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Electroosmotic Flow Hysteresis for Dissimilar Anionic Solutions

An Eng Lim, Chun Yee Lim, and Yee Cheong Lam*

School of Mechanical and Aerospace Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798

AUTHOR INFORMATION

Corresponding Author

*Phone: +65 6790 6874. Fax: +65 6792 4062. E-mail: myclam@ntu.edu.sg.

Author Contributions

All authors contributed equally.

Notes

The authors declare no competing financial interest.

ABSTRACT: Electroosmotic flow (EOF) with two or more fluids is often encountered in various microfluidic applications. However, no investigation has hitherto been conducted to investigate the hysteretic or flow direction-dependent behavior during displacement flow of solutions with *dissimilar anion species*. In this investigation, EOF of dissimilar anionic solutions was studied experimentally through current monitoring method and numerically through finite element simulations. As opposed to other conventional displacement flows, EOF involving dissimilar anionic solutions exhibits counter-intuitive behavior, whereby the current-time curve does not reach the steady-state value of the displacing electrolyte. Two distinct mechanics have been identified as the causes for this observation: (a) ion concentration adjustment when the displacing anions migrate upstream against EOF due to competition between the gradients of electromigrative and convective fluxes, and (b) ion concentration re-adjustment induced by the static diffusive interfacial region between the dissimilar fluids which can only be propagated throughout the entire microchannel with the presence of EOF. The resultant ion distributions lead to the flow rate to be directional-dependent, indicating that the flow conditions are asymmetric between these two different flow directions. The outcomes of this investigation contribute to the in-depth understanding of flow behavior in microfluidic systems involving inhomogeneous fluids, particularly dissimilar anionic solutions. The understanding of EOF hysteresis is fundamentally important for the accurate prediction of analytes transport in microfluidic devices under EOF.

INTRODUCTION

Electroosmotic flow (EOF), driven by electrokinetic phenomenon, arises from the spontaneous surface charge formation at the solid-liquid interface. Negative charges are usually developed on a solid surface upon contact with aqueous solutions when common materials such as glass or polydimethylsiloxane (PDMS) are employed for the fabrication of microfluidic channels. The negatively-charged surface attracts positive ions and repels negative ions in the solution, forming a thin layer of net positive charges (nanometer thickness) known as electrical double layer (EDL). Application of an electric field parallel to the microchannel drives the positively-charged EDL in the applied field direction, thereby inducing bulk movement of the fluid.

EOF has been used for various applications in microfluidics including separation of chemical species/particles,^{1,2} pumping of fluid,^{3,4} mixing of analytes,^{5,6} sample trapping,⁷ and pre-concentration.⁸ In many of these practical applications, two or more types of fluids with different properties such as concentration and conductivity are frequently involved. Examples are sample pre-concentration techniques such as field amplified sample stacking (FASS) and isotachopheresis (ITP),⁹ and electrokinetic

instability (EKI) micromixing of electrolytes with different electrical conductivities.¹⁰

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).^{11,12} Upon application of a constant voltage, the sample region acts as a high-electrical-resistance zone which results in locally high electric field. Under the influence of electric field, sample ions migrate from the high to low drift velocity region. This leads to a local accumulation/stacking of sample ions near the interface between regions of high and low conductivity.

In contrast, ITP is typically conducted under constant current which is critical in ensuring dynamic equilibrium: all ions are moving at the same speed.¹³⁻¹⁵ ITP requires a leading electrolyte (LE) and a terminating electrolyte (TE) that has a higher and a lower ionic mobility than the sample components respectively, so that the sample components form focused/concentrated zones in order of ionic mobility behind the LE and ahead of the TE.

Dispersion of samples due to EOF is undesirable for both FASS,¹¹ and ITP.¹⁴ The non-uniform EOF velocities generate internal pressure gradients resulting in sample dispersion that strongly affects the sensitivity and resolution of such applications.

Dispersion can be reduced by suppressing EOF through coating of water-soluble polymers,¹⁶ e.g. acidified poly(ethylene oxide) (PEO).¹⁷ Differing from these pre-concentration techniques, our investigation focuses on the hysteretic flow behavior during transport of ionic analytes with EOF.

Electroosmotic displacement flow of two fluids with different concentrations exhibits hysteretic behavior experimentally, whereby the time for one fluid to fully displace the other (defined as displacement time) differs depending on the flow direction,¹⁸ i.e. the displacement time for fluid A displacing fluid B is different from fluid B displacing fluid A. Several investigations in the literature have reported the application of EOF with large concentration difference,^{19–21} but failed to consider that this hysteresis effect might lead to incorrect interpretation of results. For example, the flow rate/velocity for fluid A displacing fluid B cannot be assumed to be the same as that for fluid B displacing fluid A. The hysteretic effect is exhibited because the ions do not retrace their trajectories when the electric field is reversed. The flow velocity and the final content in the microchannel can only be determined accurately by considering appropriately the interplay between ionic migration, EOF and electric field distribution.

We have investigated EOF hysteresis involving similar solutions with different concentration; this hysteresis was demonstrated to originate from the accumulation or depletion of pH-governing minority ions due to the imbalance of electromigrative flux, which alters the pH and flow velocity for different flow directions.²² In this investigation, we focus on EOF hysteresis involving a solution pair with *dissimilar anion species*. The minority pH-governing ions is neglected to simplify the numerical model and better elucidate the concentration evolution of the main constituent ions. From our previous investigation²², it is estimated that this simplification induces an approximate error of 10% in flow rate, given that the conductivity ratio of the solution pair in this investigation (i.e. 0.5mM of NaCl and Na₂SO₄) is 1:2.

We have also explored EOF hysteresis for solutions with *dissimilar cation species* (e.g. NaCl–CaCl₂ solution pair).²³ Unlike previous investigations,^{19,20} we have included ionic mobilities of the species in our theoretical/numerical model to better comprehend the flow behavior of solution pair with dissimilar ionic species.²³ Two distinct mechanisms for this type of EOF hysteresis have been identified, namely the widening/sharpening of interfacial region induced by conductivity difference and the difference in adjusted ion distributions (thus average zeta potentials and flow velocities) for different flow directions.

Despite the abundance of investigations on EOF with dissimilar solutions, there is yet any investigation examining specifically the displacement flow behavior for solution pair involving *dissimilar anions* (e.g. NaCl–Na₂SO₄ solution pair). This investigation demonstrates for the first time EOF hysteresis for dissimilar anionic solutions. The outcomes of this investigation contribute to the in-depth understanding of flow behavior in microfluidic systems involving inhomogeneous fluids, particularly dissimilar anionic solutions. The understanding of EOF hysteresis is fundamentally important for the accurate prediction of analytes transport in microfluidic devices under EOF.

EXPERIMENTAL SECTION

Solutions preparation. Two types of ionic solutions with the same cation but dissimilar anions, namely sodium chloride (NaCl) and sodium sulfate (Na₂SO₄), were prepared for the experiments. The solution pair is chosen as a model to study the flow behavior involving solutions with similar cation but dissimilar anions. Stock solutions of 0.01M were prepared by dissolving the salts (Sigma-Aldrich) in deionized (DI) water. Subsequently, the stock

solutions were diluted to concentrations of 0.475mM (95% of 0.5mM, required for EOF velocity measurement) and 0.5mM. The solution properties, such as conductivity and pH, were measured respectively with the conductivity meter (IONCheck 65, Radiometer Analytical) and pH meter (AccumetAR20, Fisher Scientific).

The measured conductivities for 0.475mM and 0.5mM of NaCl are $60.4 \pm 0.1 \mu\text{S}\cdot\text{cm}^{-1}$ and $63.8 \pm 0.2 \mu\text{S}\cdot\text{cm}^{-1}$ respectively, and for 0.475mM and 0.5mM of Na₂SO₄ solutions are $123.5 \pm 0.4 \mu\text{S}\cdot\text{cm}^{-1}$ and $130.6 \pm 0.3 \mu\text{S}\cdot\text{cm}^{-1}$ respectively. Low concentration/conductivity solutions were employed to minimize Joule heating as a result of an applied electric field during EOF (refer to EOF velocity measurement for a discussion on the effects of Joule heating). The measured average pH for all solutions is 5.57 ± 0.09 . All solutions have been equilibrated with the atmosphere and their pHs do not change with time. The acidity of the solutions is caused by the diffusion of carbon dioxide into water.

Figure 1. Experimental setup of current monitoring method with displacement of residing electrolyte (RE) in microchannel from (A) anode and (B) cathode reservoirs. (C) Simulation domain (not drawn to scale), where L is the length of microchannel.

Electroosmotic flow velocity, mobility and zeta potential measurements. EOF velocity, mobility and zeta potential are critical parameters that characterize EOF. For the solutions employed in our experiments, these parameters were measured with current monitoring method (see Fig. 1A), instead of using a neu-

tral marker.^{24,25} The current monitoring method enables the quantification of EOF behavior during displacement flow of fluids with small concentration difference.²⁶⁻²⁸ Huang *et al.*²⁶ has compared the current monitoring method with the neutral marker method for determining the EOF velocity, and concluded that there is no significant difference between them.

EOF experiments were conducted in polyimide coated fused silica microcapillaries (Polymicro Technologies) with a nominal internal diameter of 100 μ m; these microcapillaries were cut to 8cm by Shortix Column Cutter (SGT Ltd). The microcapillaries were flushed with acetone followed by DI water, and lastly the experimental solutions. The capillary was inserted between two Teflon reservoirs with both diameter and depth of 2cm.

The electric field for inducing EOF was supplied by a high voltage power supply (CZE1000R, Spellman) through platinum electrodes. Electric potential of 1000V was applied across the reservoirs. The microcapillary and cathode reservoir were filled with 0.5mM solution, and the anode reservoir was filled with 0.475mM solution (see Fig. 1A). As the displacing electrolyte (DE) flowed from the anode towards the cathode to displace the residing electrolyte (RE), conductivity in the microcapillary varied and the current change was monitored over time by connecting a picoammeter (Keithley 6458) in series to the capillary. A Labview program was written to control the two devices, as well as to record the voltage and current readings through a data acquisition card (PCI-6052E, National Instrument). Experiments were conducted five times for each solution type to ensure consistency and reliability of the results.

The time for the current to reach a steady value, i.e. displacement time, was determined from the current-time curve. The EOF velocity was calculated by dividing the length of microchannel with the displacement time:

$$v_{eo} = \frac{L}{t_d}, \quad (1)$$

where L is the length of microchannel and t_d is the displacement time. The measured EOF velocities for 0.5mM of Na_2SO_4 and NaCl are $(3.59 \pm 0.31) \times 10^{-4} \text{ m.s}^{-1}$ and $(7.44 \pm 0.20) \times 10^{-4} \text{ m.s}^{-1}$ respectively.

Subsequently, the EOF mobility and zeta potential were obtained by substituting the measured EOF velocity into the Helmholtz-Smoluchowski slip velocity equation:

$$v_{eo} = -\frac{\epsilon_r \epsilon_0 \zeta E}{\mu} = u_{eo} E, \quad (2)$$

where ϵ_0 is the permittivity of free space, ϵ_r is the relative permittivity of liquid, ζ is the zeta potential, E is the electric field, μ is the viscosity of fluid and u_{eo} is the EOF mobility. The measured EOF mobilities for 0.5mM of Na_2SO_4 and NaCl are $(2.43 \pm 0.25) \times 10^{-8} \text{ m}^2.\text{V}^{-1}.\text{s}^{-1}$ and $(4.32 \pm 0.12) \times 10^{-8} \text{ m}^2.\text{V}^{-1}.\text{s}^{-1}$ respectively. The measured ζ for the solutions are $-36.1 \pm 3.1 \text{ mV}$ and $-54.3 \pm 1.5 \text{ mV}$ respectively.

Applying electric field across electrolyte might induce Joule heating,²⁹ which would affect EOF by affecting the solution properties such as viscosity and EOF mobility. 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.³⁰ For the chosen experimental parameters, the worst case scenario has an estimated temperature rise of 0.16°C, which is insignificant for causing any Joule heating effects.

In microfluidic devices with small reservoirs, application of electric field through the electrodes might cause undesirable electrolytic reactions which might induce local pH change due to the production of hydronium (H_3O^+) and hydroxide (OH^-) ions.³¹

Therefore, various precautions have been adopted to maintain pH stability throughout the experiments. Large-volume reservoirs (both diameter and depth of 2cm) were employed to significantly dilute the concentrations of H_3O^+ and OH^- ions produced at the electrodes from electrolysis. Employing large reservoirs can also ensure negligible liquid level changes in the reservoirs, hence minimizing the back pressure generated.³² The electrodes were placed far ($\sim 2\text{cm}$) from the microcapillary inlet/outlet to prevent unwanted pH change in the microcapillary.²⁷ Furthermore, the experimental duration was kept short (less than 10min) and fresh solutions were employed for each experimental run.

Displacement flow of dissimilar anionic solutions. The anion species experience competing gradients of electromigrative and convective fluxes (see Eq. (7) under governing equations). The ionic mobilities $u_{m(i)}$ for common anions (e.g. NO_3^- , Cl^- and SO_4^{2-}) range from $-7.41 \times 10^{-8} \text{ m}^2.\text{V}^{-1}.\text{s}^{-1}$ to $-8.30 \times 10^{-8} \text{ m}^2.\text{V}^{-1}.\text{s}^{-1}$,³³ and the EOF mobilities u_{eo} for unbuffered solutions typically range from $2.43 \times 10^{-8} \text{ m}^2.\text{V}^{-1}.\text{s}^{-1}$ to $4.53 \times 10^{-8} \text{ m}^2.\text{V}^{-1}.\text{s}^{-1}$.²³

From the perspective of a visualization experiment, the bulk EOF takes place from the anode towards the cathode. However, the anions experience a net migration in a direction which opposes EOF because the magnitudes for the ionic mobilities of anions are higher than the EOF mobilities in our experiments. Here, DE is defined as the electrolyte with dissimilar ion species that migrates into the microchannel and displaces the initial ions residing in it. The definition is consistent even in the case of the displacement flow of dissimilar cationic solutions²³ where the cations migrate in the same direction as EOF.

The DE was placed in the cathode reservoir so that the displacing anions can replace the initial anions residing in the microchannel; the anode reservoir and microchannel were filled with the RE (see Fig. 1B). For example, for NaCl displaces Na_2SO_4 , NaCl is placed in the cathode reservoir (and is the DE) and Na_2SO_4 is residing in the microchannel (and is the RE). Except for these differences, the experimental setup was similar to that described for EOF velocity measurement. The current-time responses of displacement flows involving NaCl– Na_2SO_4 solution pair in two different directions (i.e. NaCl displaces Na_2SO_4 , and Na_2SO_4 displaces NaCl) were compared to reveal the hysteresis phenomenon.

Numerical simulations. Numerical studies were performed with finite element method (FEM) based on Poisson-Nernst-Planck (PNP) model with modified boundary conditions,^{18,23} which considers the changes of ion concentrations near EDL due to diffusion, electromigration and convection. To capture the variations of zeta potential and ion distributions, the major characteristics of two-fluid displacement flow, the zeta potential and ion distributions are not specified *a priori*; this is a significant and necessary departure from the existing conventional models.

Simple 1-D electrophoresis model is a good premise to investigate the evolution of ion distributions under negligible fluid flow or constant EOF.^{34,35} However, in a displacement flow involving multiple electrolytes (such as in our investigations), the EOF is not constant over time. The spatial and temporal variation of zeta potential can only be captured with a model that includes all the necessary governing equations (Eqs. 5 to 9) which describe the important phenomena.

In capillary zone electrophoresis (CZE), system peaks are additional peaks in electropherogram which do not correspond to any species in the sample of interest.^{36,37} One of the causes of system peaks is H_3O^+ , OH^- or other ions in the buffered solutions. In our numerical simulations, the pH-governing ions, such as H_3O^+ and OH^- , along with their acid-base equilibrium equations have been excluded in our simulations. Firstly, buffered solution is not used

in our experiments. Furthermore, the solutions ($\text{pH} = 5.57 \pm 0.09$) are in the “safe” pH range³⁷ (5 to 9), where $[\text{H}_3\text{O}^+]$ and $[\text{OH}^-]$ ($2.07\mu\text{M}$ and 4.83nM respectively) can be disregarded as their concentrations are several order of magnitude lower than the main constituent ions (0.5 to 1mM). Due to the low concentrations of H_3O^+ and OH^- ions, the surface charge density is mainly compensated by the main constituent ionic species, as dictated by the Poisson equation (Eq. 6). Secondly, a series of precautions to maintain pH stability throughout the experiment are adopted, as mentioned in the experimental section.

The simulations were implemented on COMSOL Multiphysics software. The simulation domain is a straight cylindrical microchannel with diameter of $100\mu\text{m}$ and length of 0.5cm (see Fig. 1C). The fluid flow is assumed to be axisymmetric about the center axis. The reservoirs are omitted in our numerical model for simplicity; the justification for this simplification has been detailed in the Supporting Information.

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 is set to 1nm and the size of each adjacent element further from the wall increases by 1.2 times. The mesh is sufficiently fine to resolve the steep changes of the variables across the EDL. The applied potential, electrostatic potential and ion concentrations were discretized with second order elements, while the pressure and velocity were discretized with linear elements. Convergence test was performed with higher number of elements for steady-state solutions and the numerical error is found to be negligible for this mesh selection. The convergence criterion is based on relative tolerance of less than 0.01 between subsequent iterations. The maximum integration time step is set to 0.005s , while the total simulated time is 30s .

Governing equations. Application of external electric field generates current across the microchannel. Assuming that there is no source or sink in the medium, charge conservation requires the divergence of current density $\mathbf{J}$ equals to zero,

$$\nabla \cdot \mathbf{J} = 0. \quad (3)$$

The relationship between the current density $\mathbf{J}$ and transport of ions is given as

$$\nabla \cdot [\sigma \nabla \phi + F \sum z_i D_i \nabla c_i - \mathbf{u} F \sum z_i c_i] = 0, \quad (4)$$

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, $z_{(i)}$ is the charge number, D_i is the diffusion coefficient, $u_{m(i)}$ is the ionic mobility and c_i is the concentration of ionic species. Eq. (4) consists of electromigrative, diffusive and convective current densities whereby the first term is the major contributor; the other two terms are neglected due to their small magnitudes. Hence, Eq. (4) is reduced to the Laplace equation which governs ϕ ,

$$\nabla \cdot (\sigma \nabla \phi) = 0. \quad (5)$$

The electrostatic potential distribution ψ is given by the Poisson equation

$$\nabla \cdot \nabla \psi = -\frac{\rho_e}{\epsilon_r \epsilon_0}, \quad (6)$$

where the net charge density $\rho_e = F \sum z_i c_i$. As such, zeta potential ζ varies according to the local ρ_e that changes with c_i during two-fluid displacement flow. To capture this variation, constant surface charge density is specified at the wall instead of constant ζ .

The transports of all ionic species are simulated with the Nernst-Planck equation. The change of ion concentration with time is governed by the gradients of diffusive, electromigrative

and convective fluxes. Their relationship may be described by the Nernst-Planck equation as

$$\frac{\partial c_i}{\partial t} + \nabla \cdot [-D_i \nabla c_i - u_{m(i)} c_i \nabla (\phi + \psi)] = -\mathbf{u} \cdot \nabla c_i, \quad (7)$$

where the first term represents the change of ion concentration with time, the gradient of diffusive and electromigrative fluxes are described by the second and third terms on the left of Eq. (7) respectively; the gradient of convective flux is described by the term on the right of Eq. (7).

The fluid flow for an incompressible Newtonian fluid is described by the Navier-Stokes and continuity equations, i.e. Eqs. (8) and (9) respectively,

$$\rho \frac{\partial \mathbf{u}}{\partial t} + \rho \mathbf{u} \cdot \nabla \mathbf{u} = -\nabla p + \mu \nabla^2 \mathbf{u} + \rho_e [-\nabla (\phi + \psi)], \quad (8)$$

$$\nabla \cdot \mathbf{u} = 0, \quad (9)$$

where ρ is the fluid density and p is the pressure. The electric body force (third term on the right of Eq. (8)) consists of the product of ρ_e and electric field $\mathbf{E}$. In microfluidics, the Reynolds number is typically less than 1, thus the inertial term (second term on the left of Eq. (8)) is usually ignored and Stokes flow is assumed.

Table 1. Symbols and values of parameters employed in numerical simulations. Diffusion coefficient of the ionic species are obtained from Newman and Thomas-Alyea³³, and ionic mobility of a ion species is calculated through the formula $(z_i D_i F)/(RT)$.

Parameter	Symbol (Unit)	Value
Permittivity of free space	ϵ_0 ($\text{C} \cdot \text{V}^{-1} \cdot \text{m}^{-1}$)	8.85×10^{-12}
Relative permittivity	ϵ_r	80
Viscosity of water	μ ($\text{kg} \cdot \text{m}^{-1} \cdot \text{s}^{-1}$)	8.90×10^{-4}
Density of water	ρ ($\text{kg} \cdot \text{m}^{-3}$)	1000
Faraday constant	F ($\text{C} \cdot \text{mol}^{-1}$)	96485
Gas constant	R ($\text{J} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$)	8.314
Boltzmann constant	k_b ($\text{m}^2 \cdot \text{kg} \cdot \text{s}^{-2} \cdot \text{K}^{-1}$)	1.381×10^{-23}
Temperature	T (K)	298
Electron charge	e (C)	1.602×10^{-19}
Avogadro constant	N_a (mol^{-1})	6.022×10^{23}
Solution concentration	c_0 ($\text{mol} \cdot \text{m}^{-3}$)	0.5
Diffusion coefficient of Na^+	D_{Na^+} ($\text{m}^2 \cdot \text{s}^{-1}$)	1.334×10^{-9}
Diffusion coefficient of Cl^-	D_{Cl^-} ($\text{m}^2 \cdot \text{s}^{-1}$)	2.032×10^{-9}
Diffusion coefficient of SO_4^{2-}	$D_{\text{SO}_4^{2-}}$ ($\text{m}^2 \cdot \text{s}^{-1}$)	1.065×10^{-9}
Ionic mobility of Na^+	$u_{m(\text{Na}^+)}$ ($\text{m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$)	5.194×10^{-8}
Ionic mobility of Cl^-	$u_{m(\text{Cl}^-)}$ ($\text{m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$)	-7.919×10^{-8}
Ionic mobility of SO_4^{2-}	$u_{m(\text{SO}_4^{2-})}$ ($\text{m}^2 \cdot \text{V}^{-1} \cdot \text{s}^{-1}$)	-8.296×10^{-8}
Ionic charge number of Na^+	z_{Na^+}	+1
Ionic charge number of Cl^-	z_{Cl^-}	-1
Ionic charge number of SO_4^{2-}	$z_{\text{SO}_4^{2-}}$	-2

Eqs. (8) and (9) have to be solved simultaneously with Eqs. (5), (6) and (7) to obtain the fluid flow field. This is because ϕ , ψ , c_i and $\mathbf{u}$ are strongly coupled in EOF. High computational resources are required for solving these governing equations. Therefore, the simulated channel length was reduced to 0.5cm (as compared to 8cm 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 symbols and values of parameters used in the numerical simulations are given in Table 1.

Boundary and initial conditions. The ion distributions in EDL are disturbed during EOF involving two or more fluids. This causes the local zeta potential along the microchannel to vary according to the local ion distributions. To capture this change, constant surface charge density was assumed on the channel wall.^{18,23} Relationship between the surface charge density S and ζ for a general electrolyte is given by the Grahame equation³⁸

$$S(\zeta) = \text{sgn}(\zeta) \left[2N_a \varepsilon_o \varepsilon_r k_b T \sum c_{o(i)} \left\{ \exp\left(\frac{-z_i e \zeta}{k_b T}\right) - 1 \right\} \right]^{\frac{1}{2}}, \quad (10)$$

where $\text{sgn}(\zeta)$ extracts the sign of ζ , N_a is the Avogadro constant, T is the temperature, $c_{o(i)}$ is the bulk concentration of ionic species and e is the electron charge. Surface charge densities were obtained by substituting the experimental zeta potentials (refer to zeta potential measurement) into Eq. (10), which yields $-3.03 \pm 0.28 \text{ mC.m}^{-2}$ and $3.35 \pm 0.12 \text{ mC.m}^{-2}$ for 0.5mM of Na_2SO_4 and NaCl respectively.

To solve the time-dependent solution of two-fluid displacement flow, steady-state solution of single fluid EOF has to be set as the initial condition. Boundary conditions for the steady-state numerical model of single fluid EOF are listed in Table 2. A voltage of 62.5V was set at the anode (see Fig. 1C) to establish electric field of 125V.cm^{-1} similar to the experiments. Surface charge density of the wall was specified as -3.19mC.m^{-2} ; average value of the measured S for 0.5mM of Na_2SO_4 and NaCl. It was assumed constant regardless of the ion concentrations during two-fluid displacement flow.

Table 2. Boundary conditions for steady-state numerical model of single fluid electroosmotic flow.

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

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

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

Electroneutrality condition is imposed on the middle of the microchannel (along the line of symmetry, see Fig. 1C). 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 follow Boltzmann distribution under the effect of wall potential ψ . The electric body force in Eq. (8) is non-linear since both ρ_e and $\mathbf{E}$ are functions of ψ . The wall potential induced body force is supposed to be balanced by the pressure gradient in the radial direction.³⁹ Therefore, ψ was removed from $\mathbf{E}$ in the body force term, and pressure p at the cathode and anode were set to zero.

Figure 2. Experimental current-time curves for displacement of (A) 0.5mM NaCl- Na_2SO_4 (via cathode reservoir) and (B) 0.5mM NaCl- CaCl_2 (via anode reservoir) (Lim *et al.*²³) in two different directions. (C) Comparison between simulated and experimental current-time curves for displacements of 0.5mM NaCl- Na_2SO_4 . Experimental/numerical currents and times are normalized by $I^* = (I - I^0_{\text{NaCl}}) / (I^0_{\text{Na}_2\text{SO}_4} - I^0_{\text{NaCl}})$ and $T^* = T / T_{\text{NaCl} \rightarrow \text{Na}_2\text{SO}_4}$ respectively, where I^0 with corresponding subscripts NaCl and Na_2SO_4 represent the initial currents of the solution pair, and T with subscript $\text{NaCl} \rightarrow \text{Na}_2\text{SO}_4$ represents the time for NaCl displaces Na_2SO_4 to reach steady-state current.

Figure 3. Simulated ion concentrations at axis of symmetry along microchannel when (A) 0.5mM Na_2SO_4 from cathode reservoir displaces 0.5mM NaCl in Phase 1 ($t = 5\text{s}$) and (B) Phase 2 ($t = 15\text{s}$); and (C) 0.5mM NaCl from cathode reservoir displaces 0.5mM 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.

For solving the time-dependent displacement flow, boundary conditions at the cathode were modified. Ion concentrations at the cathode were ramped from RE to DE in an arbitrarily short time of 0.01s, to simulate the initiation of the displacement process. Simulations for displacement of 0.5mM NaCl – Na_2SO_4 solution pair in two different directions were carried out to elucidate the mechanics of EOF hysteresis.

RESULTS AND DISCUSSION

Current behaviors observed in experiments. As mentioned in the experimental section, the anions experience electromigration in the direction opposing EOF. The anions of DE from the cathode reservoir are expected to fill up the microchannel, displacing the residing anions. However, the current-time curves for displacements of 0.5mM NaCl – Na_2SO_4 solution pair never approach the steady-state values of DE (see Fig. 2A), which contradicts with the conventional displacement flow experiments. The incongruity is evidenced through comparison with the displacements of 0.5mM NaCl – CaCl_2 solution pair (dissimilar cations, but similar anion) from Lim *et al.*²³, whereby the current-time curves equilibrate at the steady-state values of DE upon completion of the displacement processes (see Fig. 2B).

Similar to EOF of solutions with dissimilar cation species, current-time curves for EOF of solutions with dissimilar anions also exhibit an abrupt gradient change before reaching the equilibrium values. This gradient change allows the displacement process to

be separated into two distinct phases, namely Phase 1 and Phase 2 (see Figs. 2A and B). To explain the counter-intuitive current behaviors as observed in Fig. 2A, the evolution of ion distributions is examined through numerical simulations (see Figs. 3 and 4). The detailed time evolutions of ion concentrations, electric field, zeta potential and EOF velocity along the microchannel are contained in the Supporting Information (Figs. S4 and S5) for better illustration of the displacement process.

The experimental and numerical current-time curves are compared by normalizing the currents and times (see Fig. 2C), and their good agreement confirms the validity of our numerical model. The experimental/numerical currents and times are normalized by Eqs. (11) and (12) respectively,

$$I^* = \frac{I - I_{\text{NaCl}}^o}{I_{\text{Na}_2\text{SO}_4}^o - I_{\text{NaCl}}^o}, \quad (11)$$

$$T^* = \frac{T}{T_{\text{NaCl} \rightarrow \text{Na}_2\text{SO}_4}}, \quad (12)$$

where I^o with corresponding subscripts NaCl and Na_2SO_4 represent the initial currents of the solution pair, and T with subscript $\text{NaCl} \rightarrow \text{Na}_2\text{SO}_4$ represents the time for NaCl displaces Na_2SO_4 to reach steady-state current.

Concentration adjustment according to Kohlrausch regulating function. The anion species from DE migrate upstream against EOF from the cathode reservoir (see Figs. 3A and C). This is 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 experimental section). However, the DE concentration is adjusted from 0.5mM to 0.25mM for Na_2SO_4 (when Na_2SO_4 displaces NaCl , see Fig. 3A), and from 0.5mM to 0.98mM for NaCl (when NaCl displaces Na_2SO_4 , see Fig. 3C). The concentration adjustment fulfills both current continuity and electroneutrality (dictated by Eqs. (5) and (6) respectively), and can be predicted by the Kohlrausch regulating function⁴⁰ (KRF):

$$\text{KRF}(x) = \sum \frac{z_i c_i(x, t)}{u_{m(i)}}, \quad (13)$$

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 0.5mM of Na_2SO_4 and NaCl are 3.13×10^7 mol.V.s.m⁻⁵ and 1.59×10^7 mol.V.s.m⁻⁵ respectively. As anions from the cathode reservoir migrate towards the anode, the DE concentration is adjusted such that its KRF matches the KRF of RE. When Na_2SO_4 displaces NaCl , the concentration of Na_2SO_4 is decreased spontaneously from 0.5mM to 0.25mM. This is because the initial KRF value of Na_2SO_4 is higher than NaCl . The concentration of Na_2SO_4 is lowered so that its KRF value matches the KRF value of NaCl . On the contrary, when NaCl displaces Na_2SO_4 , the concentration of NaCl is increased from 0.5mM to 0.98mM. The KRF value of NaCl is thus increased to match the higher KRF value of Na_2SO_4 .

The KRF presented in Eq. (13) bears the following simplifications: (i) diffusion of ions is neglected, and (ii) EOF is absent. We merely use KRF to illustrate the concentration adjustment mechanics in Phase 1 and estimate the adjusted concentration for DE. The spatial and temporal evolution of ion distributions along the microchannel during the displacement process (see Figs. S4A and S5A) was obtained through the numerical simulation. Our numerical results on the adjusted DE concentrations agree well with the concentrations predicted by KRF.

At the end of Phase 1, the RE in microchannel is 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 deviates from the initial/original conductivity in the reservoir. This is one of the factors which prevent the steady-state current reaching the expected value based on the original properties of DE.

For pre-concentration techniques such as FASS and ITP, the electric field similarly induces the propagation of sample concentration shock wave and rarefaction wave,^{41,42} while the plateau concentration is governed by KRF as well.^{11,43} However, no study has hitherto been attempted to relate the focusing/depletion of ions to the change in EOF flow rate for both directions. This is because EOF is often suppressed^{11,14} to improve the sensitivity and resolution of these pre-concentration applications. Our investigation relates the adjustment of ion concentrations to the flow rate and demonstrates the hysteretic flow effect, which will be detailed in the “electroosmotic flow hysteresis loop” section.

Interestingly, a second concentration adjustment occurs in Phase 2, which is marked by the abrupt change of gradient in current-time curve.

Diffusive-interface-induced concentration adjustment. In Phase 2, despite the fact that almost the entire microchannel is filled with DE, the anions of RE are still found near the vicinity of the anode reservoir. For example, when Na_2SO_4 displaces NaCl in

Phase 1, the vicinity near the anode reservoir only contained two types of ions, namely Na^+ and Cl^- . This is because the anode reservoir and microchannel were initially filled with 0.5mM NaCl (RE) (see Fig. 4A). In Phase 2, when SO_4^{2-} ions (from DE) reach the vicinity of the anode reservoir (see Fig. 4B), a region with a mixture of three types of ions, namely Na^+ , Cl^- and SO_4^{2-} is developed.

At first glance, Cl^- ions (of RE) which migrate towards the anode should have been completely displaced by SO_4^{2-} ions (of DE) and disappear from the mixture region. However, Cl^- is retained in the mixture zone because the anode reservoir of NaCl serves as an infinite source and maintains a sharp diffusive gradient to oppose the complete displacement of Cl^- ions. In addition, the anode reservoir of NaCl also acts as an infinite sink for the incoming SO_4^{2-} , thus maintaining a sharp concentration gradient at the junction. The conditions experienced by these two ions at the anode reservoir junction are reflected in the simulation by the boundary conditions (refer to “boundary and initial conditions” section).

Subsequently, the three ions in the diffusive mixture zone reorganize themselves to fulfill both current continuity and charge neutrality (dictated by Eqs. (5) and (6) respectively). Although this diffusive interface is typically very small ($\sim 15\mu\text{m}$) as compared to the microchannel length, it has a significant impact on the final equilibrium content of the entire channel due to its difference in conductivity.

Current continuity dictated by Eq. (5) requires that current I has to obey the equation:

$$I = \int E_a \sigma_a dA = \int E_L \sigma_L dA, \quad (14)$$

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 Figs. 4B and D).

To determine the equilibrium concentration in the microchannel, Eq. 14 is re-arranged to solve for c_L :

$$c_L = \frac{\int E_a \sigma_a dA}{RE_L F \sum n_i z_i u_{m(i)}} = \frac{I_{\text{Steady-state}}}{RE_L F \sum n_i z_i u_{m(i)}}, \quad (15)$$

where R is the cross sectional area, F is the Faraday constant, n_i is the molecular formula, $z_{(i)}$ is the charge number and $u_{m(i)}$ is the ionic mobility of ionic species. While E_L can be approximated as 125V.cm^{-1} for a long channel, $I_{\text{Steady-state}}$ can be obtained from the experiments.

The numerical simulation of Na_2SO_4 displacing NaCl shows that the concentration of Na_2SO_4 decreases from 0.5mM to 0.25mM in Phase 1 and increases to 0.29mM in Phase 2. The re-adjusted DE concentration is propagated downstream throughout the entire microchannel via EOF (see Fig. 3B). As a comparison, the equilibrium concentration of Na_2SO_4 calculated with Eq. 15 based on the experimental results is 0.28 ± 0.02 mM, which is close to the simulated concentration of 0.29mM.

Similarly, for the case of NaCl displacing Na_2SO_4 , the concentration of NaCl increases from 0.5mM to 0.98mM in Phase 1 and decreases to 0.85mM in Phase 2. The equilibrium concentration of NaCl obtained based on the experimental results is 0.94 ± 0.08 mM, which is reasonably close to the simulated value of 0.85mM.

Figure 4. Simulated ion distributions at the junction between anode reservoir and microchannel when (A) 0.5mM Na_2SO_4 from cathode reservoir displaces 0.5mM NaCl in Phase 1 ($t = 5\text{s}$) and (B) Phase 2 ($t = 30\text{s}$, steady-state); and (C) 0.5mM NaCl from cathode reservoir displaces 0.5mM Na_2SO_4 in Phase 1 ($t = 5\text{s}$) and (D) Phase 2 ($t = 30\text{s}$, 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.

The diffusive current density at the channel/reservoir border (second term on the left of Eq. (4), which is in the range of 10^{-4} to 10^1 A.m^{-2}) is neglected because it is several orders of magnitude smaller than the electromigrative current density (first term on the left of Eq. (4), which is 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.

Although all the solutions have the same initial concentration of 0.5mM, the equilibrium DE concentration is 0.29mM for Na_2SO_4 and 0.85mM for NaCl . Two distinct and spontaneous concentration adjustments during the displacement process cause the steady-state current to deviate from the expected value based on the initial DE properties (see Fig. 2A). It should be highlighted that although the concentration adjustment in Phase 1 is well described by KRF, the concentration re-adjustment 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.

Difference between dissimilar cations and anions systems.

The ion concentration adjustment according to Kohlraush regulating function (KRF) applies to Phase 1 of both NaCl - CaCl_2 (dissimilar cations²³) and NaCl - Na_2SO_4 (dissimilar anions) systems. The difference between these two systems lies in Phase 2 of the displacement flow. During Phase 2 of NaCl - CaCl_2 system, the ion distribution achieved in Phase 1 is simply displaced by the content in the anode reservoir under EOF. This is because the cations in the anode reservoir migrate in the same direction as EOF. The detailed simulation results for the displacements of 0.5mM NaCl - CaCl_2 solution pair are documented in our previous work²³.

However, during Phase 2 of NaCl - Na_2SO_4 system, the ion distribution achieved in Phase 1 is *not* displaced by the content in the anode reservoir. This is because the anions in the anode reservoir migrate in an opposite direction to EOF. 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, we find that the content in the anode reservoir does have an effect on the final state of the system.

A diffusive interface which contains the anions in the anode reservoir and the displacing anions is formed at the junction between the microchannel and the reservoir. This diffusive zone causes another ion concentration adjustment and the content is propagated throughout the entire microchannel by EOF. This is the reason why the final ion concentration is different from that in the anode or cathode reservoir.

Figure 5. Variations of numerical flow rate with percentage of NaCl in microchannel for displacement of (A) 0.5mM NaCl–Na₂SO₄ and (B) 0.5mM NaCl–CaCl₂ (constructed from data in Lim *et al.*²³) solution pairs in two different directions from cathode and anode reservoirs respectively.

Electroosmotic flow hysteresis loop. The flow directional reversal is achieved experimentally here by changing the position 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 leads to a different ion distribution adjustment along the microchannel, and thus causes the flow rate to be directional-dependent, resulting in a hysteric flow behavior. To demonstrate this hysteric flow effect, the EOF hysteresis loop relating the adjustment of ion concentrations to the flow rate is plotted in Fig. 5A.

The value of zeta potential cannot be interpreted from the current-time curve for experiments involving dissimilar solutions because the two solutions have different zeta potentials. However, the change of flow rate over time can be inspected from the numerical simulation to provide insight on the hysteric behavior of EOF involving dissimilar anionic solutions. Our numerical model calculates the flow rate based on the average surface charge density which is specified as the boundary condition (refer to Table 2). The value of the surface charge density is derived from the experimentally obtained zeta potentials of NaCl and Na₂SO₄ solutions. Our numerical model is validated by the good agreement between the normalized experimental and numerical current-time curves (see Fig. 2C).

The variations of numerical flow rate with percentage of NaCl along the microchannel are shown in Fig. 5A. The EOF flow rate follows different paths (as indicated by the two lines in Fig. 5A) depending on the displacement directions, forming an open-ended hysteresis loop. This shows that the flow rate cannot be determined at a given RE–DE interface position without knowing the displacement direction.

To highlight the peculiar behavior of open-ended hysteresis loop, a typical closed hysteresis loop for the displacements of 0.5mM NaCl–CaCl₂ solution pair (dissimilar cations, but similar anion) constructed from the numerical data in Lim *et al.*²³ is shown in Fig. 5B for comparison. The EOF flow rate in both directions approach the flow rates of the respective DE. For example, when 0.5mM NaCl displaces CaCl₂, the microchannel is eventually filled with 0.5mM NaCl. In contrast, the open-ended hysteresis loop shows that the flow rate cannot approach that of DE. For example, when 0.5mM NaCl displaces Na₂SO₄, the microchannel will never be filled with 0.5mM NaCl. This is due to the two distinct and spontaneous adjustments of DE concentration during the displacement process.

CONCLUSION

In this investigation, EOF hysteresis for dissimilar anionic solutions has been demonstrated in experiments and elucidated through numerical simulations. As opposed to other conventional displacement flows (e.g. with different concentrations or cation species), EOF involving dissimilar anion species exhibits an unusual behavior, whereby the steady-state concentration of displacing electrolyte in the microchannel deviates from its original value at the end of the displacement process. This counter-intuitive behavior is attributed to the ion concentration adjustment when the displacing anions migrate against EOF. Subsequently, a second ion concentration adjustment is induced by the diffusive interface at the junction between the anode reservoir and microchannel, which is convected throughout the entire channel by EOF.

The flow direction reversal effected by reversing the electric field direction leads to a different ion distribution adjustment along the microchannel; this causes the flow rate to be directional-dependent, resulting in a hysteric flow behavior. This effect can be visualized by plotting the flow rate against the two-fluid interface displacement. The resultant hysteresis loop suggests 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 loop clearly shows that the EOF flow rate of micro-channel cannot approach the flow rate of displacing electrolyte upon completion of the displacement process.

Our findings suggest that the transport of analytes such as drugs, proteins, or other negatively charged ionic species of interest in an EOF microfluidic system requires careful considerations. 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 micro-channel and the reservoirs.

ASSOCIATED CONTENT

The Supporting Information is available free of charge on the ACS Publications website at DOI: XXXX.

Additional simulations investigating the effect of geometrical expansion at the reservoir junction; two figures illustrating the time evolutions of ion concentrations, electric field, zeta potential and electroosmotic flow velocity along the microchannel (PDF)

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Supporting Information

Electroosmotic Flow Hysteresis for Dissimilar Anionic Solutions

An Eng Lim, Chun Yee Lim & Yee Cheong Lam*

School of Mechanical and Aerospace Engineering, Nanyang Technological
University, 50 Nanyang Avenue, Singapore 639798

Corresponding Author

*Phone: +65 6790 6874. Fax: +65 6792 4062. E-mail: myclam@ntu.edu.sg.

Author Contributions

All authors contributed equally.

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Effect of Geometrical Expansion at the Reservoir Junction

We had performed axisymmetric simulations with 10x and 60x geometrical expansions (see Fig. S1) 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 S1. 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 is significantly affected at the reservoir junction (see Fig. S2), but the concentration adjustment in Phase 2 is not affected (see Fig. S3). Although the local electric field distribution at the reservoir junction differs with the addition of the reservoirs, the integral across the cross section (refer to Eq. 14 in the main text) remains the same for the preservation of current continuity, the reason for the negligible effect on the concentration adjustment in Phase 2.

The equilibrium concentration is dependent on the steady-state current ($I_{Steady-state}$) and electric field in the microchannel (E_L) (refer to Eq. 15 in the main text). In the experiments, the reservoirs are large as compared to the microchannel and contribute little electrical resistance to the system. Thus, $I_{Steady-state}$ and E_L are 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 Fig. S3). Therefore, there is negligible effect on the concentration adjustment in Phase 2 despite the significant effect on electric field due to the sharp change in geometry at the reservoir junction. As such, the geometrical expansion at the reservoir junction is not included in our numerical simulations for simplicity, as well as to minimize computation time.

Figure S2. Steady-state axial electric field when 0.5mM NaCl from cathode reservoir displaces 0.5mM Na₂SO₄ 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.

Figure S3. Steady-state Cl⁻ ion concentration when 0.5mM NaCl from cathode reservoir displaces 0.5mM Na₂SO₄ at (A) axis of symmetry and (B) channel wall for numerical simulations with no reservoirs, 10x and 60x geometrical expansions. Na⁺ and SO₄²⁻ ions are not shown. Dimensionless $x^* = x/L$, where x is the axial coordinate and L is the length of microchannel.

Time Evolutions of Ion Concentrations, Electric Field, Zeta Potential and Electroosmotic Flow Velocity along Microchannel

Figure S4. Simulated time evolutions of (A) ion concentrations, (B) electric field, (C) zeta potential and (D) electroosmotic flow velocity when 0.5mM Na_2SO_4 from cathode reservoir displaces 0.5mM NaCl in Phase 1 ($t = 0s$, $2s$ and $5s$) and Phase 2 ($t = 12s$, $15s$ and $30s$). Dimensionless $x^* = x/L$, where x is the axial coordinate and L is the length of microchannel.

Figure S5. Simulated time evolutions of (A) ion concentrations, (B) electric field, (C) zeta potential and (D) electroosmotic flow velocity when 0.5mM NaCl from cathode reservoir displaces 0.5mM Na₂SO₄ in Phase 1 ($t = 0s$, 2s and 5s) and Phase 2 ($t = 12s$, 15s and 30s). Dimensionless $x^* = x/L$, where x is the axial coordinate and L is the length of microchannel.