{H_3O^+}}, \quad (3.8)$$

where e is the elementary charge, N_{Total} is the charge site density for SiOH groups, K_A is the equilibrium constant for deprotonation of SiOH and $c_{H_3O^+}$ is the concentration of H_3O^+ ions.

The Poisson equation (Equation 3.7), along with the specified surface charge density (Equation 3.8) at the wall boundary, allows the local zeta potential to vary as functions of the concentration and pH of the solution. These have a direct impact on the EOF flow velocity depending on the displacement directions for a two-fluid displacement flow.

3.2.4 Nernst-Planck equation

The transport of all ionic species is described by the Nernst-Planck equation. Unlike the conventional PNP model, the generalized theoretical model includes the transport of pH-governing minority ions, which is hypothesized here as a possible origin of EOF hysteresis for solutions with different concentrations.

The change of ion concentration with time is governed by the overall reaction rate and the gradients of three types of fluxes, namely diffusive, electromigrative and convective fluxes, which may be written as:

$$\frac{\partial c_i}{\partial t} + \nabla \cdot \left[-D_i \nabla c_i - u_{m(i)} c_i \nabla (\phi + \psi) \right] + \mathbf{u} \cdot \nabla c_i = R_i, \quad (3.9)$$

where R_i is the total reaction rate of each of the pH-governing ionic species. The total reaction rate R_i is defined as the summation of reaction rate r from the reversible acid-base chemical reactions (see Section 3.2.5). For the main constituent ions which do not participate in any reversible acid-base chemical reactions, R_i will be zero.

3.2.5 Reversible acid-base chemical reactions

The minority pH-governing ions are dependent on one another via a set of reversible acid-base reactions. The generalized theoretical model is formulated in terms of the kinetics of forward and reverse reactions for the dissociation of the weak acids/bases (Musvoto et al. 2000).

For instance, consider the auto-ionization of water (H₂O) (Musvoto et al. 2000):

The rate of the forward reaction r_f is:

$$r_f = K_{fw} [\text{H}_2\text{O}]^2, \quad (3.11)$$

where K_{fw} is the apparent specific rate constant for the forward reaction. The value of $[\text{H}_2\text{O}]$ is assumed to be a constant, and takes the value of 1. The rate of the reverse reaction r_r is:

$$r_r = K_{rw} [\text{OH}^-] [\text{H}_3\text{O}^+] = K_{fw} / K_w [\text{OH}^-] [\text{H}_3\text{O}^+], \quad (3.12)$$

where K_{rw} is the apparent specific rate constant for the reverse reaction, which can be derived by dividing K_{fw} with the water ionization constant K_w .

With the forward and reverse reaction rates, the reaction rate r can then be computed from the acid-base equilibria and summed to obtain the total reaction rate R_i , which will be included into the Nernst-Planck equation (Equation 3.9) for determining the concentration of a particular species.

The HCO_3^- ions exist in the unbuffered solutions (e.g. potassium chloride (KCl) solution) as a result of dissolution of atmospheric carbon dioxide (CO₂) in water. For this reason, the water and carbonate systems have to be considered when unbuffered solutions are employed for two-fluid displacement flow. The dissolved CO₂ in

equilibrium with the carbonic acid (H_2CO_3), represented by H_2CO_3^* , undergoes deprotonations through the following acid-base equilibria (Musvoto et al. 2000):

To obtain the fluid flow field for two-fluid electroosmotic displacement flow, the Navier-Stokes and continuity equations (Equations 3.5 and 3.6) have to be solved simultaneously with the Laplace equation (Equation 3.3), Poisson equation (Equation 3.7), Equation 3.8 governing the charged-regulated wall condition, and the Nernst-Planck equation (Equation 3.9) which has to include the reversible acid-base chemical reactions for the pH-governing ionic species, i.e. H_3O^+ , OH^- , HCO_3^- and CO_3^{2-} ions.

The differences between the formulated generalized theoretical model for two-fluid electroosmotic displacement flow and the conventional EOF models, as well as its importance for analysis in the subsequent chapters are summarized in Table 3.1.

Table 3.1: Comparison of the generalized theoretical model for two-fluid electroosmotic displacement with the conventional EOF models, and its importance for analysis in the subsequent chapters.

Capability	Slip-velocity model	Poisson-Boltzmann (PB) model	Poisson-Nernst-Planck (PNP) model	Generalized theoretical model	Importance for analysis in subsequent chapters
Allow electric field variation due to evolving solution conductivity.	✗	✗	✓	✓	This accounts for the electromigrative flux imbalance which results in the accumulation/depletion of the pH-governing minority ions (Chapters 4 and 5), and the concentration adjustment of the main constituent ionic species (Chapters 6 and 7).
Account for transportation of main constituent ionic species during two-fluid electroosmotic displacement flow.	✗	✗	✓	✓	For capturing the transportation of main constituent ionic species under diffusive, electromigrative and convective effects, which in turn affect the zeta potential variation during two-fluid electroosmotic displacement flow (Chapters 6 and 7).
Include transportation of minority pH-governing ionic species with their reversible acid-base chemical reactions.	✗	✗	✗	✓	To capture the transportation of minority pH-governing ionic species under diffusive, electromigrative, convective and reaction effects, which influence the zeta potential variation due to pH changes during two-fluid electroosmotic displacement flow (Chapters 4 and 5).
Capture zeta potential variation with concentrations of main constituent ionic species.	✗	✗	✗	✓	To describe zeta potential variation with the concentration adjustment of main constituent ionic species responsible of the EOF hysteresis (flow discrepancies) for solutions with dissimilar ionic species (Chapters 6 and 7).
Revelation of interplay between changes of zeta potential and pH changes due to minority pH-governing ions.	✗	✗	✗	✓	To capture the relationship between zeta potential and pH changes attributed by the accumulation/depletion of the minority pH-governing ions, which accounts for the origin of EOF hysteresis for solutions with different concentrations (Chapters 4 and 5).

3.3 Simplification of generalized model for displacement flow of solutions with different concentrations

The governing equations of the generalized theoretical model (see Section 3.2) for the applied electric potential, electrostatic wall potential, multi-ion species interaction and fluid flow are strongly coupled. Hence, substantial computational resources are required when the model is fully implemented for numerical simulation. For the investigations in this thesis, the computational resources needed for the full numerical simulation are far beyond the capability of the customized computer available, namely Intel® Xeon® CPU E5-2687W 0 @ 3.10GHz 3.10GHz (2 processors) with 512GB of RAM. Appropriate simplifications are desirable to reduce the computation effort for the investigation of the origin of EOF hysteresis for solutions with different concentrations, which is hypothesized to be caused by the pH-governing minority ions. The validation of the hypothesis is to be examined in Chapters 4 and 5.

When the EDL thickness is small compared to the characteristic size of the microchannel, flow within the EDL can be excluded from the model. As such, only the flow of the bulk fluid (with zero net charge density) will be modelled, and the electric body force (third term on the right of Equation 3.6) can be disregarded. This simplifies the Navier-Stokes equation (Equation 3.6) to:

$$\rho \frac{\partial \mathbf{u}}{\partial t} = -\nabla p + \mu \nabla^2 \mathbf{u} . \quad (3.15)$$

As such, the generalized model is simplified to a slip-velocity model, resulting in the loss of information within the EDL. However, this does not affect the integrity of the model in describing the origin of EOF hysteresis for solutions with different concentrations, which is caused by the pH-governing minority ions which do not reside in the EDL.

Unlike the conventional slip-velocity model, the electric field variation during two-fluid displacement flow is described by the Laplace equation (Equation 3.3), which is required to specify the slip wall boundary condition (see Equation 2.7 in Chapter 2) for solving the simplified Navier-Stokes (Equation 3.15) and continuity (Equations 3.2) equations.

The exclusion of EDL means that the Poisson equation (Equation 3.7) for describing the charge-regulated wall condition and its relationship with zeta potential can no longer be employed. A different equation has to be formulated to govern the zeta potential, which is required for specifying the slip wall boundary condition (see Equation 2.7 in Chapter 2). During two-fluid displacement flow, the local zeta potential along the microchannel varies according to the local ion distributions. The surface charge density which governs this is determined by the Grahame equation (for a symmetric electrolyte) (Kirby 2010):

$$S_{(\text{Grahame})} = \sqrt{8c_o \varepsilon_o \varepsilon_r RT} \sinh\left(\frac{zF\zeta}{2RT}\right), \quad (3.16)$$

where c_o is the concentration of electrolyte solution, R is the gas constant, T is the temperature and z is the absolute charge number of main constituent ionic species.

The interplay between the changes of zeta potential and pH changes due to the minority pH-governing ions is described by the surface charge density with charge-regulation characteristic (Equation 3.8) with a simple modification:

$$S_{(Charge-regulated)} = \frac{-eN_{Total}K_A}{K_A + c_{H_3O^+} \exp\left(\frac{-F\zeta}{RT}\right)}, \quad (3.17)$$

where the H_3O^+ concentration at the solid/liquid interface follows the Boltzmann distribution (see Equation 2.2 in Chapter 2).

Conjoining the Grahame equation (Equation 3.16) and the modified charge-regulated equation (Equation 3.17) yields:

$$\sqrt{8c_o\epsilon_o\epsilon_rRT} \sinh\left(\frac{zF\zeta}{2RT}\right) = \frac{-eN_{Total}K_A}{K_A + c_{H_3O^+} \exp\left(\frac{-F\zeta}{RT}\right)}, \quad (3.18)$$

which allows the local zeta potential to vary as functions of the solution concentration and pH, and alters the EOF flow velocity depending on the displacement directions for a two-fluid displacement flow. Equation 3.18 is in its simplest form in describing these standard conditions, i.e. a symmetric electrolyte (see Equation 3.16) and an absence of SiOH protonation as it only occurs at extremely acidic conditions (see Section 2.2.3 in Chapter 2).

The Nernst-Planck equation (Equation 3.9), which includes the reversible acid-base chemical reactions of the pH-governing ionic species (see Section 3.2.5), can be simplified as well due to the exclusion of EDL. The electrostatic wall potential can be removed from the Nernst-Planck equation (third term on the left of Equation 3.9), which reduces it to:

$$\frac{\partial c_i}{\partial t} + \nabla \cdot \left[-D_i \nabla c_i - u_{m(i)} c_i \nabla \phi \right] + \mathbf{u} \cdot \nabla c_i = R_i. \quad (3.19)$$

3.4 Simplification of generalized model for displacement flow of solutions with dissimilar ionic species

As opposed to the displacement flow of solutions with different concentrations, a hypothesis is proposed which states that EOF hysteresis for solutions with dissimilar ionic species is mainly driven by the ionic behavior of the main constituent ions. The validation of the hypothesis is to be examined in Chapters 6 and 7.

The minority pH-governing ions along with their reversible acid-base chemical reactions (see Section 3.2.5) are not necessary to be included for the simplification of the generalized theoretical model, to ease the demand on computational resources. This simplification inevitably causes the details on flow rate discrepancy induced by the minority pH-governing ions to be lost, but nevertheless sufficient in elucidating the concentration evolution of the main constituent ions, which is the driving force for this type of EOF hysteresis.

The specified charge-regulated wall condition (Equation 3.8) for the Poisson equation (Equation 3.7) will not be applicable if the minority pH-governing ions are omitted from the theoretical model. Hence, to simulate the zeta potential variation along the microchannel during two-fluid electroosmotic displacement flow, a constant surface charge density has to be prescribed at the wall boundary, which is calculated from the experimental zeta potentials of the solutions obtained by the current monitoring method (refer to Section 2.4.1 in Chapter 2 for more details). The relation between the surface charge density and the zeta potential for a general electrolyte is given by the Grahame equation (Kirby 2010):

$$S = \text{sgn}(\zeta) \left[2N_a \varepsilon_o \varepsilon_r k_b T \sum c_{o(i)} \left\{ \exp\left(\frac{-z_i e \zeta}{k_b T}\right) - 1 \right\} \right]^{\frac{1}{2}}, \quad (3.20)$$

where $\text{sgn}(\zeta)$ extracts the sign of the zeta potential, N_a is the Avogadro constant, k_b is the Boltzmann constant and $c_{o(i)}$ is the bulk concentration of ionic species.

3.5 Summary

In this chapter, a generalized theoretical model had been formulated to describe the two-fluid electroosmotic displacement flow. The generalized model reveals the electric field variation during the displacement flow process due to the evolving conductivity of the solution. This is different from the existing models for single fluid EOF, such as the slip-velocity and PB models, which cannot describe the variation of

electric field. Unlike the conventional PNP model which only accounts for the transportation of main constituent ionic species during a two-fluid displacement flow, the generalized model includes the transportation of minority pH-governing ionic species along with their reversible acid-base chemical reactions. The significant departures from the traditional models allows for a proper description of local zeta potential variation as functions of the concentration and pH of the solution, which have a direct impact on the EOF velocity depending on the displacement directions for a two-fluid electroosmotic displacement flow.

However, substantial computational resources are required when the generalized model is fully implemented for numerical simulation because the governing equations are strongly coupled. As such, the model has to be simplified for specific experimental conditions to ease the demand on computation effort. For the investigation of the origin of EOF hysteresis for solutions with different concentrations, which is hypothesized to be caused by the minority pH-governing ions (validation of the hypothesis is to be examined in Chapters 4 and 5), the fluid flow within the EDL is omitted from the generalized model to simplify it to a modified slip-velocity model. In contrast, a hypothesis is proposed which states that EOF hysteresis for solutions with dissimilar ionic species is mainly driven by the ionic behavior of the main constituent ions (validation of the hypothesis is to be examined in Chapters 6 and 7). Therefore, the minority pH-governing ions along with their reversible acid-base chemical reactions are excluded for the simplification of the generalized model.

Chapter 4 Ionic origin of electroosmotic flow hysteresis for solutions with different concentrations^{#1}

4.1 Introduction

Electroosmotic flow (EOF) has been experimentally observed to exhibit hysteretic behavior when the flow involves the displacement of two fluids with different concentrations (Gan et al. 2006, Lim and Lam 2012). Contrary to the prediction of prevailing EOF theory, the flow behavior is found to be different, and dependent on the flow direction. For example, the flow of a lower concentration potassium chloride (KCl) solution displacing a higher concentration KCl solution is slower than the flow in the reverse direction (Lim and Lam 2012). Hitherto, the origin for such hysteretic effect is not well understood.

As EOF is a surface-driven phenomenon, intuitively the surface-related effects will be examined for the cause of EOF hysteresis. Contrary to this obvious notion, this investigation demonstrates rather counter-intuitively that EOF hysteresis originates from the ionic behavior in the bulk of the fluid. Even more surprising, it is the minority pH-governing ions such as the hydronium ions (H_3O^+) in the aqueous solutions that induce such EOF hysteresis.

^{#1}Reproduced in part from [Lim, C. Y., A. E. Lim and Y. C. Lam (2016). "Ionic origin of electro-osmotic flow hysteresis." *Scientific Reports* **6**: 22329.], which was published under a CC BY license (Creative Commons Attribution 4.0 International License) that allows for maximum dissemination and re-use of the content.

4.2 Experimental details

4.2.1 Experimental setup and materials

EOF hysteresis is hypothesized to be caused by the presence of pH-governing minority ions such as the H_3O^+ and hydroxide (OH^-) ions which are typically neglected in the conventional EOF studies. The mechanics of EOF hysteresis was examined through the electroosmotic displacement flow of three types of dilute ionic solutions, namely potassium chloride (KCl , Fluka), sodium bicarbonate (NaHCO_3 , Sigma-Aldrich) and potassium dihydrogen phosphate (KH_2PO_4 , Sigma-Aldrich), with concentrations of 0.2 mM and 1 mM for each ionic solution type.

KCl was selected as a model for a simple salt solution. NaHCO_3 and KH_2PO_4 were selected because they consist of ion species which could act as pH buffers, namely bicarbonate ions (HCO_3^-) and dihydrogen phosphate ions (H_2PO_4^-). These buffer solutions had been employed to verify if pH changes are responsible for causing the EOF hysteresis. The solution properties, such as conductivity and pH, were measured with a conductivity meter (IONCheck 65, Radiometer Analytical) and pH meter (FEP20, Mettler Toledo). The conductivity and pH measurements can be found in Table 4.1. All solutions were equilibrated with the atmosphere and their pH values do not change with time.

Table 4.1: Measured conductivities and pH values of solutions employed in the experiments.

Solution	Conductivity ($\mu\text{S}.\text{cm}^{-1}$)	pH
0.2mM KCl	30.8 ± 0.1	5.62 ± 0.02
1mM KCl	147.9 ± 0.1	5.73 ± 0.01
0.2mM NaHCO ₃	21.0 ± 0.1	7.49 ± 0.01
1mM NaHCO ₃	93.8 ± 0.3	7.90 ± 0.02
0.2mM KH ₂ PO ₄	22.8 ± 0.2	5.53 ± 0.01
1mM KH ₂ PO ₄	108.8 ± 0.1	5.19 ± 0.01

Polyimide coated fused silica microcapillaries (Polymicro Technologies) with nominal internal diameter of 100 μm and length of 8 cm were cut with a Shortix Column Cutter (SGT Ltd). The microcapillaries were flushed with acetone followed by DI water, and lastly the experimental solutions before commencing the displacement flows. The same microcapillary was used for the experiments of a particular solution pair in both flow directions.

To ensure high accuracy and repeatability, the capillary was flushed with fresh solution through a syringe after each experimental run before it was used for the next run. This was a critical step to reset the pH and ionic conditions in the capillary. As a comparison, four additional runs were performed with different pieces of capillary columns for the case of 1 mM KCl displaced 0.2 mM KCl. The displacement times were found to be 115.0 ± 2.9 s. The average and standard deviation obtained from conducting experiments with different capillaries were not significantly different from

that of the experimental runs repeated with a single capillary, which were 118.1 ± 3.9 s. The microcapillary was replaced with a new one for different solution pairs to avoid cross contamination.

Current monitoring method was employed to investigate the behavior of two-fluid electroosmotic displacement flow. Figure 4.1 shows the schematic diagram of the experimental setup employed for monitoring the current change during EOF. The microcapillary was inserted between two Teflon reservoirs with both diameter and depth of 2 cm. The electric field for inducing EOF was supplied by a high voltage power supply (CZE1000R, Spellman) through platinum electrodes. The current across the microchannel was monitored by connecting a picoammeter (Keithley 6458) in series to the microcapillary. A Labview program was written (included in Appendix B.1) to control the two devices and to record the voltage and current readings through a data acquisition card (PCI-6052E, National Instrument).

Figure 4.1: Schematic diagram of experiment setup for current monitoring method.

4.2.2 Experimental procedures

The microcapillary and reservoir connecting to the cathode were filled with the solution to be displaced, while reservoir connecting to the anode was filled with the displacing solution (Figure 4.1). Electric potential of 1000 V was applied across the reservoirs to generate displacement flows of two opposing directions, i.e. 0.2 mM KCl displaced 1 mM KCl and 1 mM KCl displaced 0.2 mM KCl. These experiments were conducted for the three solution pairs. For every solution pair, displacement flow in two different directions was conducted five times each to ensure consistency and reliability of the results.

As the solution with a different conductivity (due to the difference in ion concentration) flowed into the capillary, the solution which initially residing in it was displaced and the total resistance of the capillary was changed. The change of resistance was captured as the current change, from which the flow behavior was examined. When the displacement process was completed, the current became constant and the time to reach a steady current was termed as the displacement time. Details for the determination of displacement time from the experimental current-time curve can be found in Appendix B.2.

The Teflon reservoirs (4ml in volume) were filled with the same amount of fluid, i.e. same liquid level, before beginning the experiment. The reservoirs were sufficiently large to ensure negligible liquid level changes during the displacement process, thereby minimized the back pressure generated (Yan et al. 2007). The application of large-volume reservoirs significantly diluted the concentrations of H_3O^+

and OH^- ions produced at the electrodes due to electrolysis (Rodríguez and Chandrasekhar 2005). The short experimental times (less than 500 s) and small electric currents (0.206-1.48 μA) as a result of the small microchannel cross section ($7860 \mu\text{m}^2$) and low solution conductivities (21.0-147.9 $\mu\text{S}\cdot\text{cm}^{-1}$), also limited the production of H_3O^+ and OH^- ions (Corstjens et al. 1996). Furthermore, the electrodes were positioned far from the inlet/outlet of the capillary to further prevent unwanted pH changes in the microchannel due to electrolysis (Almutairi et al. 2009).

The effect of Joule heating, which would affect the EOF behavior by affecting the solution properties such as viscosity and EOF mobility (see Equation 2.7 in Chapter 2), was insignificant because the concentrations and conductivities of the solutions were low (see Table 4.1) (Tang et al. 2006). A conservative estimate of Joule heating can be calculated from the energy balance between the energy generation E_g and the energy storage ΔE_{st} in the fluid (Arulanandam and Li 2000). For the chosen experimental parameters, the worst case scenario (applied electric field = 125 V/cm, solution conductivity (1 mM KCl) = 147.9 $\mu\text{S}\cdot\text{cm}^{-1}$, capillary diameter = 100 μm and experimental duration = 300 s) has an estimated temperature rise of 0.06 °C, which is negligible.

4.3 Experimental results

Figures 4.2a and 4.2d show that when 1 mM KCl displaced 0.2 mM KCl, the displacement time (T_{HL}) was approximately 30% less than the displacement time for

the reverse flow direction (T_{LH}), i.e. 0.2 mM KCl displaced 1 mM KCl. The observed hysteretic behavior defied the conventional understanding of EOF. Based on the Helmholtz-Smoluchowski equation (see Equation 2.7 in Chapter 2), the EOF flow velocity is proportional to the applied electric field. While the electric field distribution across the microchannel is dependent on the ion concentration distribution, which dictates the electrical resistance (see Equation 3.3 in Chapter 3).

When the EOF was reversed due to the reversal of applied electric field, one would expect that the ions will retrace the same trajectories as the forward flow, with the same flow time. However, the experimental results had shown that the EOF flow rates for the displacement flow of 0.2 mM and 1 mM KCl in two opposing directions were in fact different (see Figures 4.2a and 4.2d).

For the same experiments repeated with 1 mM and 0.2 mM NaHCO_3 , the hysteretic behavior surprisingly vanished; the displacement times for both flow directions, i.e. 1 mM NaHCO_3 displaced 0.2 mM NaHCO_3 and 0.2 mM NaHCO_3 displaced 1 mM NaHCO_3 , were identical (see Figures 4.2b and 4.2d). As for the experiments conducted with 1 mM and 0.2 mM KH_2PO_4 , the displacement time difference for the two flow directions, i.e. 1 mM KH_2PO_4 displaced 0.2 mM KH_2PO_4 and 0.2 mM KH_2PO_4 displaced 1 mM KH_2PO_4 , were approximately 25% (see Figures 4.2c and 4.2d), slightly lower than that for the KCl solution pair.

Figure 4.2: Current-time curves obtained from displacement flows of 0.2 mM and 1 mM for (a) KCl, (b) NaHCO₃ and (c) KH₂PO₄ solutions. (d) Displacement time difference in two directions for KCl, NaHCO₃ and KH₂PO₄ solution pairs. Five experimental runs ($n = 5$) were performed for each displacement direction to obtain the error bars of displacement time. T_{LH} = time for low concentration solution displaced high concentration solution. T_{HL} = time for high concentration solution displaced low concentration solution.

These experimental results had shown that the employment of buffer NaHCO₃ and KH₂PO₄ solution pairs can suppress the hysteresis effect. Since a buffer solution is defined as one which resists pH changes when a small amount of acid/alkali is added,

the suppression of EOF hysteresis by the buffer solutions suggest that the hysteresis effect could be related to pH changes during the displacement flow process. To further prove the hypothesis that the minority pH-governing ions such as H_3O^+ and OH^- cause the hysteresis phenomenon, numerical simulations based on the simplified theoretical formulation in Section 3.3 of Chapter 3 were performed in Section 4.4.

4.4 Numerical simulations

4.4.1 Numerical model

Numerical studies were performed with COMSOL Multiphysics finite element software (FEM) based on the simplified theoretical formulation in Section 3.3 of Chapter 3. The simplified theoretical model is a slip-velocity model. For the experiments, the Debye lengths were in the range of 10-30 nm (as calculated by Equation 2.1 in Chapter 2), which were much smaller than the microcapillary diameter (100 μm). Since there is no overlapping of electrical double layer (EDL), the slip-velocity model can be adopted for the investigation. Unlike the conventional slip-velocity model with a constant zeta potential, this model allows the revelation of the interplay between changes of zeta potential and pH changes due to the minority pH-governing ions, as well as the concentration change of the main constituent ions.

The full model simulated the occurrence/suppression of EOF hysteresis to match the experimental observations (see Figures 4.2, 4.4, and B.2 in Appendix B.4). Other simulations to demonstrate the mechanics of EOF hysteresis were performed

without the convective term in the Nernst-Planck equation (fourth term on the left of Equation 3.19 in Chapter 3), and convective-related governing equations such as the Navier-Stokes and continuity equations (Equations 3.5 and 3.15 in Chapter 3). The governing equations can be found in Chapter 3. Other than the simple salt KCl and buffer NaHCO_3 , the buffer KH_2PO_4 was also employed for the investigation (see Section 4.2.1). Reversible acid-base reactions of the phosphate system for the numerical simulations can be found in Appendix B.3.

The diverse diffusion coefficient values of hydrogen ion (H^+), or more correctly H_3O^+ which is formed due to H^+ joined to a water molecule (H_2O), available in the literature range from $7.0 \times 10^{-10} \text{ m}^2.\text{s}^{-1}$ to $9.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ (Roberts and Northey 1972, Wraight 2006, Venkatnathan et al. 2007). It was assumed that the ordinary diffusive or electromigration movement of H_3O^+ contributes to its diffusion coefficient, without the Grotthuss mechanism (Agmon 1995, Wraight 2006) which involves the rapid exchange of proton H^+ between adjacent H_3O^+ and H_2O . Therefore, instead of using the typically cited value of $9.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$, the diffusion coefficient of H_3O^+ was set as $2.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$,^{#2} which is similar to the diffusion coefficient of H_2O because H_3O^+ was expected to exhibit similar diffusive properties as H_2O in the absence of Grotthuss mechanism. The excellent agreement between the numerical and experimental results for H_3O^+ diffusion coefficient value of $2.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ in Figure 5.5 of Chapter 5

^{#2}The diffusion coefficient value of $2.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ for H_3O^+ is different from the value used in [Lim, C. Y., A. E. Lim and Y. C. Lam (2016). "Ionic origin of electro-osmotic flow hysteresis." *Scientific Reports* 6: 22329.], which was $7.0 \times 10^{-10} \text{ m}^2.\text{s}^{-1}$. However, the same conclusions were reached as there were only slight differences in the simulation results (less than 5%) by using these two different diffusion coefficient values, which were $2.3 \times 10^{-10} \text{ m}^2.\text{s}^{-1}$ and $7.0 \times 10^{-10} \text{ m}^2.\text{s}^{-1}$.

justifies the choice of this diffusion coefficient value. The symbols and values of constants used for the simulations are listed in Table A.1 of Appendix A.

4.4.2 Simulation domains, initial and boundary conditions

The simulation domains were straight cylindrical microchannels with diameter of 10 μm and length of 0.05 cm or 0.5 cm (see Figure 4.3). It was assumed that the center axis of the microchannel was axisymmetric. The governing equations of the simplified theoretical model (see Chapter 3 and Appendix B.3) for the applied electric potential, electrostatic wall potential, multi-ion species interaction and fluid flow are strongly coupled. As such, substantial computational resources are required when the model is implemented for numerical simulation. Hence, the diameter and length of the microchannel in the simulations were reduced as compared to the experiments (microcapillaries of 100 μm diameter and 8 cm length). Since it had been shown that EOF hysteresis for solutions with different concentrations was independent of the voltage applied, length and diameter of the microcapillary (Lim and Lam 2012), the scaled simulations would provide good representations of the experimental flow behaviors.

Both the full simulation (with convective effect) (Figure 4.3a) and the static simulation (without convective effect) (Figure 4.3b) were meshed with 14400 quadrilateral elements (24 and 600 edge elements in the radial and axial directions respectively). Convergence test performed using higher number of elements confirmed

that there was negligible numerical error for this mesh selection. The convergence criterion was based on a relative tolerance of less than 0.001 between subsequent iterations. For simulation domain of length 0.05 cm, the maximum time step and total simulated time were set to 0.005 s and 6 s respectively. As for simulation domain of length 0.5 cm, the maximum time step and total simulated time were set to 0.05 s and 10 s respectively.

The initial and boundary conditions for the simulations with/without flow are shown in Figure 4.3. The initial conditions for the full simulation (with flow) were set to that of the solution to be displaced (see Figure 4.3a). As for the simulation without flow, initial conditions of the left and right halves of the domain were set to the corresponding solution pair for the displacement flow (see Figure 4.3b). The voltage at the inlet for 0.05 cm and 0.5 cm domains were set to 6.25 V and 62.5 V respectively to establish an electric field of 125 V/cm across the microchannel, which was similar to the experiments.

Figure 4.3: Simulation domains, initial and boundary conditions for (a) full simulation (with convective effect) and (b) static simulation (without convective effect). $\mathbf{n}$ is the unit vector normal to the boundary.

4.5 Numerical results

4.5.1 Origin of electroosmotic flow hysteresis

To verify that the minority pH-governing ions are indeed responsible for the EOF hysteresis involving solutions with different concentrations, another set of simulations without the minority ions had also been conducted as a comparison. The simulations without the minority ions for displacement flows of 0.2 mM and 1 mM KCl predicted similar displacement time in both flow directions (see Figure 4.4). As such, hysteresis effect was absent, which deviated significantly from the experimental results. In contrast, the simulations that include the minority pH-governing ions showed

good agreement with the experimental results (see Figure 4.4), which supports the hypothesis that EOF hysteresis for solutions with different concentrations is caused by the pH-governing minority ions.

Figure 4.4: Comparison between the experimental and simulated current-time plots for displacement flow of 0.2 mM and 1 mM KCl in two different flow directions.

To understand the mechanics of EOF hysteresis, comprehending the behaviors of majority and minority ions is critical. The migration flux of ionic species induced by electric field is proportional to its concentration c_i and the electric field strength E . The electromigrative flux is given by $c_i u_{m(i)} E$, where $u_{m(i)}$ is the ionic mobility of each species i . The solution with a lower conductivity experiences a higher electric field, and vice versa. This is given by the expression $E = J/\sigma$, where J is the axial current density and σ is the conductivity of solution. However, the conductivity of a solution is proportional to its ion concentration and given by $F \sum z_i u_{m(i)} c_i$, where F is the Faraday constant and z_i is the ion charge number of each species i .

The electromigrative flux of the main constituent species i for a symmetric electrolyte can be written as:

$$\text{Electromigrative flux} = \frac{Ju_{m(i)}}{F \sum z_i u_{m(i)}}, \quad (4.1)$$

where the ion concentration c_i has been cancelled out and the flux is a constant. Therefore, the incoming and outgoing fluxes for the main constituent ions (i.e. K^+ and Cl^- ions in KCl solution) will be exactly balanced (see Figure 4.5). As such, the main constituent ions will never experience accumulation or depletion at the interface between the two solutions with different concentrations.

Figure 4.5: Electromigrative flux balance of K^+ ions (main constituent ions) in displacement flow of 0.2 mM and 1 mM KCl. E -field = electric field and σ = conductivity of solution.

However, the same explanation cannot be applied for the minority ions (H_3O^+ , HCO_3^- , OH^- etc), which have negligible/no effect over the conductivities of the solutions. The electric field distribution is dictated by the main constituent ions (which determine the conductivities). These minority ions have been forced to migrate

according to the local electric field. Therefore, the incoming and outgoing fluxes of these minority ions at the interface between the two solutions will not be balanced, i.e. there is a spatial gradient of ion flux across the interface. This leads to and causes the depletion/accumulation of ions.

The pH values for 0.2 mM and 1 mM KCl were identical (numerical pH of 5.68) and thus both solutions had the same concentration of H_3O^+ ions. When 1 mM KCl displaced 0.2 mM KCl, the incoming flux of H_3O^+ was lower than the outgoing flux. The H_3O^+ ions were depleted at the interface (concentration decreased, see Figure 4.6a). In contrast, when 0.2 mM KCl displaced 1 mM KCl, the incoming flux of H_3O^+ was higher than the outgoing flux, causing the H_3O^+ ions to be accumulated at the interface (concentration increased, see Figure 4.6b).

Figure 4.6: (a) Depletion and (b) accumulation of H_3O^+ ions (minority ions) due to the imbalance of electromigrative flux across the interface of 0.2 mM and 1 mM KCl. E -field = electric field and σ = conductivity of solution.

When the H_3O^+ ions were depleted or accumulated at the interface, the pH of the solution at the interface was changed. The value of zeta potential, which governs the direction and velocity of the EOF (see Equation 2.7 in Chapter 2), is sensitive to the pH of the solution. The development of zeta potential at the glass/silica surface is caused by the deprotonation (proton removal) of silanol (SiOH) groups when it is placed in contact with an aqueous solution: $\text{SiOH} + \text{H}_2\text{O} \rightleftharpoons \text{SiO}^- + \text{H}_3\text{O}^+$ (refer to Section 2.2.5 in Chapter 2 for more details).

If the H_3O^+ concentration is increased, the equilibrium will be shifted to the left, resulting in lesser SiO^- groups. This effect lowers the concentration of negatively-charged groups on the glass surface and reduces the magnitude of zeta potential. On the contrary, when the concentration of H_3O^+ is reduced, the equilibrium is shifted to the right, increasing the concentration of SiO^- groups and the magnitude of zeta potential of the surface. The theoretical formulation for the numerical simulations relates the H_3O^+ concentration to the reversible chemical reaction of SiOH groups and zeta potential formation (see Equation 3.18 in Chapter 3), which accurately captures the changes of zeta potential in response to the pH changes.

If the accumulation or depletion of H_3O^+ ions only occurs at the interface, the changes of zeta potential over the entire channel would be negligible when the channel is sufficiently long. Interestingly, this accumulation or depletion zone did not stop at the interface, but widened and spread into the bulk of the solutions. To examine the widening of the depletion zone, the spatial gradient of electromigrative flux for ions can be separated into two components:

$$-u_{m(i)} \frac{d(c_i E)}{dx} = -u_{m(i)} \left(E \frac{dc_i}{dx} + c_i \frac{dE}{dx} \right), \quad (4.2)$$

where x is the spatial coordinate. The first and second terms on the right of Equation 4.2 represent the flux gradient due to concentration and electric field gradients respectively.

Figure 4.7a shows the H_3O^+ depletion zone when 1 mM KCl displaced 0.2 mM KCl. The initial drop of H_3O^+ concentration profile at the interface consisted of two edges. The movements of the two edges due to electromigration were examined relative to the interface. Convective effect due to the fluid flow and the reversible acid-base reactions were neglected. At the left edge, the flux gradient due to concentration and electric field gradients were balanced. Therefore, the left edge was a ‘stationary’ edge which did not move relative to the interface. On the contrary, the right edge had only one component, i.e. the electromigrative flux gradient due to concentration gradient, which caused the right edge of the H_3O^+ depletion zone to move continuously to the right, widening the depletion zone.

Similarly, the negatively charged minority ions experienced the widening of accumulation zone in the reverse direction (towards the left, see Figure 4.7b). Only HCO_3^- ions were shown here because the concentration of OH^- ions was very low for KCl solutions with pH of 5.68. The concentrations of all the ion species were not independent, but obeyed a set of reversible acid-base equilibria (see Section 3.2.5 in Chapter 3). Therefore, the accumulation of HCO_3^- ions also leads to the depletion of H_3O^+ ions through the acid-base equilibrium reactions.

Figure 4.7: Numerical simulations showing imbalance of electromigrative flux gradient that widens (a) H_3O^+ depletion and (b) HCO_3^- accumulation zones when 1 mM KCl displaced 0.2 mM KCl. Convective effect and buffering chemical reactions were not included to highlight the role of electromigration. Electromigrative flux gradient is defined as $-u_{m(i)} (Edc_i/dx + c_i dE/dx)$ where $u_{m(i)}$ = ionic mobility, E = electric field strength, c_i = ion concentration, with corresponding subscript of H_3O^+ and HCO_3^- . The coefficient outside of the bracket was not shown in the figure for conciseness. All ion concentrations were normalized with the maximum and minimum concentrations.

For the case of 1 mM KCl displaced 0.2 mM KCl, the pH of 0.2 mM KCl was increased due to the depletion of H_3O^+ ions to the right (see Figure 4.8a). At the same time, on the left side, the accumulation of HCO_3^- ions led to the reduction of H_3O^+ as they reacted to form carbonic acid (H_2CO_3) (see Equation 3.13 in Section 3.2.5 of Chapter 3), increasing the pH of 1 mM KCl as well. Consequently, the magnitude of the average zeta potential was increased due to an overall higher pH along the microchannel which generated a higher flow rate, resulting in a shorter displacement time as observed experimentally (see Figure 4.2a).

When the electric field was reversed, i.e. for the case of 0.2 mM KCl displaced 1 mM KCl, the overall pH was decreased due to the accumulation of H_3O^+ ions (see Figure 4.8b). The magnitude of the average zeta potential was decreased and translated to a lower flow rate, which led to a longer displacement time as shown in the experiments (see Figure 4.2a). The electromigrative flux imbalance of the minority pH-governing ions results in the pH and zeta potential changes along the microchannel. This flux imbalance is the origin of EOF hysteresis for solutions with different concentrations.

Figure 4.8: Numerical simulations showing pH changes due to widening of ion accumulation/depletion zone when (a) 1 mM KCl displaced 0.2 mM KCl and (b) 0.2 mM KCl displaced 1 mM KCl. Concentration of H_3O^+ and HCO_3^- ions were related through the carbonate acid-base reactions (see Section 3.2.5 in Chapter 3). Convective effect was not included. All ion concentrations were normalized with the maximum and minimum concentrations.

4.5.2 Suppression of electroosmotic flow hysteresis

The full simulations (boundary conditions for flow stated in Figure 4.3a) for displacement flow of KCl, NaHCO₃ and KH₂PO₄ solution pairs were performed to simulate the two-dimensional (2-D) flow field with the pH accumulation/depletion effect (as discussed in Section 4.5.1), for the revelation of the EOF hysteresis phenomenon. To maintain flow continuity over the entire microchannel during the displacement flow process, a pressure gradient is generated and the flow velocity profile deviates from the usual plug-like EOF profile. The flow field is a mixed electroosmotic and pressure driven flow as shown in Figure 4.9, which is commonly known as M-flow because of its unique flow profile. Such M-flow flow field had been observed and imaged by Minerick et al. (2002), where a pH gradient was artificially imposed between the cathode and the anode wells during EOF of red blood cells in a microcapillary.

Figure 4.9: Simulated fluid flow velocity profile for (a) KCl, (b) NaHCO₃ and (c) KH₂PO₄ solution pairs in two flow directions.

The variations of average zeta potential along the microchannel with the interface displacement for KCl, NaHCO₃ and KH₂PO₄ solution pairs are shown in Figure 4.10. The interface displacement is defined as the position at which the concentration of the main constituent ions coincides with the average concentration of the two solutions. In this case, the interface displacement was the position where the main constituent ion concentration was 0.6 mM, which was the average concentration of 0.2 mM and 1 mM.

Figure 4.10: Variations of average zeta potential with the interface displacement for (a) KCl, (b) NaHCO₃ and (c) KH₂PO₄ solution pairs in two flow directions. The interface displacement is defined as the position at which the concentration of main constituent ions coincides with the average concentration of the two solutions.

For KCl solution pair, the average zeta potentials for the two flow directions followed different paths, forming a hysteresis loop (see Figure 4.10a). The hysteresis loop suggested that the average zeta potential and EOF flow rate cannot be determined at a given interface position without knowing the flow direction. The two vertical lines at the start and end of the microchannel showed that the zeta potential continued to change even when the displacement of the main constituent ions had been completed. This was due to the widened depletion/accumulation zone of the pH governing ions.

EOF hysteresis with the associated pH and zeta potential variations occurs as long as there is a conductivity or ion concentration gradient between the two solutions during electroosmotic displacement flow. It affects the EOF flow rate in microfluidic devices and can even be detrimental to the chemical or biological systems which are sensitive to pH variation.

To investigate the effect of pH buffering on EOF hysteresis, experiments and numerical simulations with NaHCO_3 and KH_2PO_4 solutions had been performed. The experimental results showed that EOF hysteresis vanished for the displacement flow involving 1mM and 0.2mM of NaHCO_3 (see Figure 4.2b). The suppression of EOF hysteresis for NaHCO_3 solution pair had been shown in numerical simulations as well (see Figure B.2a in Appendix B.4), with almost identical average zeta potentials in both flow directions (see Figure 4.10b). However, both the experiments and numerical simulations demonstrated that the EOF hysteresis persisted for the displacement flows of KH_2PO_4 solution pair (see Figures 4.2c, 4.10c, and B.2b in Appendix B.4). Nonetheless, the displacement time and average zeta potential differences had been reduced with the presence of buffer KH_2PO_4 solutions.

The competitions between pH buffering and accumulation/depletion of H_3O^+ ions due to electromigrative flux are shown in Figure 4.11. When 0.2 mM NaHCO_3 displaced 1 mM NaHCO_3 , the accumulation rate of H_3O^+ ions was swiftly balanced by H_3O^+ depletion through the acid-base equilibrium reaction (see Equation 3.14 in Section 3.2.5 of Chapter 3) to form HCO_3^- ions (see Figure 4.11a). Therefore, the net change of H_3O^+ was negligible (see Figure B.3a in Appendix B.4) and the zeta

potential was not altered significantly. Similarly for 1 mM NaHCO_3 displaced 0.2 mM NaHCO_3 (see Figure B.3b in Appendix B.4), the depletion rate of H_3O^+ ions was being compensated by H_3O^+ production through the dissociation of HCO_3^- ions to carbonate ions (CO_3^{2-}) (see Figure 4.11b).

Figure 4.11: Numerical simulations showing rate of change of H_3O^+ concentration due to electromigration and acid-base reactions for (a) 0.2 mM NaHCO_3 displaced 1 mM NaHCO_3 , (b) 1 mM NaHCO_3 displaced 0.2 mM NaHCO_3 (c) 0.2 mM KH_2PO_4 displaced 1 mM KH_2PO_4 and (d) 1 mM KH_2PO_4 displaced 0.2 mM KH_2PO_4 . Convective effect was not included. The rate values were extracted at the interface between the two solutions.

For KH_2PO_4 solution pair, the H_3O^+ ion depletion/accumulation rates due to electromigration and buffering reactions did not balance each other (see Figures 4.11c and 4.11d). The net change of H_3O^+ ion concentration (see Figures B.3c and B.3d in Appendix B.4) caused a zeta potential difference between the two flow directions and generated noticeable EOF hysteresis. The buffer solutions employed in this study were not optimized for pH buffering, but rather for demonstrating that the proper selection of buffer is crucial to suppress EOF hysteresis.

4.6 Summary

The origin of EOF hysteresis for solutions with different concentrations had been demonstrated in the current monitoring experiments, and elucidated through the numerical simulations based on the simplified theoretical formulation in Chapter 3. The electric field gradient at the interface of the two aqueous solutions generated electromigrative flux imbalance of the minority pH-governing ions such as H_3O^+ and HCO_3^- ions. These ions were thus accumulated or depleted at the interface. Subsequently, the ion depletion and accumulation zones widened and spread from the interface to the bulk of the solutions. The resultant pH changes altered the zeta potential and EOF flow rate in the microchannel, and were dependent on the flow direction. When a high concentration/conductivity solution displaced a lower one, the pH, magnitude of zeta potential and EOF flow rate were increased. In contrast, when a

lower concentration/conductivity solution displaced a higher one, the pH, magnitude of zeta potential and EOF flow rate were decreased.

The EOF hysteresis was visualized by plotting the average zeta potential (or EOF flow rate) against the displacement of interface between the two solutions. The flow behaviors such as the zeta potential, pH and flow rate can only be ascertained at a given interface position or time if the flow direction is known. EOF hysteresis can adversely affect the accuracy and consistency of flow control in microfluidic devices involving two or more fluids. Furthermore, the induced pH changes in this EOF hysteresis can be detrimental to the chemical and biological systems. It had been demonstrated that such hysteresis effect can be suppressed by the careful selection of pH buffering solutions. The ion accumulation or depletion rate due to electromigration can be compensated by the buffering acid-base reactions.

Although EOF is driven by surface phenomenon and the main constituent ion species, rather counter-intuitively that EOF hysteresis for solutions with different concentrations was predicted to originate from the ionic behavior of the minority pH-governing ions in the bulk of the fluid. To provide conclusive evidence on the pH changes in such EOF hysteresis, direct experimental observation of the pH changes and their quantifications for comparison with the numerical simulations were performed in Chapter 5.

Chapter 5 pH changes in electroosmotic flow hysteresis for solutions with different concentrations^{#3}

5.1 Introduction

Electroosmotic flow (EOF) for two solutions with different concentrations induces accumulation or depletion of minority pH-governing ions at the bulk interface between the two solutions, as elucidated in Chapter 4. This effect causes significant pH changes that bring about EOF hysteresis, which is manifested by an observable flow velocity difference between the two opposing flow directions.

The prediction of pH changes in the bulk solution is rather counter-intuitive as EOF in a microchannel is believed to be caused by surface phenomenon and the main constituent ions. Without direct experimental observation of the pH changes in EOF hysteresis, this prediction will remain as an unproven hypothesis because it is only deduced through numerical simulations.

This chapter, therefore, focuses on the experimental observation of pH changes in EOF hysteresis involving two solutions with different concentrations, and their quantifications for comparison with the numerical simulations based on the theoretical model formulated in Chapter 3.

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5.2 Experimental details

5.2.1 Current monitoring experiments

Current monitoring technique was employed for the experiments (see Figure 4.1 in Chapter 4), and the experimental setup was similar to that in Section 4.2.1 of Chapter 4. A voltage of 1800 V was applied across a glass microcapillary (Polymicro Technologies, inner diameter of 250 μm and length of 15 cm) to induce displacement flow in two opposing directions, i.e. 1 mM potassium chloride (KCl, Fluka) displaced 0.2 mM KCl and 0.2 mM KCl displaced 1 mM KCl.

The conductivities of 0.2 mM and 1 mM KCl were measured to be 30.8 $\mu\text{S}\cdot\text{cm}^{-1}$ and 147.9 $\mu\text{S}\cdot\text{cm}^{-1}$ respectively (see Table 4.1 in Chapter 4). As the solution with a different concentration flowed into the capillary, the solution which initially resided in it was displaced and the total resistance of the capillary was changed. The change of resistance was reflected as an observable current change. The current values I enabled the positions of the two-fluid interface along the capillary X to be determined and non-dimensionalized with the capillary length L as P (see Figure 5.1, refer to Appendix C.1 for the detailed derivation):

$$P = \frac{\sigma_1 \sigma_2}{\sigma_2 - \sigma_1} \left(\frac{ER_c}{I} - \frac{1}{\sigma_2} \right), \quad (5.1)$$

where $\sigma_{1/2}$ is the conductivities of the two fluids, R_c is the capillary cross-sectional area and E is the applied electric field.

Electroosmotic displacement flow was stopped at various interface locations ($P = 0.2, 0.6$ and 1) (see Figure 5.1) for both flow directions, i.e. 1 mM KCl displaced 0.2 mM KCl and 0.2 mM KCl displaced 1 mM KCl , to investigate their difference in pH changes. The methodology for the quantification of the pH changes will be discussed in the subsequent section.

Figure 5.1: (a) Dimensionless interface positions during displacement flow process at $P = 0.2, 0.6$ and 1 . Current-time curves showing currents I for $P = 0.2, 0.6$ and 1 when (b) 1 mM KCl displaced 0.2 mM KCl , and (c) 0.2 mM KCl displaced 1 mM KCl .

Precautionary measures had been adopted (similar as Section 4.2.2 of Chapter 4) to minimize unnecessary pH variation in the microcapillary due to the undesirable electrolytic reactions at the electrodes in the reservoirs, which produce hydronium

(H_3O^+) and hydroxide (OH^-) ions (Rodríguez and Chandrasekhar 2005). The microcapillaries used for the experiments (inner diameter of 250 μm and length of 15 cm) resulted in currents in the range of 1.81-8.63 μA , which are higher than that in Chapter 4. However, the current values were still considered low, thus restricting the production of H_3O^+ and OH^- ions at the electrodes.

The effect of Joule heating was negligible because the concentrations and conductivities of the solutions were kept low (30.8 $\mu\text{S}\cdot\text{cm}^{-1}$ and 147.9 $\mu\text{S}\cdot\text{cm}^{-1}$ for 0.2 mM and 1 mM KCl respectively) (Tang et al. 2006). A conservative estimate of Joule heating can be calculated from the energy balance between the energy generation E_g and the energy storage ΔE_{st} in the liquid (Arulanandam and Li 2000). For the worst case scenario (applied electric field = 120 V/cm, solution conductivity (1mM KCl) = 147.9 $\mu\text{S}\cdot\text{cm}^{-1}$, capillary diameter = 250 μm and experimental duration = 400 s), the temperature increase is estimated to be 0.16 $^{\circ}\text{C}$, which is insignificant.

5.2.2 Quantification of pH changes

After the displacement flow was stopped (voltage switched off) at a specific interface location P , the capillary was detached from the current monitoring setup and connected to a syringe for the pumping of fluid into a white nylon female luer lock coupler (Cole-Parmer), which was capped with a male luer plug at one end to form a small fluid container (see Figure 5.2). Each capillary only contained approximately 7 μl of fluid. Thus, the EOF experiment (refer to Section 5.2.1) was conducted eleven times

for each interface position ($P = 0.2, 0.6$ and 1) to generate 70 to 80 μl of sample in the container, in order to obtain “snapshots” of the pH in the capillary at various time instances for the two displacement flow directions, i.e. 1 mM KCl displaced 0.2 mM KCl and 0.2 mM KCl displaced 1 mM KCl.

Figure 5.2: pH quantification of sample collected at different time instances during the displacement flow process.

Due to potential fluid loss from the capillary when executing the aforesaid procedure, the exact amount of sample was measured with a chemical balance (A&D GR-200). Thereafter, a pH-sensitive dye, 0.04 wt% bromocresol purple (Sigma-Aldrich), was added to the sample collected (2% based on the volume of sample) through a 2.0 μl microsyringe (Hamilton). The color change was captured with a color camera (Olympus SC30) attached to a stereo microscope (Olympus SZX7) (see Figure 5.2). The magnification used was 5.6x and the exposure time was 70 ms. The entire

process was repeated two times (i.e. a total of three times) for each interface position to ensure consistency and reliability of results.

The pH values of the samples were quantified by performing image analysis on the color images. A program was written in MATLAB (see Appendix C.2) to extract the RGB (red, green and blue) values from the images (see Figure 5.3). Similar approaches had been adopted for non-destructive evaluation of chlorophyll content in leaves (Kawashima and Nakatani 1998, Yadav et al. 2010, Riccardi et al. 2014).

Figure 5.3: Evaluation area for the extraction of RGB (red, green, blue) values.

A pH calibration curve was constructed for the quantification of the sample pH. It was produced based on the color images captured when bromocresol purple was added to seven calibration solutions (pH = 4.33, 5.06, 5.6, 6.02, 6.35, 6.96 and 7.43), under the same optical conditions and dye-sample ratio. The calibration solutions were pH-adjusted with hydrochloric acid (HCl) and sodium hydroxide (NaOH) as shown in

Table 5.1. The pH values of these solutions were pre-determined by a pH meter (FEP20, Mettler Toledo).

Table 5.1: Preparation of calibration solutions.

Acidic adjustment		Alkaline adjustment	
pH	Volume of 1 mM HCl (ml) added to 95 ml of 1 mM KCl	pH	Volume of 1 mM NaOH (ml) added to 98 ml of 1mM KCl
4.33	5.0	6.02	0.5
5.06	1.0	6.35	2.0
5.6	NIL	6.96	5.7
-	-	7.43	8.5

There was a strong correlation between the R, G and B values as observed from Figure 5.4. The R and G values were positively correlated to each other (correlation coefficient of 0.996), while B value was negatively correlated to R and G values (correlation coefficients of -0.977 and -0.961 respectively). This indicates that they are not independent variables. Instead, their combination will represent one independent variable. Therefore, a new variable, $C = (R+G-B)$ was employed to represent the RGB values. C was regressed on pH with a cubic curve (see Figure 5.4d), which yielded a coefficient of determination (R^2) of 0.975. The resultant calibration curve allowed the quantification of the average pH values for the samples collected at different time instances during the displacement flow in two opposing directions.

Figure 5.4: pH versus (a) R (red), (b) G (green) and (c) B (blue) values curves. (d) Calibration curve for pH quantification, where $C = (R+G-B)$ represents RGB values.

A control experiment was conducted with steady-state single fluid EOF of 0.6 mM KCl (average concentration of 1 mM and 0.2 mM) for 300 s (longest displacement time observed). The control experiment demonstrated a low pH increase of 0.1, i.e. the average pH increased from 5.64 to 5.74, which was potentially due to electrolysis at the electrodes in the reservoirs (refer to Section 5.2.1). This slight pH increase will not affect the integrity of the experiments.

The employment of pH-sensitive fluorescent dyes for the measurement of pH changes in microchannel had been reported in several literatures (Ng et al. 2009, Gencoglu et al. 2011, An et al. 2014). Due to the electrophoretic movement and stacking/depletion of the dye, the addition of another control dye, which is insensitive

to pH changes, is necessary to ensure accurate pH quantification (Gencoglu et al. 2011). For the experiments in this chapter, the pH-sensitive dye was neither added into the reservoirs nor the capillary as it could disrupt the experimental conditions and affect the pH measurement.

Instead, content of the capillary was harvested in a separate container (EOF stopped) for the addition of pH-sensitive dye, which allowed the measurement of average pH without the interference from the stacking/depletion of the dye concentration. Therefore, the dye color changes observed in the experiments were solely due to the pH changes, and not related to the stacking/depletion of the dye concentration.

5.3 Numerical simulations

5.3.1 Numerical model

To understand the trend and mechanics of the observed pH changes, numerical simulations were conducted by COMSOL Multiphysics finite element software (FEM) based on the formulated theoretical model in Section 3.3 of Chapter 3 for EOF of two solutions with different concentrations. For computational efficiency but without sacrificing much accuracy, in this investigation, the theoretical model was slightly simplified to perform one-dimensional (1-D) simulations for obtaining the pH variation along the axial direction (x -axis) of the capillary.

The fluid velocity profile in the radial direction was not simulated by the Navier-Stokes and continuity equations (Equations 3.5 and 3.15 in Chapter 3) in this 1-D model. The electric field, the main driving force of EOF, has a negligible radial component when it is applied parallel to the straight microcapillary. Thus, the fluid velocity was replaced by the Helmholtz-Smoluchowski slip velocity equation (Equation 2.7 in Chapter 2), which was calculated based on the average zeta potential ζ_{ave} along the entire capillary at every time instance.

Instead of modeling the KCl solution with K^+ and Cl^- ions separately, a single variable of conductivity was adopted (Lin et al. 2004) because the diffusion coefficients and ionic mobilities for both ions are almost identical. The evolution of conductivity due to convection and diffusion is given as:

$$\frac{d\sigma}{dt} - D_{K^+} \frac{d^2\sigma}{dx^2} + U_{ave} \frac{d\sigma}{dx} = 0, \quad (5.2)$$

where $\sigma = 2Fc_0u_{m(K^+)}$ is the conductivity of KCl solution, F is the Faraday constant, c_0 is the bulk concentration of KCl solution, U_{ave} is the average EOF velocity and $u_{m(K^+)}$ is the ionic mobility and D_{K^+} is the diffusion coefficient of K^+ .

The rest of the governing equations can be found in Chapter 3. The symbols and values of constants employed for the numerical simulations are listed in Table A.1 of Appendix A.

The diffusion coefficient values of hydrogen ion (H^+), or more correctly H_3O^+ , range from $7.0 \times 10^{-10} \text{ m}^2.\text{s}^{-1}$ to $9.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ (Roberts and Northey 1972, Wraight 2006, Venkatnathan et al. 2007). As such, the numerical simulations were performed with H_3O^+ diffusion coefficient values of $9.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ (typically cited value) and $2.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$ (diffusion coefficient of water molecule (H_2O) due to its similar size to H_3O^+), to evaluate the appropriateness of these two coefficients in describing the observed experimental behaviors.

5.3.2 Simulation domain, initial and boundary conditions

The 1-D simulation domain was a straight line with a length of 1 cm. Although the 1-D simulation domain was 1 cm, the simulation results were related to the experimental results (length of 15 cm) through the dimensionless interface position p as defined in Equation 5.1. The line was meshed with 2000 elements. The convergence criterion was based on a relative tolerance of less than 0.001 between subsequent iterations. Convergence test performed using higher number of elements confirmed that there was negligible numerical error for this mesh selection. The maximum time step and total simulated time was set to 0.005 s and 22 s respectively.

The inlet and outlet boundary conditions, and the initial conditions are listed in Table 5.2. The applied voltage was set to 120 V over the 1 cm domain to generate similar electric field magnitude (120 V/cm) as the experiments. The initial concentrations of the minority species such as H_3O^+ , OH^- , HCO_3^- (bicarbonate ion),

H_2CO_3 (carbonic acid) and CO_3^{2-} (carbonate ion) were calculated based on their respective chemical equilibria (see Appendix C.3), and set as the boundary and initial conditions. The conductivity conditions were set according to the flow scenario based on the experiments.

Table 5.2: Boundary and initial conditions for 1-D numerical simulations for 0.2 mM displaced 1 mM KCl (LH) and 1 mM displaced 0.2 mM KCl (HL).

Variables	Boundary conditions		Initial conditions
	Inlet	Outlet	Domain
V	120 V	0	$120(L - x)/L$
c_i	Initial concentrations of minority species for KCl		
$LH: 0.2\text{ mM displaced }1\text{ mM KCl}$			
σ	Conductivity of 0.2 mM KCl	Conductivity of 1 mM KCl	
$HL: 1\text{ mM displaced }0.2\text{ mM KCl}$			
σ	Conductivity of 1 mM KCl	Conductivity of 0.2 mM KCl	

5.4 Experimental results

The experimental average pH at various interface positions ($P = 0.2, 0.6$ and 1) for the two opposing flow directions are shown in Figure 5.5. EOF was conducted eleven times to collect enough sample fluid for a single pH quantification run. The entire process was performed three times for each interface location (three interface locations for one displacement direction) in two different displacement directions,

which resulted in a total of 198 EOF experimental runs. This amount of work is realistically manageable for obtaining adequate data to confirm the hypothesis that EOF hysteresis for solutions with different concentrations is related to pH changes in the bulk fluid.

For 1 mM KCl displaced 0.2 mM KCl, the average pH increased from 5.64 (initial pH of KCl solutions) to 6.07 throughout the displacing process. In the reverse flow direction, i.e. 0.2 mM KCl displaced 1 mM KCl, the average pH decreased from 5.64 to a minimum of 5.24 before increasing to 5.44 at the end of the displacement. Despite the increase from the minimum pH, the final pH was still below the initial pH of 5.64.

Figure 5.5: Experimental and numerical average pH at various dimensionless interface positions p when 1 mM KCl displaced 0.2 mM KCl (HL), and 0.2 mM KCl displaced 1 mM KCl (LH). Three experimental runs ($n = 3$) were performed for each interface position to obtain the error bars of average pH. $D_{H_3O^+}$ is the diffusion coefficient of hydronium ion.

To put it briefly, the experimental results demonstrated the definite dichotomic pH changes in the microcapillary depending on the displacement flow directions, i.e. pH increased when a higher concentration solution displaced a lower concentration solution, while pH decreased when a lower concentration solution displaced a higher concentration solution.

5.5 Numerical results

5.5.1 Mechanics for pH changes in electroosmotic flow hysteresis

The numerical simulations revealed the mechanics for the pH changes during EOF of two solutions with different concentrations (more details can be found in Chapter 4). The electric field distribution across the two solutions was determined by the conductivity ratio between the two solutions. The pH-governing minority ions were forced to migrate across the interface between the two solutions under the applied electric field. When a high concentration solution displaced a low concentration solution, the pH increase (see Figure 5.5) was caused by the depletion of H_3O^+ and accumulation of HCO_3^- across the interface.

In contrast, when a low concentration solution displaced a high concentration solution, the pH decrease (see Figure 5.5) was caused by the accumulation of H_3O^+ and depletion of HCO_3^- across the interface. The distributions of these pH-governing minority ions were determined by the interplay between acid-base equilibria,

electromigration, diffusion and convection effects. Subsequently, the pH depletion or accumulation zone expanded outward to other parts of the solution, driven by the electromigration effect.

5.5.2 Simulation with H_3O^+ diffusion coefficient as $9.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$

With the diffusion coefficient of H_3O^+ set as $9.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$, it was found that the average pH curves obtained from the numerical simulations (averaged along the capillary) had large discrepancies with the experimental data points, beyond the limits of experimental error (see Figure 5.5).

Indeed, the anomalously high value of diffusion coefficient for hydrogen ions has been a topic for intense research and debate for decades (Hynes 1999, Marx et al. 1999), where various theories had been proposed to explain the phenomenon. The Grotthuss mechanism (Agmon 1995, Wraight 2006) (also known as structural diffusion) suggests that the high value of diffusion coefficient for hydrogen ions is mainly contributed by the rapid exchange of proton (H^+) between adjacent H_3O^+ and water molecule (H_2O). However, the carriers, i.e. H_3O^+ , also experience ordinary diffusive or electromigration movement and partially contribute towards the apparent movement of H^+ in water.

5.5.3 Simulation with H_3O^+ diffusion coefficient as $2.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$

In this investigation, a lower value of diffusion coefficient was employed as the formation of ion accumulation or depletion zone was assumed to be caused by the ordinary electromigrative movement of H_3O^+ (without contribution by the Grotthuss mechanism), which was expected to exhibit similar diffusive properties as H_2O , i.e. the diffusion coefficient of H_3O^+ was set as $2.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$. Interestingly, the numerical results obtained with this H_3O^+ diffusion coefficient value agreed very well with the experimental results (see Figure 5.5). This explained and justified the choice of this H_3O^+ diffusion coefficient value for the numerical simulations in Chapter 4.

When 1 mM KCl displaced 0.2 mM KCl, the numerical average pH increased initially up to 6.06 at $P = 0.8$ before decreasing to 5.98 at $P = 1$ (see Figure 5.5). In the reverse flow direction, the numerical average pH decreased initially to 5.21 at $P = 0.53$ before increasing to 5.57 at $P = 1$ (see Figure 5.5). The initial pH increase/decrease was due to the expansion of H_3O^+ depletion or accumulation zone which was leading the interface of the two KCl solutions. Thus, the higher mobility value of H_3O^+ ($D_{\text{H}_3\text{O}^+} = 9.3 \times 10^{-9} \text{ m}^2.\text{s}^{-1}$, as discussed in Section 5.5.2) predicted the accelerated expansion of ion depletion or accumulation zone, and resulted in the average pH variations indicated in Figure 5.5 which deviate noticeably from the experimental results. As the H_3O^+ depletion or accumulation zone reached the end of the capillary, the trend of the pH changes was reversed due to the convective displacement of the unaffected fluid from the inlet reservoir.

The numerical simulations also provided further insight on the local pH distribution and evolution, which cannot be captured through the experiments. The local pH changes were much higher than the average pH changes which were approximately ± 0.4 (see Figure 5.5). When 1 mM KCl displaced 0.2 mM KCl, the maximum local pH was 7.73 while the minimum local pH in the reverse flow direction was 4.86 (see Figure 5.6). The significant local pH change therefore affected the zeta potential and EOF velocity which brought about the EOF hysteresis (as reported in Chapter 4).

Figure 5.6: Simulated local pH distribution along the microcapillary at various interface positions, which are marked with short vertical lines (at $P = 0.2$, 0.6 and 1), for 1 mM KCl displaced 0.2 mM KCl (HL) and 0.2 mM KCl displaced 1 mM KCl (LH), assuming $D_{H_3O^+} = 2.3 \times 10^{-9} \text{ m}^2 \cdot \text{s}^{-1}$.

5.6 Summary

This investigation presents conclusive evidence for the pH changes in EOF hysteresis involving two solutions with different concentrations. The average pH in the microcapillary was quantified with a pH-sensitive dye through color image processing. When 1 mM KCl (a higher concentration solution) displaced 0.2 mM KCl (a lower concentration solution), an increase in pH was observed, while the flow in the reverse direction was observed to have a decrease in pH. The pH-governing minority ions such as H_3O^+ and HCO_3^- were accumulated or depleted at the interface between the two solutions due to electromigrative flux imbalance, which caused significant pH changes during the displacement flow. The experimental results agreed well with the 1-D numerical simulations based on the formulated theoretical model (Section 3.3 of Chapter 3) with slight simplifications (see Section 5.3.1). The pH changes might be detrimental to chemical and biological systems in lab-on-a-chip devices. Appropriate buffer solutions should be employed to minimize the pH changes to achieve accurate flow control and maintain a constant pH in the microfluidic chip under EOF.

Chapter 6 Electroosmotic flow hysteresis for dissimilar cationic solutions^{#4}

6.1 Introduction

Electroosmotic flow (EOF) with two or more types of fluids is commonly encountered in various microfluidic applications. For example, in ion pre-concentration techniques such as field amplified sample stacking (FASS) (Giordano et al. 2012) and isotachopheresis (ITP) (Smejkal et al. 2013), the electric field is typically applied in one direction throughout the whole experiment, which favors the accumulation of the desired species at the interface between the dissimilar fluids.

Hitherto, no investigation has been conducted to investigate the difference in flow behaviors between opposing flow directions for electroosmotic displacement flow of solutions with *dissimilar ionic species*.

Hence, in this chapter, EOF hysteresis for solution pair with dissimilar ionic species, i.e. *dissimilar cation species*, was demonstrated for the first time through the current monitoring experiments. The mechanics for this type of EOF hysteresis were elucidated via the numerical simulations, based on the theoretical model formulated in Chapter 3.

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6.2 Experimental details

6.2.1 Experimental setup and materials

Current monitoring method was used for the experiments (see Figure 4.1 in Chapter 4), and the experimental setup was similar to that in Section 4.2.1 of Chapter 4. Three types of ionic solutions were prepared for the experiments, namely potassium chloride (KCl, Fluka), sodium chloride (NaCl, Sigma-Aldrich) and calcium chloride (CaCl₂, Sigma-Aldrich). The concentrations for all the solutions were set to be identical (0.5 mM) so that the effect of ionic species type on EOF behavior could be examined. The measured average pH (AccumetAR20, Fisher Scientific) for all solutions was 5.53 ± 0.09 . The acidity of the solutions was caused by the dissolution of atmospheric carbon dioxide (CO₂) into water. The measured conductivities (IONCheck 65, Radiometer Analytical) of the solutions are listed in Table 6.1.

Table 6.1: Measured conductivities of solutions employed in the experiments.

Solution	Conductivity ($\mu\text{S}\cdot\text{cm}^{-1}$)
0.5 mM KCl	75.5 ± 0.2
0.5 mM NaCl	63.8 ± 0.2
0.5 mM CaCl ₂	136.7 ± 0.2

6.2.2 Experimental procedures

Two solution pairs, namely 0.5 mM KCl–NaCl and 0.5 mM CaCl₂–NaCl, were selected for the investigation of electroosmotic displacement flows between symmetric electrolytes, and asymmetric-symmetric electrolytes with dissimilar cation species respectively. Current monitoring experiments were conducted to monitor the flow behaviors of the two solution pairs in two opposing directions, i.e.

- (a) 0.5 mM KCl displaced 0.5 mM NaCl,
- (b) 0.5 mM NaCl displaced 0.5 mM KCl,
- (c) 0.5 mM CaCl₂ displaced 0.5 mM NaCl, and
- (d) 0.5 mM NaCl displaced 0.5 mM CaCl₂.

The experimental procedures were similar to that in Section 4.2.2 of Chapter 4.

The responses for the two different flow directions were compared to demonstrate the hysteresis effect. In the subsequent discussions, solution which initially resided in the microcapillary before the displacement flow is defined as the residing electrolyte (RE), and the solution which was placed in the inlet reservoir and transported into the capillary to displace the residing electrolyte is defined as the displacing electrolyte (DE).

Various precautions had been adopted (see Section 4.2.2 of Chapter 4) to prevent local pH changes in the reservoirs (due to the production of hydronium (H₃O⁺) and hydroxide (OH⁻) ions) as a result of the undesirable electrolytic reactions at the

electrodes, which would affect the EOF behavior during the displacement experiment (Rodríguez and Chandrasekhar 2005).

Since the concentrations and conductivities of the solutions used for the experiments were low (see Table 6.1), Joule heating which would affect the EOF behavior by affecting the solution properties such as viscosity and EOF mobility (see Equation 2.7 in Chapter 2) could be ignored (Tang et al. 2006). A conservative estimate of Joule heating can be calculated from the energy balance between the energy generation E_g and the energy storage ΔE_{st} in the liquid (Arulanandam and Li 2000). For the chosen experimental parameters, the worst case scenario (applied electric field = 125 V/cm, solution conductivity (0.5 mM CaCl_2) = $136.7 \mu\text{S}\cdot\text{cm}^{-1}$, capillary diameter = 100 μm and experimental duration = 500 s) has an estimated temperature rise of 0.08 °C, which is negligible.

6.3 Experimental results

As opposed to the displacement flow of similar solutions with different concentrations (see Chapters 4 and 5), the current-time curve for the displacement flow of 0.5 mM KCl–NaCl and 0.5 mM CaCl_2 –NaCl in two different directions exhibited an abrupt change of gradient (see Figures 6.1a and 6.1b). Hence, the term “turning time” was introduced to describe this abrupt change, whereby the magnitude of the second time derivative of current is maximum. The displacement time was determined by the time required for the current to reach a steady value. The turning and displacement

times were computed from the experimental data by performing curve fitting of the current-time curves through a regression analysis of SigmaPlot. The turning and displacement times of the current-time curves for the two solution pairs in two different directions are shown in Figures 6.2a and 6.2b.

Figure 6.1: Current-time curves for displacement flow of (a) 0.5 mM KCl–NaCl and (b) 0.5 mM CaCl₂–NaCl in two directions.

Figure 6.2: Turning and displacement times for displacement flow of (a) 0.5 mM KCl–NaCl and (b) 0.5 mM CaCl₂–NaCl in two directions. Five experimental runs ($n = 5$) were performed for each displacement direction to obtain the error bars of turning/displacement time.

Student's t -test (test for normality is detailed in Appendix D.2) was performed on the data to examine if the mean turning times, as well as the displacement times, between the two flow directions differ significantly for the two cases. Let $T_{LH(Turn)}$ be the turning time and T_{LH} be the displacement time for a low conductivity solution displacing a high conductivity solution, and $T_{HL(Turn)}$ be the turning time and T_{HL} be the displacement time for a high conductivity solution displacing a low conductivity solution. The null hypothesis H_o suggests that $T_{LH(Turn)}$ (or T_{LH}) is the same as $T_{HL(Turn)}$ (or T_{HL}) while the alternative hypothesis H_I suggests otherwise. The t -score is calculated by:

$$t_s = \frac{\bar{T}_{LH(Turn)/LH} - \bar{T}_{HL(Turn)/HL}}{\sqrt{\frac{s_{LH(Turn)/LH}^2}{n} - \frac{s_{HL(Turn)/HL}^2}{n}}}, \quad (6.1)$$

where $T_{LH(Turn)}$, $T_{HL(Turn)}$, T_{LH} and T_{HL} are the sample means of $T_{LH(Turn)}$, $T_{HL(Turn)}$, T_{LH} and T_{HL} respectively, $s_{LH(Turn)}$, $s_{HL(Turn)}$, s_{LH} and s_{HL} are the sample standard deviations of $T_{LH(Turn)}$, $T_{HL(Turn)}$, T_{LH} and T_{HL} respectively, and n is the number of samples.

The t -scores calculated from the experimental data are shown in Table 6.2. Since the sample size for each data group is 5, the degrees of freedom (dof) for the t -score is 8. The critical t -scores for 1% significance level in a two tails test are ± 3.36 . The magnitudes of all the t -scores in Table 6.2, except that for the displacement times of KCl–NaCl solution pair, are higher than the magnitude of the critical t -score. This indicates that the probability for both the observed turning and displacement time

differences to be caused by random error is very small (less than 1%) and thus H_o can be rejected.

Table 6.2: t -scores for turning and displacement times calculated from experimental data.

Solution pair	t-score for turning time	t-score for displacement time
0.5 mM KCl NaCl	-5.75	-1.12
0.5 mM CaCl ₂ -NaCl	8.86	-18.53

For the KCl–NaCl solution pair, the difference between the displacement times was not statistically significant. However, the mean turning time difference between the two different flow directions was statistically significant, which was 7%. In the case of CaCl₂–NaCl solution pair, the mean turning time and the mean displacement time for NaCl displacing CaCl₂ was 18% longer and 21% shorter respectively, than the flow in the reverse direction.

The coexistence of the turning and displacement times in the current-time curves indicated that EOF involving solution pair with dissimilar cationic species consisted of two distinct phases. The mechanics for the hysteretic behavior of EOF with dissimilar cationic solutions will be explained in Section 6.5 with the support of numerical simulations conducted in Section 6.4.

6.4 Numerical simulations

6.4.1 Numerical model

Numerical studies were performed with COMSOL Multiphysics finite element software (FEM) based on the theoretical model after simplifications in Section 3.4 of Chapter 3 for EOF of solution pair with dissimilar ionic species. The governing equations can be found in Chapter 3. The symbols and values of constants employed for the numerical simulations are listed in Table A.1 of Appendix A.

The minority pH-governing ions were neglected in the theoretical model to simplify the numerical simulations. For EOF hysteresis of displacement flow between 0.2 mM and 1 mM KCl solutions (conductivity ratio of 1:5), the displacement time difference for the two different flow directions was approximately 30% (see Figures 4.2a and 4.2d in Chapter 4). Given the conductivity ratios of 0.5 mM KCl–NaCl and 0.5 mM CaCl₂–NaCl solution pairs in this investigation (see Table 6.1), it was estimated that this simplification induces an approximate error of $\leq 12\%$ on the flow rate based on the investigation conducted in Chapter 4.

Even though some details will be lost from the simplification, this simplified model is nevertheless sufficient in elucidating the concentration evolution of the main constituent ions, which is the driving force for this type of EOF hysteresis. The validity of the simplified model can be further assessed if it can predict well the observed experimental results.

6.4.2 Simulation domain

The simulation domain (see Figure 6.3) was a straight microchannel with diameter of 100 μm and length of 0.5 cm. The fluid flow was assumed to be axisymmetric about the center axis of the cylindrical channel.

Figure 6.3: Simulation domain (not drawn to scale).

As the governing equations (see Chapter 3) for the applied electric potential, electrostatic wall potential, main constituent ion distributions and fluid flow are strongly coupled, substantial computational resources are required. Therefore, the domain length was reduced to 0.5 cm (as compared to 8 cm in the experiments) to minimize computational time. The reduced length induced a slight error (estimated to be less than 1%) in the simulated equilibrium current due to the presence of diffused regions at the junctions of reservoirs and microchannel upon completion of the displacement process. The results from the scaled model can thus be normalized to provide good representations of the experimental flow behaviors.

The simulation model was meshed with 24000 quadrilateral elements (24 and 1000 edge elements in the radial and axial directions respectively). The size of the

elements at the wall boundary was set to 1 nm and the size of each adjacent element further from the wall increased by 1.2 times. This mesh selection is sufficiently fine to resolve the steep changes of the variables across the electrical double layer (EDL). The applied potential, electrostatic potential and ion concentrations were discretized with second order elements, and linear elements were employed for the discretization of the pressure and velocity. For the steady-state solutions, convergence test performed using higher number of elements confirmed that there was negligible numerical error for this mesh selection. The convergence criterion was based on a relative tolerance of less than 0.01 between subsequent iterations. The maximum integration time step was set to 0.05 s, while the total simulated time was 22 s.

6.4.3 Boundary and initial conditions

The governing equations have to be solved simultaneously first to obtain the steady-state solution of a single fluid EOF, which has to be set as the initial condition for solving the time-dependent problem of two-fluid displacement flow. The boundary conditions for the steady-state numerical model of a single fluid EOF are shown in Table 6.3.

A voltage of 62.5 V was set at the inlet to establish an electric field of 125 V/cm across the microchannel similar to that in the experiment. The surface charge density S of the wall was specified as -3.46 mC.m^{-2} and -3.60 mC.m^{-2} for 0.5 mM KCl–NaCl and 0.5 mM CaCl_2 –NaCl solution pairs respectively; these values are the average values of

the calculated surface charge densities for the two solutions involved in the displacement flow process (see Table D.1 in Appendix D.1). The surface charge density was assumed constant regardless of the concentrations of solutions during the course of the two-fluid displacement flow.

Table 6.3: Boundary conditions for steady-state numerical model of single fluid electroosmotic flow.

Variable	Condition	Boundary
Applied potential ϕ	$\phi = 62.5V$	Inlet (Anode)
	$\phi = 0V$	Outlet (Cathode)
	$-\mathbf{n} \cdot \nabla \phi = 0^a$	Wall and symmetry
Electrostatic potential ψ	$\mathbf{n} \cdot \nabla \psi = 0$	Inlet, outlet and symmetry
	$\mathbf{n} \cdot \nabla \psi = \frac{S}{\epsilon_r \epsilon_o}$	Wall
Concentrations of ions c_i	$c_i = c_{o(i)} \exp\left(\frac{-z_i e \psi}{k_b T}\right)$	Inlet and outlet
	$-\mathbf{n} \cdot \mathbf{N} = 0^b$	Wall and symmetry
Flow velocity $\mathbf{u}$ and Pressure p	$\mathbf{u} = 0$	Wall
	$\mathbf{n} \cdot \mathbf{u} = 0$	Symmetry
	$p = 0$	Inlet and outlet

^a $\mathbf{n}$ is the unit vector normal to the boundary.

$$^b \mathbf{N} = \left[-D_i \nabla c_i - u_{m(i)} c_i \nabla (\phi + \psi) + \mathbf{u} c_i \right].$$

Electroneutrality condition was imposed on the middle of the microchannel (refer to Section 2.2.1 in Chapter 2). However, electroneutrality is not applied on the channel wall boundary to enable the formation of the EDL (which contains a net

positive charge) to balance the negative surface charge imposed on the boundary. The applied electric field generates an electric force in the EDL and drives the bulk fluid motion through viscous effect. Ion concentrations at the cathode and anode were set to follow Boltzmann distribution under the effect of wall potential ψ . The pressure p at the cathode and anode were set to zero.

In the experiments, the displacing electrolyte (DE) was placed in the inlet (anode) reservoir (see Figure 4.1 in Chapter 4) so that it could replace the residing electrolyte (RE) which initially resided in the microcapillary. After obtaining the steady-state solution, the boundary conditions at the inlet were modified to simulate two-fluid displacement flow with the steady-state solution as the initial condition.

For 0.5 mM NaCl (DE) displacing 0.5 mM KCl (RE), the Na^+ and K^+ concentrations at the inlet were changed by ramping (in an arbitrarily short time of 0.01 s) from 0 mM to 0.5 mM and 0.5 mM to 0 mM respectively. For 0.5 mM KCl (DE) displacing 0.5 mM NaCl (RE), the Na^+ and K^+ concentrations were ramped from 0.5 mM to 0 mM and 0 mM to 0.5 mM respectively.

For 0.5 mM NaCl (DE) displacing 0.5 mM CaCl_2 (RE), the Na^+ , Ca^{2+} and Cl^- concentrations at the inlet were changed by ramping from 0 mM to 0.5 mM, 0.5 mM to 0 mM and 1 mM to 0.5 mM respectively. For 0.5 mM CaCl_2 (DE) displacing 0.5 mM NaCl (RE), the Na^+ , Ca^{2+} and Cl^- concentrations were ramped from 0.5 mM to 0 mM, 0 mM to 0.5 mM and 0.5 mM to 1 mM respectively.

Simulations for the displacement flow of 0.5 mM KCl–NaCl and 0.5 mM CaCl₂–NaCl solution pairs in the two different directions were performed to obtain the time-dependent flow solutions.

6.5 Numerical results

6.5.1 Symmetric electrolytes with dissimilar cations: KCl–NaCl solution pair

The simulated current-time curves for the displacement flow of 0.5 mM KCl (higher conductivity) and 0.5 mM NaCl (lower conductivity) in both directions (see Figure 6.4a) indicated that $T_{HL(Turn)}$ was 15% longer than $T_{LH(Turn)}$ and T_{HL} was 4% longer than T_{LH} . However, t -test based on the experimental results suggested that the displacement time difference was not statistically significant (see Section 6.3). This small difference was not detectable in the experiments as it was within the range of experimental error.

The simulated and experimental current-time curves (see Figure 6.4b) were compared by normalizing the currents and times in both cases. The currents were normalized with the steady-state currents for the two solutions whereas the times were normalized by the time for KCl to fully displace NaCl (T_{HL}). The simulation results matched almost exactly with the experimental results and the displacement time difference was indeed not significant.

Figure 6.4: (a) Simulated current-time curves for displacement flow of 0.5 mM KCl–NaCl in both directions and (b) comparison between simulation and experimental results. Currents and displacement times were normalized with the two steady-state current values and time for KCl to fully displace NaCl (T_{HL}) respectively.

The current increased or decreased beyond the range of the equilibrium currents during the displacement flow (see Figure 6.4a). To explain this current behavior and the turning time difference, evolution of ion distributions throughout the displacement flow was examined through the simulation for the two flow directions. The displacement flow can be separated into two distinct phases with the turning time of the current-time curve; namely Phase 1 before the turning time, and Phase 2 after the turning time (see Figure 6.4a).

When KCl (DE) displaced NaCl (RE), accumulation of K^+ and Cl^- occurred at the upstream of the interface during Phase 1 (see Figure 6.5a). The ion accumulation effect was caused by the difference in conductivity of the two solutions, which arose from the ionic mobility difference between the cations in DE and RE. The ionic

mobilities $u_{m(i)}$ for K^+ and Na^+ are $7.930 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ and $5.194 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ respectively. The conductivity σ of KCl solution ($75.0 \text{ } \mu\text{S} \cdot \text{cm}^{-1}$) is higher than NaCl solution ($63.3 \text{ } \mu\text{S} \cdot \text{cm}^{-1}$) due to the higher ionic mobility.

Figure 6.5: Simulated ion concentrations at axis of symmetry along microchannel when (a) 0.5 mM KCl displaced 0.5 mM NaCl in Phase 1 ($t = 2 \text{ s}$) and (b) Phase 2 ($t = 6 \text{ s}$), and (c) 0.5 mM NaCl displaced 0.5 mM KCl in Phase 1 ($t = 2 \text{ s}$) and (d) Phase 2 ($t = 6 \text{ s}$).

The applied potential generated high electric field in RE with lower conductivity and low electric field in DE with higher conductivity. The difference in electric field in the two solutions caused the incoming electromigrative flux (third term

on the left side of Equation 3.9 in Chapter 3) of Cl^- ions from RE to be higher than the outgoing flux in DE. This electromigrative flux gradient at the interface led to the accumulation of Cl^- (with concentration increasing from 0.5 mM to 0.62 mM) as shown in Figure 6.6a. To maintain charge neutrality, K^+ ion concentration in the vicinity was increased from 0.5 mM to 0.62 mM to match the concentration of Cl^- (see Figure 6.5a). This accumulation of ions caused the total conductivity across the microchannel (and thus current) to increase beyond the equilibrium value (see Figure 6.4a).

Figure 6.6: Schematic drawings to illustrate Cl^- ion concentration adjustment when (a) 0.5 mM KCl displaced 0.5 mM NaCl and (b) 0.5 mM NaCl displaced 0.5 mM KCl. E and σ denote electric field and conductivity respectively. Positive ions are not shown.

On the contrary, when NaCl (DE) displaced KCl (RE), depletion of Na^+ and Cl^- ions occurred at the upstream of the interface between the two solutions during Phase 1 (see Figure 6.5c). The ion depletion effect was similarly caused by the difference in conductivity of DE and RE, which was induced by the ionic mobility difference between the cations. The applied potential generated lower electric field in RE with higher conductivity. Therefore, the incoming electromigrative flux of Cl^- ions from RE

was lower than the outgoing flux in DE, resulting in the depletion of Cl^- ions (with concentration decreasing from 0.5 mM to 0.4 mM) as shown in Figure 6.6b. The depletion of ions caused the total conductivity across the microchannel (and thus current) to decrease below the equilibrium value (see Figure 6.4a).

In addition to the accumulation or depletion of ions, displacement flow of dissimilar ionic solutions also caused variation of interface widths between the two solutions depending on the flow direction (see Figures 6.5a and 6.5c). Interface width is defined as the length of the region which contained both DE and RE. The interface widths obtained from the simulations for both flow directions are plotted in Figure 6.7c. Gradual widening of the interface width had been observed when KCl displaced NaCl, while the interface width remained almost constant in the reverse direction.

The interfacial widening and sharpening effects were caused by the difference in electric field between DE (displacing electrolyte) and RE (residing electrolyte) after the occurrence of ion accumulation/depletion (see Figures 6.6a and 6.6b). For KCl displaced NaCl, electric field in the ion-crowded region of DE was lower than RE due to its high conductivity (see Figure 6.7a). As K^+ ions diffused to the boundary of RE, it experienced high electric field. Therefore, K^+ ions migrated faster than the surrounding ions and penetrated into the RE region, resulting in a widened interfacial region.

In contrast, when NaCl displaced KCl, the ion-deficient region of DE had a higher electric field than RE as a result of its low conductivity (see Figure 6.7b). Thus,

as Na^+ ions diffused to the boundary of RE, they decelerated back to DE due to the low electric field in the RE region. This resulted in a sharp and constant interfacial region.

Figure 6.7: Simulated conductivity and electric field at axis of symmetry along microchannel in Phase 1 ($t = 2$ s) when (a) 0.5 mM KCl displaced 0.5 mM and (b) 0.5 mM NaCl displaced 0.5 mM KCl. (c) Simulated interface widths in both flow directions.

The time for RE, including ions in the interface, to be fully displaced out of the microchannel was reflected as the turning time in the current-time curve. The average flow velocities U_{ave} throughout Phase 1 for both flow directions were examined to explain the turning time difference observed in the simulations (see Figure 6.4a). The average flow velocity is described as:

$$U_{ave} = \frac{Q}{R_c}, \quad (6.2)$$

where Q is the flow rate obtained by integrating the x -velocity over the cross sectional area R_c of the microchannel.

For KCl–NaCl solution pair, U_{ave} for both directions during Phase 1 differed by less than 10% (see Figure 6.8a). Therefore, the dominating factor which contributed to the turning time difference was the widening/sharpening effect of the interfacial region. The flow direction which generated wider interfacial region required a longer time for the complete displacement of RE, resulting in $T_{LH(Turn)}$ shorter than $T_{HL(Turn)}$. This effect is only relevant if the difference in U_{ave} between the two flow directions is small.

The slight difference in U_{ave} between the two flow directions during Phase 1 was attributed to the small difference in length-average zeta potential $\bar{\zeta}$ (see Figure 6.8b). Tang et al. (2011) had derived an equation indicating that U_{ave} is essentially the length-average electroosmotic velocity by combining Ohm's law and fluid mass conservation in the EOF of two solutions. This condition, together with Smoluchowski slip velocity equation (see Equation 2.7 in Chapter 2), yielded the direct relation of U_{ave} and $\bar{\zeta}$. The length-average zeta potential can be defined as:

$$\bar{\zeta} = \frac{1}{L} \int_0^L \zeta(x) dx, \quad (6.3)$$

where $\zeta(x)$ is the zeta potential, which is a function of x position along the microchannel.

Figure 6.8: Simulated (a) average flow velocity U_{ave} and (b) length-average zeta potential $\bar{\zeta}$ for displacement flow of 0.5 mM KCl–NaCl in both flow directions.

After RE was completely flushed out, DE of different concentrations remained in the microchannel for both flow directions. According to the simulations, KCl with concentration ranging from 0.5-0.62 mM was formed in the channel for the case of KCl displaced NaCl, while NaCl with concentration ranging from 0.4-0.5 mM was produced in the reverse flow direction (see Figures 6.5b and 6.5d).

Subsequent current changes during Phase 2 (after the turning time) were caused by the displacement flow of DE at different concentrations which were generated in Phase 1 from ion accumulation or depletion. Figure 6.8b shows that $\bar{\zeta}$ for 0.5-0.62 mM KCl and 0.4-0.5mM NaCl differed insignificantly (by less than 10%). The resultant small difference in U_{ave} (see Figure 6.8a) thereby generated a slight displacement time difference between the two flow directions which can only be detected in the numerical simulations, but not experimentally.

6.5.2 Asymmetric-symmetric electrolytes with dissimilar cations: CaCl₂ - NaCl solution pair

The simulated current-time curves for the displacement flow of 0.5 mM CaCl₂ (higher conductivity) and 0.5 mM NaCl (lower conductivity) in both directions (see Figure 6.9a) showed that $T_{LH(Turn)}$ was 27% longer than $T_{HL(Turn)}$ while T_{HL} was 34% longer than T_{LH} . The simulated and experimental current-time curves (see Figure 6.9b) were compared by normalizing the currents and times in both cases. The currents were normalized with the maximum and minimum currents whereas the times were normalized by the time for the ascending curve to reach a steady value (T_{HL}). Figure 6.9b shows that the simulation results agreed very well with the experimental results.

Figure 6.9: (a) Simulated current-time curves for displacement flow of 0.5 mM CaCl₂-NaCl in both directions and (b) comparison between simulation and experimental results. Currents and displacement times were normalized with the maximum and minimum currents and time for the ascending curve to reach a steady value (T_{HL}) respectively.

The current change was bounded within the range of the equilibrium currents during the displacement flow (see Figure 6.9a). Similarly, the evolution of ion

distributions was examined via the numerical simulation (see Figure 6.10) to explain the current behavior, as well as the turning and displacement time differences between the two flow directions. The conductivities σ for CaCl_2 and NaCl are $135.9 \mu\text{S}\cdot\text{cm}^{-1}$ and $63.3 \mu\text{S}\cdot\text{cm}^{-1}$ respectively. When CaCl_2 (DE) displaced NaCl (RE), the applied potential generated lower electric field in CaCl_2 (higher conductivity) and higher electric field in NaCl (lower conductivity) due to the difference in conductivity.

Figure 6.10: Simulated ion concentrations at axis of symmetry along microchannel when (a) 0.5 mM CaCl_2 displaced 0.5 mM NaCl in Phase 1 ($t = 2$ s) and (b) Phase 2 ($t = 6$ s), and (c) 0.5 mM NaCl displaced 0.5 mM CaCl_2 in Phase 1 ($t = 2$ s) and (d) Phase 2 ($t = 6$ s).

The resultant electromigrative flux gradient changed Cl^- ion concentration to 0.54 mM at the upstream of the interfacial region as the ions transversed from RE to DE in Phase 1 (see Figure 6.11a). However, the adjusted Cl^- ion concentration was still lower than the Cl^- ion concentration of DE. Therefore, the current did not rise beyond the equilibrium value (see Figure 6.9a). To satisfy the electroneutrality condition, Ca^{2+} ion concentration changed from 0.5 mM to 0.27 mM to match the charges of Cl^- ions at the upstream of the interface as shown in Figure 6.10a.

Figure 6.11: Schematic drawings to illustrate Cl^- ion concentration adjustment when (a) 0.5 mM CaCl_2 displaced 0.5 mM NaCl and (b) 0.5 mM NaCl displaced 0.5 mM CaCl_2 . E and σ denote electric field and conductivity respectively. Positive ions are not shown.

Similar argument can be applied to the flow in the reverse direction, i.e. NaCl (DE) displaced CaCl_2 (RE). At the vicinity of the interface between the two solutions, concentration of Na^+ and Cl^- ions were adjusted to 0.9 mM during Phase 1 as observed in Figure 6.10c. This adjustment was similarly caused by the difference in electric field

between DE and RE, which was induced by the conductivity difference of the two solutions (see Figure 6.11b).

In addition to the adjustment of the ion concentrations, interface width broadening occurred when CaCl_2 displaced NaCl . On the contrary, the interface width remained narrow and almost constant for NaCl displaced CaCl_2 (see Figure 6.12). The interface width difference was caused by the difference in electric field between DE and RE (similar to the case of KCl – NaCl solution pair in Section 6.5.1).

Figure 6.12: Simulated conductivity and electric field at axis of symmetry along microchannel in Phase 1 ($t = 2$ s) when (a) 0.5 mM CaCl_2 displaced 0.5 mM and (b) 0.5 mM NaCl displaced 0.5 mM CaCl_2 . (c) Simulated interface widths in both flow directions.

To examine the turning time difference, the average flow velocity U_{ave} and length-average zeta potential $\bar{\zeta}$ for both flow directions are plotted in Figure 6.13. The large variation of $\bar{\zeta}$ throughout the displacement flow arose as a result of the non-uniform zeta potential along the channel due to the ion adjustment effect. As depicted in Figure 6.13b, $\bar{\zeta}$ for NaCl displaced CaCl_2 was 50% smaller than the flow in the reverse direction initially. The flow direction with higher $\bar{\zeta}$ thus generated higher U_{ave} (see Figure 6.13a) which required a shorter time for the complete displacement of RE. These provides the explanation for $T_{LH(Turn)}$ to be longer than $T_{HL(Turn)}$ predicted in the simulations. The widening/sharpening effect of the interfacial region did not significantly affect the turning time difference because the advective effect arising from the large difference in U_{ave} was more dominant than the interfacial diffusive effect.

Figure 6.13: Simulated (a) average flow velocity U_{ave} and (b) length-average zeta potential $\bar{\zeta}$ for displacement flow of 0.5 mM CaCl_2 -NaCl in both flow directions.

Upon complete displacement of RE, DE with different concentrations were left in the microchannel for the two flow directions. The simulations indicated that CaCl_2

with concentration ranging from 0.27-0.5 mM was produced in the channel for CaCl_2 displaced NaCl, while NaCl with concentration ranging from 0.5-0.9 mM was formed in the reverse flow direction (see Figures 6.10b and 6.10d).

Similar to the case of KCl–NaCl solution pair, subsequent current changes during Phase 2 were due to the displacement flow of DE at different concentrations which were produced in Phase 1 from the ion adjustment effect. Figure 6.13b clearly shows that $\bar{\zeta}$ for 0.27-0.5 mM CaCl_2 was at least 35% smaller than that for 0.5-0.9 mM NaCl. Consequently, U_{ave} for 0.5-0.9 mM NaCl was much faster than that for 0.27-0.5 mM CaCl_2 (see Figure 6.13a), which provides the explanation for T_{HL} being longer than T_{LH} .

6.6 Summary

EOF involving solution pair with dissimilar cationic species was investigated both experimentally and numerically. Current monitoring experiments were carried out to monitor the displacement flow behaviors of different solution pairs through the current-time curves. Finite element simulations based on the proposed theoretical model in Chapter 3 were then performed to elucidate the movement of ions and flow behaviors.

Two important times which exhibited hysteretic or flow direction-dependent behavior were identified: (a) turning time which marks the abrupt gradient change of

current-time curve and (b) displacement time which is the time for one solution to completely displace the initial solution (indicated by a steady current). The turning time divided the electroosmotic displacement flow process into two distinct phases: namely Phase 1 where the residing (initial) electrolyte is displaced and Phase 2 which involves the displacement flow of the displacing electrolyte and its adjusted concentration. Both the experimental and simulation results demonstrated that the turning and displacement times for a particular solution pair differed with the flow direction.

Two different mechanisms had been identified as the causes of EOF hysteresis for dissimilar cationic solutions. The first mechanics was the widening/sharpening effect of the interfacial region induced by conductivity difference between the two solutions. This effect is weak and is only significant if the average flow velocity difference between the two directions is small, e.g. NaCl–KCl solution pair. The second mechanics involved the difference in length-averaged zeta potentials for the two flow directions. This effect is pronounced in the case whereby the zeta potentials of the two solutions differ significantly, e.g. CaCl₂–NaCl solution pair.

Chapter 7 Electroosmotic flow hysteresis for dissimilar anionic solutions^{#5}

7.1 Introduction

Even though there are various investigations on electroosmotic flow (EOF) with different types of fluids (Ren et al. 2001, Ren et al. 2002, Ren et al. 2003), there is yet any investigation examining specifically the electroosmotic displacement flow behavior of solution pair with *dissimilar anion species*. For the first time, the EOF hysteresis phenomenon for dissimilar anionic solutions demonstrated via the current monitoring experiments was explained through the numerical simulations based on the theoretical formulation in Chapter 3.

7.2 Experimental details

7.2.1 Experimental setup and materials

Current monitoring technique was used for the experiments. The experimental setup was similar to that described in Section 4.2.1 of Chapter 4, except for the electrolyte solutions employed and the placement of displacing electrolyte in the reservoir (see Figure 7.1). Explanation for the choice of placement for the displacing electrolyte is detailed in Section 7.2.2.

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Figure 7.1: Experimental setup of current monitoring method for displacement of residing electrolyte (RE) in microchannel from cathode reservoir.

Two types of ionic solutions with the same cation but dissimilar anions, namely sodium chloride (NaCl) and sodium sulfate (Na_2SO_4), were prepared for the experiments. The solution pair was chosen as a model to study the electroosmotic displacement flow behavior for dissimilar anionic solutions. The concentrations for the solutions were set to be identical (0.5 mM) so that the effect of ionic species type on EOF behavior could be examined.

The measured conductivities (IONCheck 65, Radiometer Analytical) for 0.5 mM NaCl and 0.5 mM Na_2SO_4 were $63.8 \pm 0.2 \mu\text{S}\cdot\text{cm}^{-1}$ and $130.6 \pm 0.3 \mu\text{S}\cdot\text{cm}^{-1}$ respectively. The measured average pH (AccumetAR20, Fisher Scientific) for the solutions was 5.57 ± 0.09 . Since the solutions employed were unbuffered solutions, the acidity of the solutions was caused by the dissolution of atmospheric carbon dioxide (CO_2) into water.

7.2.2 Experimental procedures

From the perspective of a visualization experiment, the bulk EOF takes place from the anode towards the cathode. However, the anions experienced a net migration in a direction which opposed the EOF because the magnitudes for the ionic mobilities $u_{m(i)}$ of anions ($u_{m(Cl^-)} = -7.92 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ and $u_{m(SO_4^{2-})} = -8.30 \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$, refer to Table A.1 in Appendix A) were higher than the EOF mobilities u_{eo} in the experiments ($u_{eo(Na_2SO_4)} = (2.87 \pm 0.25) \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$ and $u_{eo(NaCl)} = (4.32 \pm 0.12) \times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$, see Appendix E.1).

Similar to previous definitions (see Chapter 6), the displacing electrolyte (DE) is the electrolyte with dissimilar ion species that migrated into the microchannel and displaced the initial ions of the residing electrolyte (RE).

The anode reservoir and microchannel were filled with the RE (see Figure 7.1) for the displacement flow experiment. The DE was placed in the cathode reservoir so that the displacing anions could migrate upstream against the EOF, replacing the initial anions of the RE in the microchannel. For example, for NaCl displacing Na₂SO₄, NaCl was placed in the cathode reservoir (DE) and Na₂SO₄ was residing in the microchannel (RE). The current-time responses of displacement flows involving NaCl–Na₂SO₄ solution pair in two different directions, i.e. NaCl displaced Na₂SO₄ and Na₂SO₄ displaced NaCl, were compared to reveal the hysteresis phenomenon.

Several precautions had been adopted (see Section 4.2.2 of Chapter 4) to maintain pH stability throughout the experiments, as the application of electric field

through the electrodes might cause undesirable electrolytic reactions which induce local pH changes in the reservoirs due to the production of hydronium (H_3O^+) and hydroxide (OH^-) ions (Rodríguez and Chandrasekhar 2005).

The concentrations and conductivities of the solutions employed for the experiments were low (see Section 7.2.1). As such, Joule heating which would affect the EOF behavior by affecting the solution properties such as viscosity and EOF mobility (see Equation 2.7 in Chapter 2) could be ignored (Tang et al. 2006). A conservative estimate of Joule heating can be calculated from the energy balance between the energy generation E_g and the energy storage ΔE_{st} in the liquid (Arulanandam and Li 2000). For the chosen experimental parameters, the worst case scenario (applied electric field = 125 V/cm, solution conductivity (0.5 mM Na_2SO_4) = $130.6 \mu\text{S}\cdot\text{cm}^{-1}$, capillary diameter = 100 μm and experimental duration = 1000 s) has an estimated temperature rise of 0.16 °C, which is negligible.

7.3 Numerical simulations

Numerical studies were performed with COMSOL Multiphysics finite element software (FEM) based on the theoretical formulation after simplifications in Section 3.4 of Chapter 3, which is similar to that in Chapter 6 (see Section 6.4.1 for details on the exclusion of minority pH-governing ions). The governing equations can be found in Chapter 3. The symbols and values of constants employed for the numerical simulations are listed in Table A.1 of Appendix A.

The simulation domain, meshing and discretization of the variables were similar to that in Section 6.4.2 of Chapter 6. The convergence criterion and the integration time step were also similar to that in Section 6.4.2 of Chapter 6, except that the total simulated time was 30 s.

The steady-state solution of single fluid EOF was obtained first and set as the initial condition for solving the time-dependent solution of two-fluid displacement flow. The boundary conditions (similar to that in Section 6.4.3 of Chapter 6) can be found in Table 6.3 of Chapter 6. The surface charge density S of the wall was specified as -3.19 mC.m^{-2} , namely the average value of the calculated surface charge densities for NaCl and Na₂SO₄ solutions (see Table E.1 in Appendix E.1). The surface charge density was assumed constant regardless of the ion concentrations during the displacement process.

In the experiments, the displacing electrolyte (DE) was placed in the cathode reservoir (see Figure 7.1) so that the displacing anions could replace the initial anions of the residing electrolyte (RE) in the microchannel. To simulate that, the boundary conditions at the cathode of the simulation domain (see Figure 6.3 in Chapter 6) were modified. For 0.5 mM NaCl (DE) displacing 0.5 mM Na₂SO₄ (RE), the Na⁺, Cl⁻ and SO₄²⁻ concentrations at the cathode were changed by ramping (in an arbitrarily short time of 0.01 s) from 1 mM to 0.5 mM, 0 mM to 0.5 mM and 0.5 mM to 0 mM respectively. For 0.5 mM Na₂SO₄ (DE) displacing 0.5 mM NaCl (RE), the Na⁺, Cl⁻ and SO₄²⁻ concentrations were ramped from 0.5 mM to 1 mM, 0.5 mM to 0 mM and 0 mM to 0.5 mM respectively.

Simulations for the displacement of 0.5 mM NaCl–Na₂SO₄ solution pair in two different directions were carried out to elucidate the mechanics of EOF hysteresis for dissimilar anionic solutions.

7.4 Results and discussion

7.4.1 Current behaviors observed in experiments

As mentioned in Section 7.2.2, the anions experienced electromigration in the direction opposing EOF. The anions of DE from the cathode reservoir migrated upstream against the EOF to fill up the microchannel, displacing the residing anions. However, the current-time curves for displacements of 0.5 mM NaCl–Na₂SO₄ solution pair never approach the steady-state current values of the DE (see Figure 7.2a). This incongruity was evidenced through comparison with the experimental results for displacements of 0.5 mM NaCl–CaCl₂ solution pair (dissimilar cationic solutions) in Chapter 6, whereby the current-time curves equilibrated at the steady-state current values of the DE upon completion of the displacement processes (see Figure 7.2b).

Figure 7.2: Experimental current-time curves for displacement of (a) 0.5 mM NaCl–Na₂SO₄ (via cathode reservoir) and (b) 0.5 mM NaCl–CaCl₂ (via anode reservoir, see Chapter 6) in two different directions. (c) Comparison between simulated and experimental current-time curves for displacements of 0.5 mM NaCl–Na₂SO₄. Experimental/numerical currents and times were normalized by $I^* = (I - I_{NaCl}^0) / (I_{Na_2SO_4}^0 - I_{NaCl}^0)$ and $t^* = t / t_{NaCl \rightarrow Na_2SO_4}$ respectively, where I^0 with corresponding subscripts *NaCl* and *Na₂SO₄* represent the initial currents of the solution pair, and t with subscript *NaCl*→*Na₂SO₄* represents the time for NaCl displaced Na₂SO₄ to reach steady-state current.

Similar to EOF of solutions with dissimilar cation species, the current-time curves for EOF of solutions with dissimilar anions exhibited an abrupt gradient change before reaching the equilibrium values. The definitions of turning and displacement times are detailed in Section 6.3 of Chapter 6. Even though the turning and displacement times seem to be exceedingly weak for the observation in Figure 7.2a,

they were still able to be computed from the experimental current-time curves by performing curve fitting with a SigmaPlot regression analysis. The turning times for Na_2SO_4 displacing NaCl and the reverse direction were 191.0 ± 9.5 s and 156.7 ± 8.5 s respectively, while the displacement times were 337.5 ± 25.2 s and 321.3 ± 15.3 s respectively. The abrupt gradient change (indicated by the turning time) allowed the displacement process to be separated into two distinct phases, namely Phase 1 and Phase 2 (see Figure 7.2a). To explain the counter-intuitive current behaviors as observed in Figure 7.2a, the evolution of ion distribution was examined through numerical simulations (see Figures 7.3 and 7.4).

The experimental/numerical currents and times were normalized by Equations 7.1 and 7.2 respectively:

$$I^* = \frac{I - I_{NaCl}^o}{I_{Na_2SO_4}^o - I_{NaCl}^o}, \quad (7.1)$$

$$t^* = \frac{t}{t_{NaCl \rightarrow Na_2SO_4}}, \quad (7.2)$$

where I^o with corresponding subscripts $NaCl$ and Na_2SO_4 represent the initial currents of the solution pair, and t with subscript $NaCl \rightarrow Na_2SO_4$ represents the time for NaCl displaced Na_2SO_4 to reach steady-state current.

The experimental and numerical current-time curves were compared through these normalized currents and times (see Figure 7.2c), and their good agreement confirmed the validity of the numerical model.

7.4.2 Concentration adjustment according to Kohlrausch regulating function

From the simulation results, the anion species from DE migrated upstream against EOF from the cathode reservoir (see Figures 7.3a and 7.3c). This was caused by the stronger gradient of electromigrative flux as compared to the convective flux induced by EOF. This effect can be predicted by comparing the ionic mobilities $u_{m(i)}$ of anions and EOF mobilities u_{eo} (refer to Section 7.2.2). However, the DE concentration was adjusted from 0.5 mM to 0.25 mM for Na_2SO_4 (when Na_2SO_4 displaced NaCl , see Figure 7.3a), and from 0.5 mM to 0.98 mM for NaCl (when NaCl displaced Na_2SO_4 , see Figure 7.3c). The concentration adjustment fulfilled both current continuity and charge neutrality (dictated by Equations 3.3 and 3.7 respectively in Chapter 3), and could be predicted by the Kohlrausch regulating function (KRF) (Hruška and Gaš 2007):

$$\text{KRF}(x) = \sum \frac{z_i c_i(x, t)}{u_{m(i)}}, \quad (7.3)$$

where $c_i(x, t)$ is the concentration of ionic species as a function of axial coordinate x for the channel and time t .

The KRF for 0.5 mM of Na_2SO_4 and NaCl are $3.13 \times 10^7 \text{ mol.V.s.m}^{-5}$ and $1.59 \times 10^7 \text{ mol.V.s.m}^{-5}$ respectively. As anions from the cathode reservoir migrated toward the anode, the DE concentration was adjusted such that its KRF matched the KRF of

the RE. When Na_2SO_4 displaced NaCl , the concentration of Na_2SO_4 was decreased spontaneously from 0.5 mM to 0.25 mM. This was because the initial KRF value of Na_2SO_4 was higher than NaCl . The concentration of Na_2SO_4 was lowered so that its KRF value matched the KRF value of NaCl . In contrast, when NaCl displaced Na_2SO_4 , the concentration of NaCl was increased from 0.5 mM to 0.98 mM. The KRF value of NaCl was thus increased to match the higher KRF value of Na_2SO_4 .

Figure 7.3: Simulated ion concentrations at axis of symmetry along microchannel when (a) 0.5 mM Na_2SO_4 from cathode reservoir displaced 0.5 mM NaCl in Phase 1 ($t = 5\text{ s}$) and (b) Phase 2 ($t = 15\text{ s}$); and (c) 0.5 mM NaCl from cathode reservoir displaced 0.5 mM Na_2SO_4 in Phase 1 ($t = 5\text{ s}$) and (d) Phase 2 ($t = 15\text{ s}$). Dimensionless $x^* = x/L$, where x is the axial coordinate and L is the length of microchannel.

The KRF presented in Equation 7.3 bears the following simplifications: (i) diffusion of ions is neglected, and (ii) EOF is absent. KRF was merely employed to illustrate the concentration adjustment mechanics in Phase 1 and to estimate the adjusted concentration for the DE. The spatial and temporal evolution of ion distributions along the microchannel during the displacement process was obtained through the numerical simulation (see Figure 7.3). The numerical results on the adjusted DE concentrations agreed well with the concentrations predicted by KRF.

At the end of Phase 1, the RE in microchannel was completely replaced by the DE with the adjusted concentration based on KRF. Due to the spontaneous concentration adjustment during the displacement process, the conductivity of DE deviated from the initial/original conductivity in the reservoir. This was one of the factors which prevent the steady-state current reaching the expected value based on the original properties of DE (see Figure 7.2a).

Similarly, KRF can also be used to describe the concentration adjustment (in Phase 1) for EOF of dissimilar cationic solutions (Chapter 6). Take for example the case of EOF with 0.5 mM NaCl–CaCl₂ solution pair (KRF of CaCl₂ is 2.88×10^7 mol.V.s.m⁻⁵), KRF predicts that the DE concentration should adjust from 0.5 mM to 0.28 mM for CaCl₂ (when CaCl₂ displaced NaCl from the anode reservoir), and from 0.5 mM to 0.9 mM for NaCl (when NaCl displaced CaCl₂ from the anode reservoir). The predictions agreed well with the simulation results (see Section 6.5.2 in Chapter 6), which justifies the use of KRF in predicting the concentration adjustment during EOF of dissimilar ionic solutions.

For pre-concentration techniques such as field amplified sample stacking (FASS) and isotachophoresis (ITP), the electric field similarly induces the propagation of sample concentration shock wave and rarefaction wave (Bharadwaj and Santiago 2004, Bahga et al. 2011), while the plateau concentration is governed by KRF as well (Boček 1981, Bharadwaj and Santiago 2005). However, no study has hitherto been attempted to relate the focusing or depletion of ions to the change in EOF flow rate for different displacement directions. This is because EOF is often suppressed to improve the sensitivity and resolution of these pre-concentration applications (Bharadwaj and Santiago 2005, Garcia-Schwarz et al. 2011). The investigations in Chapters 5 and 6 relate the adjustment of ion concentrations to the EOF flow rate and demonstrate the hysteretic flow effect, which are detailed in Section 7.4.5.

Interestingly, a second concentration adjustment occurred in Phase 2 for EOF of dissimilar anionic solutions, which was marked by the abrupt change of gradient in the current-time curve (see Figures 7.2a and 7.2c).

7.4.3 Diffusive-interface-induced concentration adjustment

In Phase 2, despite the fact that almost the entire microchannel was filled with the DE, the anions of RE were still found near the vicinity of the anode reservoir. For example, when Na_2SO_4 displaced NaCl in Phase 1, the vicinity near the anode reservoir only contained two types of ions, namely Na^+ and Cl^- . This was because the anode reservoir and microchannel were initially filled with 0.5 mM NaCl (RE) (see Figure

7.4a). In Phase 2, when SO_4^{2-} ions (of the DE) reached the vicinity of the anode reservoir, a region with a mixture of three types of ions, namely Na^+ , Cl^- and SO_4^{2-} , was developed (see Figure 7.4b).

Figure 7.4: Simulated ion distributions at the junction between anode reservoir and microchannel when (a) 0.5 mM Na_2SO_4 from cathode reservoir displaced 0.5 mM NaCl in Phase 1 ($t = 5$ s) and (b) Phase 2 ($t = 30$ s, steady-state); and (c) 0.5 mM NaCl from cathode reservoir displaced 0.5 mM Na_2SO_4 in Phase 1 ($t = 5$ s) and (d) Phase 2 ($t = 30$ s, steady-state). E is the electric field, σ is the conductivity, and the differential dA indicates that integration is performed over the cross sectional area of interest. The location of integration is denoted by the subscripts a and L which represent the anode reservoir junction and other locations in the microchannel respectively. Dimensionless $x^* = x/L$, where x is the axial coordinate and L is the length of microchannel.

At first glance, Cl^- ions (of the RE) which migrated toward the anode should have been completely displaced by SO_4^{2-} ions (of the DE) and disappear from the mixture region. However, Cl^- was retained in the mixture zone because the anode reservoir of NaCl served as an infinite source and maintained a sharp diffusive gradient to oppose the complete displacement of Cl^- ions. In addition, the anode reservoir of NaCl also acted as an infinite sink for the incoming SO_4^{2-} , thus maintaining a sharp concentration gradient at the junction.

Subsequently, the three ions in the diffusive mixture zone reorganized themselves to fulfill both current continuity and charge neutrality (dictated by Equations 3.3 and 3.7 respectively in Chapter 3). Although this diffusive interface was very small ($\sim 15 \mu\text{m}$) as compared to the microchannel length, it had a significant impact on the final equilibrium content of the entire channel due to its difference in conductivity.

Current continuity requires that current I has to obey the equation:

$$I = \int E_a \sigma_a dA = \int E_L \sigma_L dA, \quad (7.4)$$

where E is the electric field, σ is the conductivity, and the differential dA indicates that integration is performed over the cross sectional area of interest. The location of integration is denoted by the subscripts a and L which represent the anode reservoir junction and other locations in the microchannel respectively (see Figures 7.4b and 7.4d).

To determine the equilibrium concentration c_L in the microchannel, Equation 7.4 was rearranged to solve for c_L :

$$c_L = \frac{\int E_a \sigma_a dA}{R_c E_L F \sum n_i z_i u_{m(i)}} = \frac{I_{Steady-state}}{R_c E_L F \sum n_i z_i u_{m(i)}}, \quad (7.5)$$

where R_c is the cross sectional area of microchannel, F is the Faraday constant, $I_{Steady-state}$ is the steady-state current, n_i is the molecular formula, $z_{(i)}$ is the charge number and $u_{m(i)}$ is the ionic mobility of ionic species. For the calculation, E_L can be approximated as 125 V/cm for a long channel and $I_{Steady-state}$ can be obtained from the experiments.

The numerical simulation for Na_2SO_4 displaced NaCl showed that the concentration of Na_2SO_4 decreased from 0.5 mM to 0.25 mM in Phase 1 and increased to 0.29 mM in Phase 2. The readjusted DE concentration was propagated downstream throughout the entire microchannel via EOF (see Figure 7.3b). As a comparison, the equilibrium concentration of Na_2SO_4 calculated with Equation 7.5 based on the experimental results was 0.28 ± 0.02 mM, which is close to the simulated concentration of 0.29 mM.

The numerical simulation for NaCl displaced Na_2SO_4 showed that the concentration of NaCl increased from 0.5 mM to 0.98 mM in Phase 1 and decreased to 0.85 mM in Phase 2. Similarly, the readjusted DE concentration was propagated downstream throughout the entire microchannel via EOF (see Figure 7.3d). The equilibrium concentration of NaCl obtained (through Equation 7.5) based on the

experimental results was 0.94 ± 0.08 mM, which is reasonably close to the simulated value of 0.85 mM.

The diffusive current density at the channel/reservoir border (second term on the left of Equation 3.2 in Chapter 3, which was in the range of 10^{-4} to 10^1 A.m⁻²) could be neglected as it was several orders of magnitude smaller than the electromigrative current density (first term on the left of Equation 3.2, which was in the range of 10^2 A.m⁻²). As such, it would not have a significant effect on the observed ion concentration adjustments in both Phase 1 and Phase 2.

In the experiments, the electrodes were placed in the wide reservoir regions (see Figure 7.1). According to Laplace equation (Equation 3.3 in Chapter 3), the electric field will be significantly affected at the location where the microchannel widens into the reservoir. Despite that, there was negligible effect on the concentration adjustment in Phase 2. Detailed explanations can be found in Appendix E.2. Therefore, the geometrical expansion at the reservoir junction was not included in the numerical simulations for simplicity, as well as to minimize computational effort.

Although all the solutions had the same initial concentration of 0.5 mM, the equilibrium DE concentration was 0.29 mM for Na₂SO₄ and 0.85 mM for NaCl. Two distinct and spontaneous concentration adjustments during the displacement process caused the steady-state current to deviate from the expected value based on the initial DE properties (see Figure 7.2a).

It should be highlighted that although the concentration adjustment in Phase 1 is well described by Kohlraush regulating function (KRF), the concentration readjustment at the reservoir junction at Phase 2 has never been described in the literature. This diffusive-interface-induced concentration adjustment affects the content in the entire microchannel when EOF convects the adjusted analytes downstream from the reservoir junction.

7.4.4 Difference between dissimilar cations and anions systems

The ion concentration adjustment according to Kohlraush regulating function (KRF) applies to Phase 1 of both NaCl–CaCl₂ (dissimilar cations) and NaCl–Na₂SO₄ (dissimilar anions) systems (see Section 7.4.2). The difference between these two systems lies in Phase 2 of the displacement flow.

During Phase 2 of NaCl–CaCl₂ system, the ion distribution achieved in Phase 1 was simply displaced by the content in the anode reservoir under EOF (see Section 6.5.2 in Chapter 6). This was because the cations in the anode reservoir migrated in the same direction as EOF.

However, during Phase 2 of NaCl–Na₂SO₄ system, the ion distribution achieved in Phase 1 was *not* displaced by the content in the anode reservoir. This was because the anions in the anode reservoir migrated in an opposite direction to the EOF (see Section 7.4.2). At first glance, this might suggest that the content in the anode reservoir

would have no effect on the final state of the system. On the contrary, the content in the anode reservoir does have an effect on the final state of the system.

A diffusive interface containing the anions in the anode reservoir and the displacing anions was formed at the junction between the microchannel and the reservoir (see Section 7.4.3). This diffusive zone caused another ion concentration adjustment and the content was propagated throughout the entire channel by EOF, which explains the difference in final ion concentration from that in the anode or cathode reservoir.

7.4.5 Electroosmotic flow hysteresis loop

The flow direction reversal was achieved experimentally by changing the positions of anode and cathode reservoirs. Conceptually, it can also be achieved by reversing the electric field direction (i.e. changing the polarity of the applied voltage). One might intuitively anticipate that reversing the flow direction would simply cause the fluid to retrace its path, i.e. the flow behavior is reversible. However, the flow direction reversal led to different ion distribution adjustments along the microchannel (see Sections 7.4.2 and 7.4.3), and thus caused the EOF flow rate to be directional-dependent, resulting in a hysteretic flow behavior.

To demonstrate this hysteresis effect, the EOF hysteresis loop relating the EOF flow rate and the percentage of NaCl along the microchannel is plotted in Figure 7.5a.

The EOF flow rate followed different paths (as indicated by the two lines in Figure 7.5a) depending on the displacement directions, forming an open-ended hysteresis loop. This showed that the flow rate cannot be determined at a given RE–DE interface position without knowing the displacement direction.

Figure 7.5: Variations of numerical flow rate with percentage of NaCl in microchannel for displacement of (a) 0.5 mM NaCl–Na₂SO₄ and (b) 0.5 mM NaCl–CaCl₂ (constructed from the numerical data in Chapter 6) solution pairs in two different directions from cathode and anode reservoirs respectively.

To highlight the peculiar behavior of the open-ended hysteresis loop, a typical closed hysteresis loop for the displacements of 0.5 mM NaCl–CaCl₂ solution pair (dissimilar cationic solutions) constructed from the numerical data in Chapter 6 is shown in Figure 7.5b for comparison. The EOF flow rate in both directions approached the flow rates of their respective DE. For example, when 0.5 mM NaCl displaced CaCl₂, the microchannel was eventually filled with 0.5 mM NaCl. In contrast, the open-ended hysteresis loop showed that the EOF flow rate cannot approach that of the

DE. For example, when 0.5 mM NaCl displaced Na₂SO₄, the microchannel will never be filled with 0.5 mM NaCl. This was due to the two distinct and spontaneous adjustments of DE concentration during the displacement process (see Sections 7.4.2 and 7.4.3).

7.5 Summary

In this investigation, EOF hysteresis for solution pair with dissimilar anion species was demonstrated by the current monitoring experiments, and elucidated through the numerical simulations based on the theoretical formulation in Chapter 3. In contrast to EOF for solution pair with different concentrations or dissimilar cation species, EOF involving dissimilar anionic solutions exhibited an unusual behavior, whereby the steady-state concentration of the displacing electrolyte in the microchannel deviated from its original value at the end of the displacement process. This counter-intuitive behavior was attributed to the ion concentration adjustment when the displacing anions migrated against the EOF. Subsequently, a second ion concentration adjustment was induced by the diffusive interface at the junction between the anode reservoir and the microchannel, which was convected throughout the entire channel by EOF.

The flow direction reversal, effected by reversing the electric field direction, led to different ion distribution adjustments along the microchannel. This caused the EOF flow rate to be directional-dependent, resulting in a hysteretic flow behavior. This

hysteresis effect can be visualized by plotting the EOF flow rate against the two-fluid interface displacement. The resultant hysteresis loop suggested that the flow behavior can only be ascertained at a given interface position or time if the displacement direction is known. Furthermore, the open-ended hysteresis loop also suggested that the EOF flow rate in the microchannel cannot approach the flow rate of the displacing electrolyte upon completion of the displacement process.

These findings imply that the transport of analytes such as drugs, proteins, or other negatively charged ionic species of interest in an EOF microfluidic system, requires careful consideration. The flow rate of fluid and concentration of the analytes which are placed into the reservoir might deviate significantly from their initial values after they are delivered into the microchannel, depending on the properties of fluid initially residing in the microchannel and the reservoirs.

Chapter 8 Effect of nanostructure orientation on electroosmotic flow in a microfluidic channel^{#6}

8.1 Introduction

Electroosmotic flow (EOF) is not desirable in certain applications. For example, EOF generates a counterflow opposite to the electrophoresis migration of negatively charged samples, e.g. DNA and sodium dodecyl sulfate (SDS) denatured proteins, which degrades the resolution of electrophoretic separation (Viovy 2000). The suppression of EOF is conventionally accomplished through the coating of water-soluble polymers (Horvath and Dolnik 2001), e.g. acidified poly(ethylene oxide) (PEO) (Preisler and Yeung 1996).

Nanoscale networks/structures are often integrated within microchannels for a broad range of applications, such as electrophoretic separation of biomolecules (Li et al. 2003, Kaji et al. 2004), high reaction efficiency catalytic microreactors (Kusakabe et al. 2001, Miyazaki et al. 2004), and enhancement of heat transfer (Nagayama et al. 2007, Li et al. 2012) and sensing (Li et al. 2013, Ng et al. 2016). They had been discovered to reduce EOF effectively when present in microchannels (Kang and Suh 2009, Messinger and Squires 2010, Yasui et al. 2011, Koga et al. 2013). Despite the vast investigations, a proper study on the orientation effect of nanostructures is yet to be conducted.

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Yasui et al. (2011) fabricated microchannels with two different types of nanopillar arrangements, i.e. the square and tilted array patterns. Both configurations had no observable EOF velocity difference between them, but both reduced EOF significantly as compared to the smooth channel. This lack of EOF velocity difference between the two configurations could well be due to their insignificant orientation difference. For a rigorous investigation on the effect of nanostructure orientation on EOF, the nanostructure designs in microchannels should demonstrate a significant orientation difference.

In this investigation, a novel fabrication process was developed, namely a hybrid LIGA-DEEMO process (LIGA stands for **L**ithographie, **G**alvanoformung, **A**bformung, which means Lithography, Electroplating, Molding in German, while DEEMO stands for **D**ry **E**tching, **E**lectroplating, **M**olding) for the fabrication of microchannels with large-area arrays of parallel and perpendicular indented nanolines, which have a significant orientation difference.

This newly developed fabrication method ensures the micro-/nanostructures produced are of good regularity, cover a relatively large area and can be mass-produced, as compared to the conventional micro- and nanofabrication techniques (see Section 2.8 in Chapter 2). The orientation effect of nanostructures on EOF (parallel versus perpendicular indented nanolines) in a microfluidic channel was then examined experimentally by current monitoring technique, and numerically by finite element simulations.

8.2 Fabrication methods

8.2.1 Microchannel design with different nanostructured surfaces

Figure 8.1a shows the exploded view of the entire micro-/nanofluidic device. The injection molded cyclic olefin copolymer (COC) chip had dimensions of 75 mm x 25 mm x 1 mm, which is similar to that of a standard microscope slide. The molded chip was sealed via thermal bonding with a thin COC foil (thickness of approximately 100 μm) to provide enclosure for the open microchannel, as well as address the requirement of short working distance for high magnification microscopy experiments. Practical inlet/outlet ports were also integrated to the system through the use of female luer lock couplers for easy fluidic access.

The schematic diagram as shown in Figure 8.1b represents design of the microchannels with different nanostructures. The rectangular microchannel had width of 100 μm and length of 4.8 ± 0.1 cm, with large-area arrays of parallel/perpendicular indented nanolines (period of 397.6 ± 4.3 nm, line width of 184.6 ± 10.7 nm and depth of 93.4 ± 8.5 nm) on the bottom wall of the channel. Characterizations of the parallel and perpendicular indented nanolines inside the respective microchannels were performed with the aid of an atomic force microscope (AFM, Park NX20). Figures 8.1c and 8.1d show the AFM images of the different nanostructured surfaces. A smooth microchannel without any nanostructure (roughness arithmetic mean value $Ra = 5.5 \pm$

0.6 nm) was also fabricated via the conventional DEEMO process (see Appendix F.1 for more details) to serve as a comparison to the microchannels with nanostructures.

The heights of the microchannels with parallel/perpendicular indented nanolines were $3.96 \pm 0.09 \mu\text{m}$, and the height of the smooth microchannel was $32.4 \pm 0.6 \mu\text{m}$, as measured by a Dektak-XT stylus surface profiler. The smooth channel served as a benchmark for the measurement of zeta potential and EOF velocity without nanostructured surface. The additional channel height allows ease of fabrication but does not affect the flow velocity as EOF generates the same flow velocity regardless of cross section area (see Section 2.2.3 in Chapter 2).

Figure 8.1: (a) Exploded view of micro-/nanofluidic device. (b) Schematic diagram of microchannels with different nanostructured surfaces (not drawn to scale). Atomic force microscope (AFM) images of (c) parallel and (d) perpendicular indented nanolines on the bottom walls of the respective microchannels.

8.2.2 Hybrid LIGA-DEEMO process for the fabrication of master structures on silicon wafers

Fabrication of Si masters for microchannels with large-area arrays of parallel and perpendicular indented nanolines involved a two-step lithography process (see Figure 8.2): (i) deep UV (DUV) lithography for the production of nanolines and (ii) standard UV lithography for the construction of microchannel. To ensure good regularity and controllability over the nanoscale patterns, DUV lithography (Burn Jeng 1975, Kawamura et al. 1982) was adopted instead of the conventional electron beam lithography (EBL) (Liu et al. 2002, Grigorescu and Hagen 2009), as EBL is costly and time consuming for large-area structuring.

The DUV lithography process started with hexamethyldisilazane (HMDS) deposition on the silicon wafers to remove native oxide. This was followed by spin coating of bottom anti-reflective coating (BARC) (DUV42P, Nissan Chemical) and then 350 nm thick positive resist (M230Y, JSR Micro). Subsequently, the wafers were prebaked at 90 °C for 130 s. Four exposures were performed using Canon FPA-3000 EX4 stepper (KrF laser as light source, wavelength of 248 nm) with dose of 24 mJ.cm⁻² to stitch (accuracy of 3 σ (standard deviations) = 35 nm) the parallel/perpendicular line grating (period of 400 nm, line width of 200 nm and grating area of 100 μ m x 1.25 cm) to form the bottom surface of the microchannel (base area of 100 μ m x 5 cm).

After the exposures and post baking at 130 °C for 1 min, the wafers were puddled in the developer (MF CD-26 developer, Rohm and Haas Electronic Materials LLC) for 1 min. To reveal the Si surface, BARC layer in the developed structures was removed by pure oxygen plasma. The wafers were then exposed to C₄F₈/SF₆ plasma (75/38 sccm) reactive ion etching (RIE) in STS Pegasus system (coil/platen power = 800/40 W, pressure = 4 mtorr, temperature = -20 °C and $t = 110$ s). Thereafter, the remaining DUV resist and BARC were stripped in TePla 300 plasma asher for 10 min with flow rate of O₂ at 400 sscm, N₂ at 70 sscm, pressure of 1 mbar and power of 1000 W. The etched parallel and perpendicular nanolines had period of 399.8 ± 5.3 nm, line width of 176.2 ± 4.1 nm and depth of 94.0 ± 3.8 nm, as characterized by Park NX20 AFM.

A second lithography step (standard UV lithography process) was performed for the construction of the microchannel. The etched Si wafers were vacuum baked at 90 °C for 5 min, followed by HMDS priming for 5 min (YES-310TA-E vapor prime oven, Yield Engineering System) to promote adhesion of the photoresist. A 4.2 μ m thick layer of positive resist (AZ 5214E, MicroChemicals) was spin coated on the wafers using a SSE Maximus 804 cluster system. The spin speed was first set at 725 rpm for 1 min, followed by 3500 rpm for 10 s. Subsequently, prebaking was conducted at 95 °C for 90 s in 1 mm proximity.

A chrome mask was used to define the flow channel pattern (width of 100 μ m and length of 5 cm). The desired microchannel pattern was aligned with the etched parallel/perpendicular nanolines through the alignment marks (created during DUV

process), and transferred onto the wafers using Karl Süss MA6/BA6 contact aligner (350 W Hg lamp with i-line filter as light source, exposure wavelength of 365 nm) in hard contact mode with exposure dose of 140 mJ.cm^{-2} . After that, the exposed wafers were immersed in developer (AZ 351B, MicroChemicals) for 1 min. The developed microchannels had width of $100 \text{ }\mu\text{m}$ and height of $4.2 \text{ }\mu\text{m}$, as measured by Dektak-XT stylus profiler.

Figure 8.2: Schematics of hybrid LIGA-DEEMO fabrication process for microchannels with large-area arrays of parallel and perpendicular indented nanolines.

8.2.3 Creation of mold inserts via electroplating

For injection molding, a metal mold insert is fabricated because silicon is too brittle for the injection molding process. Hence, negative mold inserts were fabricated by electroplating nickel (Ni) onto the Si wafers produced previously (refer to Section 8.2.2). Mold inserts produced in this manner can withstand several thousand cycles, which are ideally suited for mass production settings. For electroplating to proceed, the surface being deposited on must be conductive. As such, a seed layer of approximately 85 nm nickel/vanadium (Ni/V, 93/7 wt%) was sputtered onto the Si wafers (Custom system, Kurt J. Lesker). The vanadium served to inhibit oxidation of nickel, which would otherwise interfere with the electroplating process.

Subsequently the sputtered wafers were left in the nickel bath (Microform 200, Technotrans) for approximately 6 h to electroplate shims of 350 μm thickness (maximum current of 3.5 A and charge of 18.1 Ah). The Ni shims were loosely attached to the Si masters, and thus they can be easily removed from the wafers. Resist transferred from the Si masters to the Ni shims were stripped in TePla 300 plasma asher for 20 min with flow rate of O_2 at 400 sscm, N_2 at 70 sscm, pressure of 1 mbar and power of 1000 W. Before the injection molding process, the negative Ni mold inserts were coated with perfluorodecyltrichlorosilane (FDTS) monolayer (MVD100E, Applied Microstructures) to function as anti-stiction layer.

8.2.4 Injection molding with cyclic olefin copolymer

Replicas of the master structures were injection molded in an Engel Victory 80/45 Tech hydraulic injection molder equipped with an Engel ERC 13/1-F pick up robot. The negative Ni mold inserts were fitted into the molding tool of the machine, which was custom designed to produce polymer chips with the dimensions of a standard microscope slide (refer to Section 8.2.1). Parts were injection molded in TOPAS 5013L-10 COC from Topas Advanced Polymers GmbH. Due to the relatively high glass transition temperature of 135 °C, TOPAS 5013L-10 COC is well-suited for experiments at elevated temperatures, e.g. melting of double-stranded DNA for separation into two single strands (also known as DNA denaturation) (Persson and Tegenfeldt 2010) and polymerase chain reaction (PCR) amplification of DNA in COC microfluidic devices (Koh et al. 2003). Furthermore, it has high flowability that should facilitate the filling of nanoscale cavities on the mold inserts.

A variotherm process was employed for the injection molding, with melt temperature of 270 °C, mold temperature of 130 °C, injection pressure of 950 bar, injection speed of 40 mm.s⁻¹, holding pressure of 400 bar for 10 s and demolding temperature of 95 °C, which resulted in a overall cycle time of about 2 to 4 min. High replication quality of the molded micro- and nanostructures was observed from the characterizations of injection molded COC chips and Si masters through the use of Dektak-XT stylus profiler and Park NX20 AFM (refer to Sections 8.2.1 and 8.2.2).

8.2.5 Thermal bonding and integration of practical inlet/outlet ports

The injection molded chips were sealed to 101.6 μm thick extruded TOPAS COC foils of the same grade by thermal bonding (Specac Atlas manual hydraulic press with heated platens). Entrance and exit holes were punched on the COC foil (approximately 5 cm apart), and aligned with the open microchannel on the molded chip (see Figure 8.1a). To ensure uniform pressure across the chip surface, the molded chip and the foil were sandwiched between two thin PDMS layers (approximately 3 mm thick for each layer) for compensating slight non-uniformities in flatness. A piston force of 0.25 kN was applied for 10 min with temperature of 128 $^{\circ}\text{C}$ to bond the chip to the foil.

Practical inlet and outlet ports were integrated to the devices via the use of white nylon female luer lock couplers (Cole-Parmer) to connect the microchannels to external flow or air pressure control (see Figure 8.1a). UV adhesive, Dymax 215-CTH-LV-UR-SC exhibits excellent adhesion between the nylon coupler and COC chip. However, the UV adhesive often flows and clogs the inlet and outlet of the microchannel when it is applied directly to the chip due to its low viscosity. To overcome this, the luer lock couplers were first attached to the inlet and outlet with Pro-Spec epoxy steel adhesive (mixing ratio 1:1 and set time of 4 min), which restricted the flow of the UV adhesive into the microchannel. An UV flood curing system (UVF600, Technodigm) was employed to cure the UV adhesive for 8 min. The blue color of the UV adhesive turns colorless when it is fully cured. Subsequently, the device was left under normal room conditions for 24 h so that the adhesive can reach its full strength.

8.3 Experimental details

8.3.1 Experimental setup and materials

Current monitoring experiments (see Figure 8.3) were conducted to investigate the orientation effect of nanostructures on EOF (parallel versus perpendicular indented nanolines) in a microchannel. The experimental setup was similar to that in Section 4.2.1 of Chapter 4, except that the micro-/nanofluidic devices (see Section 8.2 for its fabrication) and the electrolyte solutions employed were different.

Figure 8.3: Schematic diagram of experimental setup for current monitoring method.

The effect of nanolines orientation on EOF was characterized by performing the experiments with sodium bicarbonate (NaHCO_3 , Sigma-Aldrich) solutions of different concentrations, i.e. 1 mM and 5 mM. Table 8.1 presents the measured conductivities

(IONCheck 65, Radiometer Analytical) and pH values (FEP20, Mettler Toledo) for the solutions used in the experiments.

Table 8.1: Measured conductivities and pH values of solutions used in the experiments.

Solution (NaHCO₃)	Conductivity ($\mu\text{S.cm}^{-1}$)	pH
0.95 mM	88.4 ± 0.2	7.74 ± 0.02
1 mM	93.8 ± 0.3	7.90 ± 0.02
4.75 mM	437.4 ± 0.5	8.41 ± 0.01
5 mM	466.6 ± 0.2	8.69 ± 0.01

8.3.2 Experimental procedures

Using a syringe pump (100DM Syringe Pump, Teledyne Isco), the microchannels (see Figure 8.3) were flushed with 1/5 mM NaHCO₃ (solution to be displaced) for 10 min through the inlet port at 20 psi (138 kPa), with the outlet port exposed to the atmosphere. Thereafter, white nylon male luer plug (Cole-Parmer) was secured on the outlet port to stop the flow, and to maintain a constant pressure of 20 psi (138 kPa) in the microchannel for another 10 min. This was to facilitate the filling of the nanostructures. In addition, the microchannels were flushed with 1/5 mM NaHCO₃ by EOF (electric potential of 500 V) for 15 min, to ensure that the nanostructures were completely filled. Subsequently, the microchannels were filled with fresh NaHCO₃ solutions in preparation for the current monitoring experiments.

The microchannel and reservoir connecting to the cathode were filled with 1/5 mM NaHCO₃, while reservoir connecting to the anode was filled with 0.95/4.75 mM

NaHCO₃ (95% concentration of the solution to be displaced) (see Figure 8.3). Electric potential of 500 V was applied across the reservoirs to induce EOF. The time for the current to reach a steady value, i.e. displacement time, was determined from the current-time curve. Experiments were conducted six times (three runs per chip) for each type of microchannel to ensure consistency and reliability of the results. The average EOF velocity can then be calculated by dividing the length of channel L with the displacement time t_d through Equation 2.22 in Chapter 2.

EOF of two solutions with large concentration difference induces pH change and exhibits hysteretic behavior (see Chapters 4 and 5), whereby the flow rate/velocity is directional-dependent, i.e. the flow rate/velocity for fluid A displacing fluid B is different from fluid B displacing fluid A. Furthermore, electrolysis at the electrodes generates hydronium (H₃O⁺) and hydroxide (OH⁻) ions which will alter the pH in the reservoirs (Rodríguez and Chandrasekhar 2005). Hence, several precautions were implemented to ensure negligible pH change during the experiments.

NaHCO₃ buffered solutions were used to keep the pH constant throughout the experiments. Small concentration difference (5% difference) between the displacing and residing fluids was employed to minimize the pH change during the displacement process. Large-volume reservoirs (200 μL) were used to significantly dilute the concentrations of H₃O⁺ and OH⁻ ions produced at the electrodes from electrolysis, which at the same time ensured negligible liquid level change to minimize the back pressure generated (Yan et al. 2007). The short experimental times (less than 150 s) and small electric currents (0.0392-1.57 μA) as a result of the small microchannel cross

sections ($400/3200 \mu\text{m}^2$) and low solution conductivities ($88.4\text{--}466.6 \mu\text{S}\cdot\text{cm}^{-1}$), also limited the production of H_3O^+ and OH^- ions (Corstjens et al. 1996). pH indicator strips (Merck 109535) were employed to measure the pH in the reservoirs, before and after the experiments. The pH changes were found to be negligible.

The effect of Joule heating, which causes EOF to deviate from its normal plug-like velocity flow profile, can be neglected because the conductivities of the solutions employed ($88.4\text{--}466.6 \mu\text{S}\cdot\text{cm}^{-1}$) were low (Tang et al. 2006). A conservative estimate of Joule heating can be calculated from the energy balance between energy generation E_g and energy storage ΔE_{st} in the liquid (Arulanandam and Li 2000). For the chosen experimental parameters, the worst case scenario (applied electric field = 104 V/cm , NaHCO_3 concentration = 5 mM , microchannel cross sectional area = $3200 \mu\text{m}^2$ and experimental duration = 150 s) has an estimated temperature rise of $0.7 \text{ }^\circ\text{C}$, which is negligible.

8.4 Experimental results

The measured displacement times of 1 mM NaHCO_3 for microchannels with parallel and perpendicular indented nanolines were $66.5 \pm 3.6 \text{ s}$ and $83.5 \pm 9.0 \text{ s}$ respectively, and for smooth microchannel was $66.7 \pm 2.7 \text{ s}$. As for the case with 5 mM NaHCO_3 , the measured displacement times for microchannels with parallel and perpendicular indented nanolines were $73.5 \pm 4.4 \text{ s}$ and $93.8 \pm 6.7 \text{ s}$ respectively, and

for smooth microchannel was 73.2 ± 2.5 s. Comparison between the experimental results for 1 mM and 5 mM NaHCO_3 is shown in Figure 8.4.

Increasing the NaHCO_3 concentration by five times (from 1 mM to 5 mM) decreased the Debye length from approximately 10 nm to 4 nm (calculated by Equation 2.1 in Chapter 2), which resulted in longer displacement times (flow velocity reduction according to Equation 2.22 in Chapter 2) (see Figure 8.4). The experimental results for 5 mM NaHCO_3 demonstrated a similar trend as the case with 1 mM NaHCO_3 .

Figure 8.4: Displacement times for microchannels with large-area arrays of parallel and perpendicular indented nanolines, in comparison to smooth microchannel, for 1 mM and 5 mM NaHCO_3 . Six experimental runs ($n = 6$) were performed for each type of microchannel with 1 mM/ 5 mM NaHCO_3 to obtain the error bars of displacement time.

For the microchannel with perpendicular indented nanolines, the displacement time was increased by approximately 25%, in comparison to the smooth microchannel

(see Figure 8.4). The results revealed that the EOF velocity was lowered with the introduction of nanostructured surface in the microchannel. This observation agrees with the existing literatures (Kang and Suh 2009, Messinger and Squires 2010, Yasui et al. 2011, Koga et al. 2013) which suggest that nanostructured surface significantly reduces EOF.

In contrast, negligible difference in the flow velocities was observed for the microchannel with parallel indented nanolines, in comparison to the smooth microchannel (see Figure 8.4).

For pressure driven flow, the fluid flow velocity is determined by the hydraulic resistance which is inversely proportional to the cross sectional area of the channel. A channel with a larger cross section yields a low hydraulic resistance and thus a higher flow velocity. However, this concept is not applicable to EOF. EOF produces the same flow velocity regardless of cross section area (refer to Section 2.2.3 in Chapter 2). Therefore, the difference in cross sectional area for smooth microchannel and microchannel with perpendicular nanolines (see Section 8.2.1) is not the cause for the observed flow velocity difference.

EOF velocity is dependent on the electric field, permittivity and viscosity of the fluid, and zeta potential (indicated by Equation 2.7 in Chapter 2). Zeta potential, a measure of the surface chemical properties, is an important parameter that dictates the direction and velocity of EOF. Since the smooth and nanostructured microchannels

were fabricated from the same material, they had the same nominal “material” zeta potential.

To describe the combined effect of nanostructures and zeta potential of the virgin material on EOF, an “effective” zeta potential concept was proposed. The introduction of an effective zeta potential exemplifies the strong correlation between the EOF velocity and the surface topography, in addition to the nominal “material” zeta potential.

Since the thickness of the electrical double layer (EDL) was thin compared to the micro-/nanostructures of the injection molded COC chips (refer to Section 8.2.1), the effective zeta potential ζ_{eff} , which includes the chemical properties and the topography of the surface, can be derived by substituting the average EOF velocity (Equation 2.22) into the Helmholtz-Smoluchowski slip velocity equation (Equation 2.7), and expressed as Equation 2.23 in Chapter 2.

The effective zeta potentials of 1 mM NaHCO₃ for microchannels with parallel and perpendicular indented nanolines were -87.3 ± 4.5 mV and -70.1 ± 7.8 mV respectively, and for smooth microchannel was -87.0 ± 3.5 mV. The effective zeta potentials of 5 mM NaHCO₃ for microchannels with parallel and perpendicular indented nanolines were -79.0 ± 4.9 mV and -62.0 ± 4.5 mV respectively, and for smooth microchannel was -79.2 ± 2.9 mV. Magnitude of effective zeta potentials of 1 mM and 5 mM NaHCO₃ for microchannels with parallel and perpendicular indented nanolines are shown in Figure 8.5, as compared to the smooth microchannel.

Figure 8.5: Magnitude of effective zeta potentials for microchannels with large-area arrays of parallel and perpendicular indented nanolines, in comparison to smooth microchannel, for 1 mM and 5 mM NaHCO₃. Six experimental runs ($n = 6$) were performed for each type of microchannel with 1 mM/ 5 mM NaHCO₃ to obtain the error bars of effective zeta potential.

Figure 8.5 indicates that the magnitude of effective zeta potential (and thus EOF velocity) was significantly reduced (approximately 20%) for microchannel with perpendicular indented nanolines. In contrast, for the smooth microchannel and the microchannel with parallel indented nanolines, their effective zeta potentials had negligible difference.

This investigation demonstrates for the first time that *the orientation of nanostructures*, and not just the presence of nanostructures, is an important factor which affects the EOF behavior. The mechanics causing the perpendicular indented nanolines in the microchannel to have a significant effect on the EOF, but not the

parallel indented nanolines, will be elucidated in Section 8.6 with the support of numerical simulations performed in Section 8.5.

8.5 Numerical Simulations

Numerical simulations were conducted for steady-state EOF of 1 mM NaHCO_3 . The case for 5 mM NaHCO_3 is not simulated as it requires very fine mesh to resolve the steep changes of variables due to the extremely thin EDL, which is computationally expensive and tedious. However, the outcomes of simulating only the steady-state EOF of 1 mM NaHCO_3 will be sufficient to demonstrate the mechanism involved. This is because the experimental results for 5 mM NaHCO_3 and 1 mM NaHCO_3 had similar trends (see Figure 8.4), indicating that the percentage reduction of flow velocity due to the nanostructured surface was not influenced by the solution concentration.

The simulations were implemented on COMSOL Multiphysics finite element software (FEM). The simulations were based on the theoretical model after simplifications in Section 3.4 of Chapter 3, which adequately describes the steady-state single fluid EOF, and is capable of capturing changes in the variables near the EDL due to the presence of nanostructures in the microchannel. The governing equations have been discussed in Chapter 3. The symbols and values of constants for the numerical simulations can be found in Table A.1 of Appendix A.

Two-dimensional (2-D) simulations were performed to investigate the effect of perpendicular indented nanolines on EOF in a microchannel, in comparison to a

smooth channel. The simulation domains and boundary conditions are shown in Figure 8.6. The period, line width and the depth of the perpendicular indented nanolines were 400 nm, 185 nm and 93 nm respectively. The domain heights for the nanostructured microchannel and the smooth channel were 4 μm and 32 μm respectively. As the governing equations (see Chapter 3) for the applied electric potential, electrostatic wall potential, main constituent ion distributions and fluid flow are strongly coupled, substantial computational effort is required. Thus, the simulated channel length was reduced to 28 μm (as compared to 4.8 cm in the experiments) to minimize computational time.

Figure 8.6: Numerical simulation domains and boundary conditions.

The boundary conditions were similar to that in Chapters 6 and 7 (for steady-state single fluid EOF), except that the voltage applied was 0.292 V to establish the experimental electric field of 104 V/cm, and the surface charge density of the channel wall was specified as $-9.86 \text{ mC} \cdot \text{m}^{-2}$ (calculated by Equation 3.20 in Chapter 3) based on

the nominal “material” zeta potential (-87.0 mV, effective zeta potential of 1 mM NaHCO_3 for smooth channel) of the COC fluidic devices.

The simulation domains were meshed with triangular elements. The size of the elements at the wall boundaries was set to 1-2.5 nm, and the size of each adjacent element further from the wall was increased by 1.05 times. The applied potential, electrostatic potential and ion concentrations were discretized with second order elements, and linear elements were employed for the discretization of the pressure and velocity. Convergence test performed using higher number of elements confirmed that there was negligible numerical error for this mesh selection. The convergence criterion was based on a relative tolerance of less than 0.001 between subsequent iterations.

8.6 Numerical results

EOF originates from the interaction between the EDL and the applied electric field. The nanostructures distorted the local electric field at the wall, and thus affected the effective zeta potential and the flow velocity. For the microchannel with parallel indented nanolines, it was deduced that the parallel nanolines did not distort the electric field as they were in the same direction as the electric field. Thus, the flow velocity was the same as the smooth microchannel, which agreed with the experimental observations (see Figure 8.4).

For the microchannel with perpendicular indented nanolines, finite element simulation indicated that the perpendicular nanolines distorted the electric field which

followed the contour of the nanostructures (see Figure 8.7a), and reduced the average electric field along the surface from 104 V/cm to 73.3 V/cm. This reduced the fluid flow velocity across the entire microchannel (see Figure 8.7b) as EOF is driven by the electric field at the surface. The simulation predicted that the average fluid velocity for 1 mM NaHCO_3 decreased from $7.23 \times 10^{-4} \text{ m.s}^{-1}$ to $6.33 \times 10^{-4} \text{ m.s}^{-1}$. This prediction agreed well with the observed velocity reduction of $(7.21 \pm 0.29) \times 10^{-4} \text{ m.s}^{-1}$ to $(5.81 \pm 0.65) \times 10^{-4} \text{ m.s}^{-1}$ in the experiments.

Figure 8.7: Simulated (a) electric field lines and (b) fluid flow velocity profile for smooth microchannel, and microchannel with perpendicular indented nanolines, for 1 mM NaHCO_3 .

The orientation of the nanostructures dictated the distortion of the local electric field, and thus the effective zeta potential. Yasui et al. (2011) fabricated microchannels

with two different types of nanopillars arrangements, i.e. the square and tilted array patterns. Both nanopillar configurations had no observable EOF velocity difference between them, but both significantly reduced EOF as compared to a smooth channel. This lack of EOF velocity difference between the two configurations could well be due to their insignificant orientation difference. However, nanolines are significantly different from nanopillars because the arrangement of nanolines determines the degree of EOF suppression. This investigation demonstrates the two extremes, the perpendicular nanolines significantly distorted the electric field, and in contrast the parallel nanolines did not distort the electric field at all. As such, the parallel nanolines had no effect on EOF, while the perpendicular nanolines significantly reduced EOF.

8.7 Summary

This investigation presents nanostructure designs (with the associated fabrication method) in microchannels that possess maximum orientation difference, i.e. parallel versus perpendicular indented nanolines, to examine the orientation effect of nanostructures on EOF.

The fabrication process consisted of four phases: fabrication of master structures on Si wafers, creation of mold inserts via electroplating, injection molding with COC, and thermal bonding and integration of inlet/outlet ports. The novel feature of this fabrication method was the demonstration of hybrid LIGA-DEEMO process for the fabrication of microchannels with large-area arrays of parallel and perpendicular

indented nanolines. The fabrication method exploited the strengths of these two fundamentally different technologies to ensure good regularity and controllability over the nanoscale patterns.

The orientation effect of nanostructures on EOF within a microchannel was examined experimentally by current monitoring technique, and numerically by finite element simulations. The proposed “effective” zeta potential concept encompassed both the effects of nanostructures and zeta potential of the virgin material to describe the EOF discrepancy between the smooth and nanostructured microchannels. The experimental results showed that the perpendicular nanolines significantly reduced the effective zeta potential (and thus EOF velocity) by approximately 20%. The numerical simulation revealed that the flow velocity reduction was due to the local electric field distortion at the nanostructured surface. On the contrary, the parallel nanolines had no effect on the EOF, as it was deduced that the parallel nanolines did not result in distortion of the electric field.

Chapter 9 Conclusions and future work

9.1 Conclusions

The conventional theoretical models for electroosmotic flow (EOF) are mainly developed to describe a single fluid EOF. They cannot reveal the critical phenomena of two-fluid electroosmotic displacement flow. Hence, in this thesis, a generalized theoretical model was formulated for a proper description of the two-fluid displacement flow process (Chapter 3). The significant departures from the traditional models allowed local zeta potential variation according to the flow conditions, which had a direct impact on the EOF velocity depending on the displacement directions for a two-fluid electroosmotic displacement flow. For numerical simulation implementation, appropriate simplifications were performed to the generalized model for specific experimental conditions to reduce the computational effort.

EOF has been experimentally observed to exhibit hysteretic behavior when the flow involves the displacement of two fluids with *different concentrations* (Gan et al. 2006, Lim and Lam 2012). The origin of this EOF hysteresis was shown to be caused by the ionic behavior of the minority pH-governing ions in the bulk of the fluid, through both experimental and numerical approaches (Chapter 4). As elucidated by the simulations, the hysteresis effect was attributed to the accumulation/depletion of pH-governing minority ions, such as hydronium (H_3O^+) and bicarbonate (HCO_3^-) ions, at the two-fluid interface, which subsequently widened and spread to the bulk of the fluid.

The resultant pH changes altered the zeta potential and EOF flow rate in the microchannel, and were dependent on the flow direction.

The prediction of pH changes in the bulk solution is rather counter-intuitive as EOF in a microchannel is believed to be driven by surface phenomenon and the main constituent ions. Without direct experimental observation of the pH changes in such EOF hysteresis, this prediction will remain as an unproven hypothesis because it is only deduced through numerical simulations. As such, experiments that focus on the direct observation of pH changes during EOF of solutions with different concentrations, and their quantifications for comparison with the numerical simulations were performed (Chapter 5). The experimental results agreed well with the simulation results which showed conclusive evidence for the pH changes in this EOF hysteresis.

Even though there are abundance investigations on electroosmotic displacement flow with different types of fluids (Ren et al. 2001, Ren et al. 2002, Ren et al. 2003), no investigation has hitherto been conducted to investigate the flow direction-dependent behavior during EOF of solutions with *dissimilar ionic species*. EOF hysteresis for solution pair with dissimilar cationic species was investigated both experimentally and numerically (Chapter 6). Two different mechanisms had been identified as the causes of this type of EOF hysteresis: (a) widening/sharpening effect of the interfacial region induced by conductivity difference between the two solutions, and (b) difference in ion distributions (and thus average zeta potentials) due to concentration adjustments in different flow directions.

EOF hysteresis involving solution pair with dissimilar anionic species was also investigated both experimentally and numerically (Chapter 7). In contrast to EOF of solutions with different concentrations or cationic species, EOF for dissimilar anionic solutions exhibited an unusual behavior, whereby the steady-state concentration of the displacing electrolyte in the microchannel deviated from its original value. This was due to the ion concentration adjustment when the displacing anions migrated against EOF, and a second ion concentration adjustment induced by the diffusive interface at the junction between the anode reservoir and microchannel which was convected throughout the entire channel by EOF. The resultant ion distributions (thus difference in average zeta potentials) led to the flow rate to be directional-dependent.

Despite the vast investigations of EOF reduction due to the presence of nanostructures in microchannels (Kang and Suh 2009, Messinger and Squires 2010, Yasui et al. 2011, Koga et al. 2013), a proper study on the orientation effect of nanostructures is yet to be conducted. A novel fabrication method was developed for microchannels with nanostructure designs possessing maximum orientation difference, i.e. parallel versus perpendicular indented nanolines, to examine the effect of nanostructure orientation on EOF (Chapter 8). The fabrication process consisted of four phases: fabrication of silicon master, creation of mold insert via electroplating, injection molding with cyclic olefin copolymer (COC), and thermal bonding and integration of practical inlet/outlet ports.

The effect of nanostructure orientation on EOF within a microfluidic channel was studied both experimentally and numerically. The experimental results showed that

the perpendicular nanolines significantly reduced the EOF velocity (by approximately 20%). The numerical simulation revealed that the flow velocity reduction was due to the local electric field distortion at the nanostructured surface. On the contrary, the parallel nanolines had no effect on the EOF, as it was deduced that the parallel nanolines did not result in distortion of the electric field.

9.2 Recommendations for future work

The investigations contained in this thesis have not investigated the EOF hysteresis and the effect of nanoline orientation angles on EOF exhaustively. Recommendations for further investigations include:

- (a) Enhancement and diminution of EOF hysteresis for solutions with different concentrations – The mixed electroosmotic and pressure driven flow (M-flow) (see Figure 4.9 of Chapter 4) can possibly introduce different hysteresis dynamics as its convection direction varies depending on the pH accumulation/depletion effect during the displacement flow process. Since M-flow occurs when there is a large surface charge change (Minerick et al. 2002), it is expected to be more pronounced at a larger time frame, which might affect the EOF hysteresis window. As such, the classical isotachophoresis configuration (Smejkal et al. 2013), i.e. an immiscible organic solvent with an aqueous electrolyte, can be adopted for future investigation. It can be employed to study if a higher conductivity jump would amplify the pH accumulation/depletion effect that produces an even larger surface

charge change, leading to a more severe M-flow for the enhancement of EOF hysteresis. Investigation on the diminution of EOF hysteresis should also be pursued via the application of higher voltages, i.e. increasing the EOF velocity, to examine when would the displacement flow cease showing flow discrepancies.

- (b) EOF hysteresis for solutions with dissimilar cation and anion species – This thesis only presents EOF hysteresis for solutions with different concentrations (Chapters 4 and 5) and dissimilar cation or anion species (Chapters 6 and 7). Thus, electroosmotic displacement flow of solutions with dissimilar cation and anion species, for example potassium chloride (KCl) and sodium sulfate (Na_2SO_4) solution pair, can be explored to investigate its flow direction-dependent behavior.
- (c) Characterizations of EOF hysteresis in nanochannels – In this thesis, the investigations on EOF hysteresis were conducted in microchannels (Chapters 4, 5, 6 and 7), where there is no overlapping of electrical double layer (EDL). Since nanofluidic devices have gained popularity due to its exclusive properties for ion/fluid transportation (Schoch et al. 2008, Zangle et al. 2010), it would be beneficial to extend the investigations of EOF hysteresis to nanochannels with overlapping EDL.
- (d) Effect of nanoline orientation angles on EOF within a micro-/nanochannel – This thesis has demonstrated without doubt the orientation effect of nanostructures through the two extreme orientations of the nanolines, i.e. parallel versus perpendicular nanolines (Chapter 8). However, the relationship on the effect of nanoline orientation on EOF is yet to be established. Therefore, investigation on the effect of nanoline angles on EOF within a microchannel should be pursued.

The effect of nanochannel dimensions and aspect ratio can also be investigated to examine its importance on manipulating EOF, especially for extreme values, in conjunction with the nanoline orientation.

- (e) EOF in microchannel with black silicon nanostructures – In this thesis, the newly proposed fabrication method for producing nanostructures within a microchannel (Chapter 8) is not cost-effective when the dimensions of the nanostructures need to be varied. This is because photomasks of different nanostructure dimensions have to be fabricated for the deep UV (DUV) lithography step, which is prohibitively expensive. As such, reactive ion etching (RIE, which is a maskless method) can be adopted to produce black silicon nanostructures (hemispheroid-like nanostructures) in a microchannel for EOF reduction, for which the dimensions of the nanostructures can be varied by simply manipulating the flow rates of the etching gas (SF_6 and CH_4) and passivating gas (O_2) (Yue et al. 2013, Telecka et al. 2016).

List of publications

Journal papers

1. Lim, A. E., C. Y. Lim and Y. C. Lam (2015). "Electroosmotic flow hysteresis for dissimilar ionic solutions." Biomicrofluidics **9**(2): 024113.
2. Lim, C. Y., A. E. Lim and Y. C. Lam (2016). "Ionic origin of electro-osmotic flow hysteresis." Scientific Reports **6**: 22329.
3. Lim, A. E., C. Y. Lim and Y. C. Lam (2016). "Electroosmotic flow hysteresis for dissimilar anionic solutions." Analytical Chemistry **88**(16): 8064-8073.
4. Lim, A. E., C. Y. Lim, Y. C. Lam, R. Taboryski and S. R. Wang (2017). "Effect of nanostructures orientation on electroosmotic flow in a microfluidic channel." Nanotechnology **28**(25): 255303.
5. Lim, C. Y., A. E. Lim and Y. C. Lam (2017). "pH change in electroosmotic flow hysteresis." Analytical Chemistry **89**(17): 9394-9399.

Conference papers

1. Lim, C. Y., A. E. Lim and Y. C. Lam (2014). "Hysteresis in two-fluid electroosmotic displacement flow." 1st International Conference on Micro & Nanofluidics, University of Twente, The Netherlands.

[Poster presentation, 18th May – 21st May 2014]

2. Lim, C. Y., A. E. Lim and Y. C. Lam (2015). “Electroosmotic flow in nanostructured microfluidic channel.” 2nd International Conference on Polymer Replication on Nanoscale, Technical University of Denmark, Denmark.

[Oral presentation, Best paper award, 18th May – 19th May 2015]

3. Lim, A. E., C. Y. Lim and Y. C. Lam (2016). “Electroosmotic flow hysteresis for dissimilar anionic solutions.” 5th International Conference on Micro/Nanoscale Heat & Mass Transfer, Biopolis, Singapore.

[Oral presentation, 4th Jan – 6th Jan 2015]

4. Lim, A. E., C. Y. Lim, R. Taboryski and Y. C. Lam (2016). “Effects of nanostructured surfaces on electro-osmotic flow.” 3rd International Conference on Polymer Replication on Nanoscale, University of Applied Sciences and Arts Northwestern Switzerland, Switzerland.

[Oral presentation, 19th May – 20th May 2016]

5. Lim, C. Y., A. E. Lim and Y. C. Lam (2017). “Observation of pH change induced by electroosmotic flow hysteresis.” 8th Australia New Zealand Nano-Microfluidics Symposium, Hobart, Tasmania, Australia.

[Oral presentation, 26th May – 29th Jun 2017]

6. Lim, A. E., C. Y. Lim, Y. C. Lam and R. Taboryski (2017). “Electroosmotic flow in microchannel with black silicon nanostructures.” 7th International Multidisciplinary Conference on Optofluidics, Grand Copthorne Hotel, Singapore.

[Oral presentation, Best paper award, 25th Jul – 28th Jul 2017]

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 - Abstract, Chapter 1, Chapter 2, Chapter 3, Chapter 8, Chapter 9, Appendix A and Appendix F.

Appendix A Symbols and values of constants employed for numerical simulations

The symbols and values of constants employed for the numerical simulations in Chapters 4 to 8 can be found in Table A.1.

Table A.1: Symbols and values of constants employed in numerical simulations.

Parameter	Symbol (Unit)	Value
Permittivity of free space	ϵ_o (C.V ⁻¹ .m ⁻¹)	8.85 x 10 ⁻¹²
Relative permittivity	ϵ_r	80
Viscosity of water	μ (kg.m ⁻¹ .s ⁻¹)	8.90 x 10 ⁻⁴
Density of water	ρ (kg.m ⁻³)	1000
Faraday constant	F (C.mol ⁻¹)	96485
Gas constant	R (J.mol ⁻¹ .K ⁻¹)	8.314
Boltzmann constant	k_b (J.K ⁻¹)	1.38 x 10 ⁻²³
Temperature	T (K)	298
Electron charge	e (C)	1.602 x 10 ⁻¹⁹
Avogadro constant	N_a (mol ⁻¹)	6.022 x 10 ²³
Diffusion coefficient ^a of K ⁺ ion	D_{K^+} (m ² .s ⁻¹)	1.957 x 10 ⁻⁹
Diffusion coefficient of Cl ⁻ ion	D_{Cl^-} (m ² .s ⁻¹)	2.032 x 10 ⁻⁹
Diffusion coefficient of Na ⁺ ion	D_{Na^+} (m ² .s ⁻¹)	1.334 x 10 ⁻⁹
Diffusion coefficient of Ca ²⁺ ion	$D_{Ca^{2+}}$ (m ² .s ⁻¹)	7.920 x 10 ⁻¹⁰
Diffusion coefficient of SO ₄ ²⁻ ion	$D_{SO_4^{2-}}$ (m ² .s ⁻¹)	1.065 x 10 ⁻⁹
Diffusion coefficient of H ₃ O ⁺ ion	$D_{H_3O^+}$ (m ² .s ⁻¹)	9.3 x 10 ⁻⁹ /2.3 x 10 ⁻⁹
Diffusion coefficient of OH ⁻ ion	D_{OH^-} (m ² .s ⁻¹)	5.26 x 10 ⁻⁹

Diffusion coefficient of H_2CO_3	$D_{H_2CO_3} (m^2.s^{-1})$	1.3×10^{-9}
Diffusion coefficient of HCO_3^- ion	$D_{HCO_3^-} (m^2.s^{-1})$	1.105×10^{-9}
Diffusion coefficient of CO_3^{2-} ion	$D_{CO_3^{2-}} (m^2.s^{-1})$	9.2×10^{-10}
Diffusion coefficient of H_3PO_4	$D_{H_3PO_4} (m^2.s^{-1})$	8.7×10^{-10}
Diffusion coefficient of $H_2PO_4^-$ ion	$D_{H_2PO_4^-} (m^2.s^{-1})$	9.59×10^{-10}
Diffusion coefficient of HPO_4^{2-} ion	$D_{HPO_4^{2-}} (m^2.s^{-1})$	7.59×10^{-10}
Ionic mobility ^b of K^+ ion	$u_{m(K^+)} (m^2.V^{-1}.s^{-1})$	7.621×10^{-8}
Ionic mobility of Cl^- ion	$u_{m(Cl^-)} (m^2.V^{-1}.s^{-1})$	-7.913×10^{-8}
Ionic mobility of Na^+ ion	$u_{m(Na^+)} (m^2.V^{-1}.s^{-1})$	5.195×10^{-8}
Ionic mobility of Ca^{2+} ion	$u_{m(Ca^{2+})} (m^2.V^{-1}.s^{-1})$	6.169×10^{-8}
Ionic mobility of SO_4^{2-} ion	$u_{m(SO_4^{2-})} (m^2.V^{-1}.s^{-1})$	-8.296×10^{-8}
Ionic mobility of H_3O^+ ion	$u_{m(H_3O^+)} (m^2.V^{-1}.s^{-1})$	3.622×10^{-7} $/8.957 \times 10^{-8}$
Ionic mobility of OH^- ion	$u_{m(OH^-)} (m^2.V^{-1}.s^{-1})$	-2.048×10^{-7}
Ionic mobility of HCO_3^- ion	$u_{m(HCO_3^-)} (m^2.V^{-1}.s^{-1})$	-4.303×10^{-8}
Ionic mobility of CO_3^{2-} ion	$u_{m(CO_3^{2-})} (m^2.V^{-1}.s^{-1})$	-7.166×10^{-8}
Ionic mobility of $H_2PO_4^-$ ion	$u_{m(H_2PO_4^-)} (m^2.V^{-1}.s^{-1})$	-3.735×10^{-8}
Ionic mobility of HPO_4^{2-} ion	$u_{m(HPO_4^{2-})} (m^2.V^{-1}.s^{-1})$	-5.912×10^{-8}
Ionic charge number of K^+ ion	z_{K^+}	+1
Ionic charge number of Cl^- ion	z_{Cl^-}	-1
Ionic charge number of Na^+ ion	z_{Na^+}	+1
Ionic charge number of Ca^{2+} ion	$z_{Ca^{2+}}$	+2
Ionic charge number of SO_4^{2-} ion	$z_{SO_4^{2-}}$	-2
Ionic charge number of H_3O^+ ion	$z_{H_3O^+}$	+1
Ionic charge number of OH^- ion	z_{OH^-}	-1
Ionic charge number of HCO_3^- ion	$z_{HCO_3^-}$	-1

Ionic charge number of CO_3^{2-} ion	$z_{\text{CO}_3^{2-}}$	-2
Ionic charge number of H_2PO_4^- ion	$z_{\text{H}_2\text{PO}_4^-}$	-1
Ionic charge number of HPO_4^{2-} ion	$z_{\text{HPO}_4^{2-}}$	-2
Equilibrium constant of SiOH deprotonation reaction ^c	K_A	6.310×10^{-8}
Total number site density of SiOH ^c	$N_{\text{Total}} (\text{m}^{-2})$	8×10^{18}
Partial pressure of carbon dioxide	$P_{\text{CO}_2} (\text{atm})$	3.5×10^{-4}
Henry's law constant	$K_H (\text{L.atm.mol}^{-1})$	29.76
Apparent specific forward rate ^d constant of water	$K_{fw} (\text{mol.dm}^{-3}.\text{s}^{-1})$	1×10^{-4}
Equilibrium constant ^d of water auto-ionization	$K_w (\text{mol}^2.\text{dm}^{-6})$	1×10^{-14}
Apparent specific forward rate constant of carbonate system 1	$K_{fC1} (\text{s}^{-1})$	4.45
Equilibrium constant of carbonate system 1	$K_{C1} (\text{mol.dm}^{-3})$	4.45×10^{-7}
Apparent specific forward rate constant of carbonate system 2	$K_{fC2} (\text{s}^{-1})$	0.469
Equilibrium constant of carbonate system 2	$K_{C2} (\text{mol.dm}^{-3})$	4.69×10^{-11}
Apparent specific forward rate constant of phosphate system 1 ^e	$K_{fP1} (\text{s}^{-1})$	1
Equilibrium constant of phosphate system 1	$K_{P1} (\text{mol.dm}^{-3})$	7.5×10^{-3}
Apparent specific forward rate constant of phosphate system 2 ^e	$K_{fP2} (\text{s}^{-1})$	1×10^{-1}
Equilibrium constant of phosphate system 2	$K_{P2} (\text{mol.dm}^{-3})$	6.2×10^{-8}

^aDiffusion coefficient of the ionic species were obtained from Newman and Thomas-Alyea (2004), Wraight (2006), Nesic et al. (1996), Zeebe (2011), Leaist (1984) and Buffle et al. (2007) respectively.

^bIonic mobility of a specific ion species was calculated by the formula: $(z_i D_i F)/(RT)$.

^cEquilibrium constant of SiOH deprotonation reaction and the total number site density of SiOH were taken from Leung et al. (2009) and Yeh et al. (2012).

^dApparent specific forward rate and equilibrium constants for various chemical equilibrium equations, such as the auto-ionization of water, equilibria of carbonic acid and triprotic phosphoric acid, were adapted from Musvoto et al. (2000).

^eApparent specific forward rate constants for phosphate system were assumed to have the same order of magnitudes with carbonate system to reduce computational effort because the rates provided by Musvoto et al. (2000) are too rapid. From a practical point of view, the assumption is reasonable as the exact values are of little importance. The reactions will reach its equilibrium faster than other dynamic processes in the simulations (Musvoto et al. 2000).

Appendix B Ionic origin of electroosmotic flow hysteresis for solutions with different concentrations

B.1 Labview program for controlling experimental devices

A Labview program was written such that the “*controls*” and “*indicators*” were the corresponding input and output terminals of the current monitoring experimental devices (see Section 4.2.1 of Chapter 4). (1) and (2) as shown in Figure B.1 will display the graphical plots of the input voltage and output current signals respectively, and (3) is for the user to directly input the supplied voltage so that there will be minimal contact with the high voltage power supply. (4) is an emergency stop button that allows the user to terminate the power supply; this is a safety feature allowing the experiment to be stopped immediately in case of any abnormality. While (5) is a save button for saving the experimental data (in text format) to a specified destination (6) set by the user, which can subsequently be retrieved for data analysis.

Figure B.1: Labview program window.

B.2 Determination of displacement time from current-time curve

Electrical current in a cylindrical microcapillary is dependent on the applied field strength and conductivity of the solution. When a solution with a different conductivity (due to difference in ion concentration) flows into the capillary, the total resistance of the capillary is changed and this causes the current to change as well. The upstream and downstream fluids can be treated analogously as two variable resistors connected in series under a constant applied electric field with equivalent variable resistances written as:

$$R_1 = \frac{X}{\sigma_1 R_c}, \quad (\text{B.1})$$

$$R_2 = \frac{L-X}{\sigma_2 R_c}, \quad (\text{B.2})$$

where $R_{l/2}$ is the instantaneous resistances, X is the interface position, $\sigma_{l/2}$ is the conductivities of the two fluids, R_c is the capillary cross-sectional area and L is the length of microcapillary. With the assumption of a sharp interface between the two fluids, the time-dependent current according to Ohm's law can be expressed as:

$$I = \frac{\Delta V}{R_T} = \frac{R_c \Delta V \sigma_1 \sigma_2}{(\sigma_2 - \sigma_1)X + L\sigma_1}, \quad (\text{B.3})$$

where ΔV is the electric potential difference and R_T is the total instantaneous resistance of the system.

To determine the displacement times from the experimental data, curve fitting of the current-time curves were performed through a regression analysis of SigmaPlot. Equation B.3 was re-written to facilitate the curve fitting function, which was:

$$I^{-1} = At^C + B, \quad (\text{B.4})$$

where I^{-1} is the inverse of experimental current, while A , B and C are the coefficients assigned for fitting. The coefficient of determination (R^2) values for the fittings were as high as 0.98, indicating that the regression function was capable of capturing the experimental trend. Once A , B and C were determined, the displacement times were then calculated by evaluating t from Equation B.4 when I was taken as the average current value after completion of the displacement process.

B.3 Reversible acid-base equilibria of phosphate system

For potassium dihydrogen phosphate (KH_2PO_4) solution, water (H_2O) (see Equation 3.10 in Section 3.2.5 of Chapter 3) and phosphate systems were considered. The triprotic phosphoric acid (H_3PO_4) undergoes several deprotonations (Musvoto et al. 2000). To reduce the computational time, dissociation of hydrogen phosphate ion (HPO_4^{2-}) to phosphate (PO_4^{3-}) was removed from the numerical simulations as it was negligible for the pH range in this investigation (see Table 4.1). The acid-base reactions employed were (Musvoto et al. 2000):

where H_2PO_4^- represents the dihydrogen phosphate anion and H_3O^+ is the hydronium ion.

B.4 Comparison between experimental and simulation results for buffer solutions

Experimental and simulated current-time curves for the displacement flow of 0.2 mM and 1 mM buffer solutions, namely sodium bicarbonate (NaHCO_3) and KH_2PO_4 , in two opposing flow directions are shown in Figure B.2. The resultant H_3O^+ concentrations as a result of the electromigration and buffering reactions during the displacement flow of the buffer solutions are shown in Figure B.3.

Figure B.2: Experimental and simulated current-time curves for the displacement flow of 0.2 mM and 1 mM buffer solutions, namely (a) NaHCO_3 and (b) KH_2PO_4 , in two opposing flow directions. Currents and displacement times were normalized with the two steady-state current values and time for 0.2 mM to fully displace 1 mM (T_{LH}) respectively.

Figure B.3: Snapshots of simulated H_3O^+ concentration for NaHCO_3 solution pair when (a) 0.2 mM displaced 1 mM ($t = 0.2\text{ s}$) and (b) 1 mM displaced 0.2 mM ($t = 0.2\text{ s}$), as well as for KH_2PO_4 solution pair when (c) 0.2 mM displaced 1 mM ($t = 0.5\text{ s}$) and (d) 1 mM displaced 0.2 mM ($t = 0.35\text{ s}$).

Appendix C pH changes in electroosmotic flow

hysteresis for solutions with different concentrations

C.1 Determination of dimensionless interface position P

Based on the assumption of a sharp interface between two fluids, the time-dependent current I according to Ohm's law may be expressed as (see Equation B.3 in Appendix B.2):

$$I = \frac{\Delta V}{R_T} = \frac{\Delta V R_c \sigma_1 \sigma_2}{(\sigma_2 - \sigma_1)X + L\sigma_1} = \frac{E R_c \sigma_1 \sigma_2}{(\sigma_2 - \sigma_1)P + \sigma_1}, \quad (\text{C.1})$$

where ΔV is the electric potential difference, R_T is the total instantaneous resistance of the system, X is the interface position, $\sigma_{1/2}$ is the conductivities of the two fluids, R_c is the capillary cross-sectional area, L is the length of the capillary, E is the applied electric field and P is the dimensionless interface position. Rearranging Equation C.1 yields Equation C.2:

$$P = \frac{\sigma_1 \sigma_2}{\sigma_2 - \sigma_1} \left(\frac{E R_c}{I} - \frac{1}{\sigma_2} \right). \quad (\text{C.2})$$

C.2 MATLAB coding for color image analysis

The MATLAB coding written to extract the RGB (red, green, blue) values from the color images for image analysis is as follows:

```
clc;
clear;
close all;

A = imread ('HL-20-3.tif');

image_handle=figure;imshow(A);

[x,y] = getpts;

// Define radius of evaluation area
r3 = 650;

aoe = [x-r3,y-r3,r3*2,r3*2];

image_handle=figure;imshow(A);rectangle('Position',aoe);

x1 = r3;
y1 = r3;

viscircles([x,y],r1);

image = imcrop(A,aoe);

mask = zeros(size(image,1),size(image,2));
%
for i = 1:size(image,1)
    for j = 1:size(image,2)
        if sqrt((i-y1)^2+(j-x1)^2)<=r1
            mask(i,j)=1;
        end
    end
end
end
```

```

%Split into RGB Channels
Red = image(:,:,1).*uint8(mask);
Green = image(:,:,2).*uint8(mask);
Blue = image(:,:,3).*uint8(mask);

%Get histValues for each channel
[yRed, x] = imhist(Red);
[yGreen, x] = imhist(Green);
[yBlue, x] = imhist(Blue);

image_handle=figure;

%Plot them together in one plot
plot(x, yRed, 'Red', x, yGreen, 'Green', x, yBlue, 'Blue');

axis([100 230 0 4e4]);

[MR,IR] = max(yRed(2:256));
[MG,IG] = max(yGreen(2:256));
[MB,IB] = max(yBlue(2:256));

IR
IG
IB

B = [2:256]';

avIR = sum(yRed(2:256).*B)/sum(yRed(2:256))
avIG = sum(yGreen(2:256).*B)/sum(yGreen(2:256))
avIB = sum(yBlue(2:256).*B)/sum(yBlue(2:256))

```

C.3 Calculation for initial concentrations of minority ions

The expression for the equilibrium constant of auto-ionization of water (H_2O) (see Equation 3.10 in Chapter 3) (also known as the water ionization constant) is:

$$K_w = [\text{OH}^-] [\text{H}_3\text{O}^+], \quad (\text{C.3})$$

where OH^- and H_3O^+ represent the hydroxide and hydronium ions, and the value of $[\text{H}_2\text{O}]$ is assumed to be a constant which takes the value of 1.

The equilibrium constant for carbonate system 1 (see Equation 3.13 in Chapter 3) is defined as:

$$K_{C1} = [\text{HCO}_3^-] [\text{H}_3\text{O}^+] / [\text{H}_2\text{CO}_3^*], \quad (\text{C.4})$$

where HCO_3^- represents the bicarbonate ion and H_2CO_3^* represents the dissolved carbon dioxide (CO_2) in equilibrium with the carbonic acid (H_2CO_3). The equilibrium constant for carbonate system 2 (see Equation 3.14 in Chapter 3) is defined as:

$$K_{C2} = [\text{CO}_3^{2-}] [\text{H}_3\text{O}^+] / [\text{HCO}_3^-], \quad (\text{C.5})$$

where CO_3^{2-} represents the carbonate ion.

To obtain the equilibrium concentrations for all the species, the concentration of H_2CO_3^* must first be calculated from Henry's law:

$$[\text{H}_2\text{CO}_3^*] = P\text{CO}_2 / K_H \quad (\text{C.6})$$

where $P\text{CO}_2$ is the partial pressure of CO_2 and K_H is the Henry's law constant under room temperature T . Equations C.3, C.4, C.5, C.6 and the charge neutrality condition (Equation C.7):

$$[\text{H}_3\text{O}^+] - [\text{OH}^-] - [\text{HCO}_3^-] - 2[\text{CO}_3^{2-}] = 0, \quad (\text{C.7})$$

were solved simultaneously to calculate the equilibrium concentrations for all species, which were:

$$[\text{H}_3\text{O}^+] = 2.313 \text{ } \mu\text{M},$$

$$[\text{OH}^-] = 4.32 \text{ nM},$$

$$[\text{H}_2\text{CO}_3^*] = 12 \text{ } \mu\text{M},$$

$$[\text{HCO}_3^-] = 2.309 \text{ } \mu\text{M},$$

$$[\text{CO}_3^{2-}] = 46.8 \text{ pM}.$$

Appendix D Electroosmotic flow hysteresis for dissimilar cationic solutions

D.1 Zeta potentials and surface charge densities for experimental solutions

Current monitoring method (see Figure 4.1 in Chapter 4) was employed to find out the zeta potentials and surface charge densities for 0.5 mM of potassium chloride (KCl), sodium chloride (NaCl) and calcium chloride (CaCl_2). The experimental setup was similar to that in Section 4.2.1 of Chapter 4.

The microcapillary and reservoir connecting to the cathode were filled with 0.5 mM solution, while reservoir connecting to the anode was filled with 0.475 mM solution (5% concentration difference) (see Figure 4.1 in Chapter 4). Electric potential of 1000 V was applied across the two reservoirs to induce electroosmotic flow (EOF). The electroosmotic displacement flow experiment was conducted five times for each type of solution to ensure consistency and reliability of results.

The time for the current to reach a steady value, i.e. displacement time, was determined from the current-time curve. The zeta potentials for the three types of solutions were calculated through the displacement times with Equation 2.23 in Chapter 2. The surface charge densities of the solutions were then obtained by substituting the calculated zeta potentials into Equation 3.20 in Chapter 3. Table D.1 shows the zeta potentials and surface charge densities for the experimental solutions.

Table D.1: Zeta potentials and surface charge densities for the experimental solutions.

Solution	Zeta potential (mV)	Surface charge density (mC.m ⁻²)
0.5 mM KCl	-56.9 ± 0.5	-3.57 ± 0.04
0.5 mM NaCl	-54.3 ± 1.5	-3.35 ± 0.12
0.5 mM CaCl ₂	-30.6 ± 0.9	-3.84 ± 0.16

D.2 Shapiro-Wilk test

The Shapiro-Wilk test (Shapiro and Wilk 1965) at 1% significance level was used to check if the experimentally obtained turning and displacement times follow the normal statistical distribution.

Let $T_{LH(Turn)}$ be the turning time and T_{LH} be the displacement time for a low conductivity solution displacing a high conductivity solution, and $T_{HL(Turn)}$ be the turning time and T_{HL} be the displacement time for a high conductivity solution displacing a low conductivity solution. The null hypothesis H_o suggests that $T_{LH(Turn)/LH/HL(Turn)/HL}$ is normally distributed, while the alternative hypothesis H_I suggests otherwise. The experimental data were arranged in ascending order so that $(T_{LH(Turn)/LH/HL(Turn)/HL})_1 < \dots < (T_{LH(Turn)/LH/HL(Turn)/HL})_n$. The test statistic is calculated as:

$$W = \frac{\left[\sum_i^m a_i \left[(T_{LH(Turn)/LH/HL(Turn)/HL})_{n+1-i} - (T_{LH(Turn)/LH/HL(Turn)/HL})_i \right] \right]^2}{\sum_i^n \left[(T_{LH(Turn)/LH/HL(Turn)/HL})_i - \bar{T}_{LH(Turn)/LH/HL(Turn)/HL} \right]^2}, \quad (D.1)$$

where $T_{LH(Turn)/LH/HL(Turn)/HL}$ is the sample mean of $T_{LH(Turn)/LH/HL(Turn)/HL}$, n is the number of samples, a_i is the weight coefficient based on n and $m = n/2$ (if n is even) or $(n-1)/2$ (if n is odd).

The test statistic w values calculated from the experimental data are shown in Table D.2. Based on the test statistic w values in Table D.2 and $n = 5$, the p -values for the turning and displacement times were obtained (see Table D.3). Since the p -values shown in Table D.3 are greater than 0.01 (alpha level), H_o which suggests that the experimental data are normally distributed can be retained.

Table D.2: Test statistic w for turning and displacement times calculated from experimental data.

Displacement direction	Test statistic w for turning time	Test statistic w for displacement time
0.5 mM NaCl displacing KCl (LH)	0.910	0.985
0.5 mM KCl displacing NaCl (HL)	0.811	0.883
0.5 mM NaCl displacing CaCl ₂ (LH)	0.949	0.749
0.5 mM CaCl ₂ displacing NaCl (HL)	0.917	0.933

Table D.3: p -values for turning and displacement times.

Displacement direction	p-value for turning time	p-value for displacement time
0.5 mM NaCl displacing KCl (LH)	0.444	0.944
0.5 mM KCl displacing NaCl (HL)	0.115	0.356
0.5 mM NaCl displacing CaCl ₂ (LH)	0.666	0.0419
0.5 mM CaCl ₂ displacing NaCl (HL)	0.467	0.550

Appendix E Electroosmotic flow hysteresis for dissimilar anionic solutions

E.1 Zeta potentials, electroosmotic flow mobilities and surface charge densities for experimental solutions

Current monitoring technique (see Figure 4.1 in Chapter 4) was used to find out the zeta potentials, electroosmotic flow (EOF) mobilities, surface charge densities for 0.5 mM of sodium chloride (NaCl) and sodium sulfate (Na_2SO_4), and the experimental setup was similar to that in Section 4.2.1 of Chapter 4. Details on the experimental procedures can be found in Appendix D.1.

The zeta potentials for the two solutions were calculated with the displacement times (displacement flow between 0.475 mM and 0.5 mM solutions) through Equation 2.23 in Chapter 2. Subsequently, the EOF mobilities for the solutions were derived by substituting the calculated zeta potentials into the Helmholtz-Smoluchowski slip velocity equation (Equation 2.7 in Chapter 2). The surface charge densities of the solutions were obtained by substituting the calculated zeta potentials into Equation 3.20 in Chapter 3. Table E.1 shows the zeta potentials, EOF mobilities and surface charge densities for the experimental solutions.

Table E.1: Zeta potentials, EOF mobilities and surface charge densities for the experimental solutions.

Solution	Zeta potential (mV)	EOF mobility ($\times 10^{-8} \text{ m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$)	Surface charge density ($\text{mC} \cdot \text{m}^{-2}$)
0.5 mM NaCl	-54.3 ± 1.5	4.32 ± 0.12	-3.35 ± 0.12
0.5 mM Na ₂ SO ₄	-36.1 ± 3.1	2.87 ± 0.25	-3.03 ± 0.28

E.2 Effect of geometrical expansion at reservoir junction

Axisymmetric simulations had been performed with 10x and 60x geometrical expansions (see Figure E.1) to investigate their effect on electric field distribution across the reservoir and microchannel. Except for the addition of reservoirs, the numerical simulations were similar to that described in the main text. For simulation efficiency and availability of computational resources, an axisymmetric simulation, including the geometry of reservoir, was conducted. The reservoir was modeled as a spherical object instead of the actual cylindrical one. However, this would have negligible or no impact on the conclusions obtained as the key characteristic of the sharp change of geometry between the reservoir and the channel was still captured by an axisymmetric simulation.

Figure E.1: Simulation domain with reservoirs (not drawn to scale), where L is the length of microchannel. DE and RE represent the displacing and residing electrolytes respectively.

Indeed, the electric field was significantly affected at the reservoir junction (see Figure E.2), but the concentration adjustment in Phase 2 was not affected (see Figure E.3). Although the local electric field distribution at the reservoir junction differed with the addition of the reservoirs, the integral across the cross section remained the same for the preservation of current continuity (refer to Equation 7.4 in Chapter 7), which explains the negligible effect on the concentration adjustment in Phase 2.

Figure E.2: Steady-state axial electric field when 0.5 mM NaCl from cathode reservoir displaced 0.5 mM Na_2SO_4 at (a) axis of symmetry and (b) channel wall for numerical simulations with no reservoirs, 10x and 60x geometrical expansions. Dimensionless $x^* = x/L$, where x is the axial coordinate and L is the length of microchannel.

The equilibrium concentration is dependent on the steady-state current ($I_{Steady-state}$) and electric field in the microchannel (E_L) (refer to Equation 7.5 in Chapter 7). In the experiments, the reservoirs were large as compared to the microchannel and contribute little electrical resistance to the system. Thus, $I_{Steady-state}$ and E_L were not significantly affected by the presence of reservoirs. With the addition of the reservoirs, the equilibrium DE concentration was found to differ by less than 1% as compared to the original simulation without reservoirs (see Figure E.3). Despite the significant effect on electric field due to the sharp change in geometry at the reservoir junction, there was negligible effect on the concentration adjustment in Phase 2. As such, the geometrical expansion at the reservoir junction was not included in the numerical simulations for simplicity, as well as to minimize computation time.

Figure E.3: Steady-state Cl^- ion concentration when 0.5 mM NaCl from cathode reservoir displaced 0.5 mM Na_2SO_4 at (a) axis of symmetry and (b) channel wall for numerical simulations with no reservoirs, 10x and 60x geometrical expansions. Na^+ and SO_4^{2-} ions are not shown. Dimensionless $x^* = x/L$, where x is the axial coordinate and L is the length of microchannel.

Appendix F Effect of nanostructure orientation on electroosmotic flow in a microfluidic channel

F.1 Fabrication of smooth microchannel

The smooth microchannel was fabricated via the conventional DEEMO (Dry Etching, Electroplating, MOlding) process as shown in Figure F.1. The detailed process parameters are presented in Table F.1.

Figure F.1: Schematics of DEEMO fabrication process for smooth microchannel.

Table F.1: Process parameters of DEEMO fabrication process for smooth microchannel.

Process	Parameters for smooth microchannel
Spin coating of photoresist on Si	HMDS priming. 2 μm AZ MiR 701 positive resist, spin-off 30 s at 2600 rpm. Prebaking: 90 °C for 1 min.
Photolithography	350 W Hg lamp with i-line filter as light source, 365 nm wavelength. $W/A = 7 \text{ mW.cm}^{-2}$; $t = 25 \text{ s}$. Post exposure bake: 110 °C for 1 min, then puddled in TMAH developer for 1 min.
Deep reactive ion etching (DRIE)	Passivation>>Etch: $\text{C}_4\text{F}_8/\text{SF}_6/\text{O}_2 = 150/275/15$ sccm; Coil/Platen power = 2000/2500 W; Pressure = 20/26 mtorr; $t = 1/2.2 \text{ s}$; Cycles = 75; Temperature = 0 °C.
Resist stripping	$\text{O}_2/\text{N}_2 = 400/70$ sccm; Pressure = 1 mbar; Power = 1000 W; $t = 10 \text{ min}$.
Electroplating	Sputtering of 85 nm Ni/V seed layer (93/7 wt%). Electroplating Ni shim of 350 μm thickness: Maximum current = 3.5 A; Charge = 18.1 Ah; $t = 6 \text{ h}$.
KOH etching	25 wt% KOH: Temperature = 80 °C; $t = 8 \text{ h}$.
Injection molding	Mold temperature = 90 °C; Melt temperature = 270 °C; Injection pressure = $950 \pm 25 \text{ bar}$; Injection velocity = 37 mm.s^{-1} ; Holding pressure = $600 \pm 25 \text{ bar}$ for 3 s; cooling time = 30 s; Pre-drying (Permanent) = 90 °C; Cycle time = 30 s to 1 min.

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