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Experimentally and theoretically observed native pH shifts in a nanochannel array

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Abstract

Lab-on-a-chip (LOC) technology provides a powerful platform for simultaneous separation, purification, and identification of low concentration multicomponent mixtures. As the characteristic dimension of LOC devices decreases down to the nanoscale, the possibility of containing an entire lab on a single chip is becoming a reality. This research examines one of the unique physical characteristics of nanochannels, in which native pH shifts occur. As a result of the electrical double layer taking up a significant portion of a 100 nm wide nanochannel, electroneutrality no longer exists in the channel causing a radial pH gradient. This work describes experimentally observed pH shifts as a function of ionic strength using the fluorescent pH indicator 5-(and-6)-carboxy SNARF®-1 and compares it to a model developed using Comsol Multiphysics. At low ionic strengths (~ 3 mM) the mean pH shift is approximately 1 pH unit whereas at high ionic strengths (~ 150 mM) the mean pH shift is reduced to 0.1 pH units. An independent analysis using fluorescein pH indicator is also presented supporting these findings. Two independent non-linear simulations coupling the Nernst-Planck equation describing transport in ionic solutions subjected to an electric field and Poisson's equation to describe the electric field as it relates to the charge distribution are solved using a finite element solver. In addition, the effects of chemical activities are considered in the simulations. The first numerical simulation is based on a surface ζ -potential which significantly underestimates the experimental results for most ionic strengths. A modified model assuming that SNARF and fluorescein molecules are able to diffuse into the hydrolyzed SiO₂ phase, and in the case of the SNARF molecule, able to bind to neutral regions of the SiO₂ phase agrees *quantitatively* with experimental results.

1 Introduction

Over the past decade, lab-on-a-chip (LOC) technology has produced simple devices that would potentially offer an efficient means for simultaneous separation and detection of important biological molecules.¹ Due to many advantages associated with the LOC technology, such as low reagent consumption, high-throughput, and low retention times, the research in micro/nanoscale technology has drastically evolved. Novel applications such as single molecule interactions,² DNA analysis,^{3–6} and preconcentration⁷ contribute to the recent advancements in nanofluidic research. However, also associated with nanoscale devices are problems such as fouling and detection that become more difficult to deal with than in their microfluidic counter-parts. Despite these drawbacks, nanofluidics offers

insights into new and novel science and will continue to evolve because different physical phenomena begin to emerge due to wall effects that now dominate the molecular environment.⁸ In addition, interfacial effects become increasingly more important at the nanoscale.⁹ Two such phenomena that occur as the transport limiting dimension is decreased to the molecular level are the electrical double layer (EDL) and the resulting ionic distributions.

The EDL theory dates back to the early 1900s when Gouy (1910) and Chapman (1913) described how charges on a solid surface influence the electrolyte ion distribution near that surface. The EDL consists of two layers (Fig. 1). The inner layer is the Stern layer where immobilized, solvated positive ions are attracted to the negatively charged wall. In the outer diffuse layer, assuming a negatively charged wall, there is an influx of positive counter ions which forms a space charge region compared to the bulk solution where electroneutrality exists. The potential between the Stern layer and diffuse layer is commonly known as the ζ -potential. The distance between the Stern layer and diffuse layer is the Debye Length, κ^{-1} , and represents the length of the EDL. As a result of the ion distribution, there exists a potential field, Ψ , across the EDL which is a function of the charge on the wall and subsequent ζ -potential, the electrolyte concentration, and the pH.

In microfluidics, the EDL and its effect on the electrolyte distribution can be ignored because it takes up only a small fraction of the microchannel and does not affect the bulk volume. However, at the nanoscale, the EDL can no longer be ignored because the dimensions of the channel are the same order of magnitude as those of the EDL. This can lead to ion exclusion and selectivity commonly known as concentration polarization.⁸ Various texts have used EDL theory to predict potential distributions near flat surfaces,^{10,11} near fixed spherical charges,^{11–13} or across narrow capillaries.¹¹ The ionic distribution of the electrolyte is predicted from the potential distribution using a Boltzmann relationship as described in the theory section of this paper. Important to note is the resulting hydronium ion distribution which leads to pH changes across the channel.

Various researchers have considered pH changes due to electrical potential differences caused by the presence of fixed charges in nanoscopic locations such as hollow polyelectrolyte surfaces,¹⁴ metal oxide surfaces¹⁵ and ion exchange resins.^{16,17} Sukhorukov *et al.* show theoretically and experimentally how the interiors of hollow polyelectrolyte shells have in excess of a 1 pH unit difference in the interior compared with the bulk solution outside the shell due to excess charges in both the external solution and interior of the shell.¹⁴ Further, electroneutrality is not satisfied, resulting in a pH difference between the interior and exterior of the shell which is inversely proportional to the ionic strength of solution.¹⁴ The pH changes at oxide interfaces, in particular SiO₂ surfaces, due to intrinsic buffer capacity, was shown in ion sensitive field effect transistors due to the charging behavior of electrolyte solutions in contact with metal hydroxides.¹⁵ In anion exchange resins, phosphate ions adsorbed to the resin and hydronium ions were excluded from the resin by a Donnan equilibrium resulting in a pH difference between the resin and bulk solution.¹⁶ Further, work done in our laboratory confirms that the resin phase pH shift was inversely proportional to buffer concentration using a classical Donnan equilibrium approach.¹⁷ The research presented here describes experimentally how observed pH shifts resulting from the aforementioned potential distributions in nanochannels vary as a function of ionic strength and addresses how the pH of the solution in the nanochannels can be controlled by ζ -potential manipulation.

Native pH shifts and the control or modulation of these pH shifts in nanochannels is an important aspect of nanofluidic research and could open the field to new and novel separation strategies. Researchers have already begun using separation strategies such as

electrokinetic transport in nanochannels or nanostructures to separate ions according to their valence^{18–20} or size.^{3,21} More recently, Xuan *et al.* have separated charged solutes on the basis of mobility ratio and valence using pressure driven-flow, electric field driven-flow, and a combination of the two in nanochannels.²²

The nanochannel array here includes an external electrode applied to the semiconductor silicon (Si) substrate surrounding the nanochannels but separated from the fluid by an insulating silicon dioxide (SiO₂) phase. Theoretically, pH modulation of solution could be accomplished by applying an external voltage perpendicular to the nanochannel array. Previous work using external electric fields has demonstrated that the ζ -potential can be manipulated to control the electro-osmotic flow in capillary zone electrophoresis.²³ This technique known as fluidic-FET, (analogous to field effect transistors in integrated circuits, where an electric current is manipulated by a perpendicular field generated by a third electrode or gate electrode), was also demonstrated more recently to control the magnitude and direction of electro-osmotic flow in microfluidic²⁴ as well as nanofluidic networks by applying an external electric field to control the ζ -potential on the channel wall. By manipulating the ζ -potential, one could also control the local pH of the solution in a nanochannel array. Therefore, pH modulation in nanochannels offers two potential separation applications, isoelectric focusing (IEF) without ampholytes and nanoelectrochromatography.

IEF separates amphoteric molecules according to isoelectric point, or point of zero net charge in a pH gradient. Historically, the pH gradient has been formed by synthetic carrier ampholytes which can be problematic when an additional purification step is required to remove the carrier ampholytes from the solute of interest.^{25,26} Fluidic-FET techniques at the nanoscale may enable pH gradients to form without the need for ampholytes and facilitate the use of IEF as a viable separation strategy at the nanoscale. Nanoelectrochromatography could be used to bind target proteins to chromatographic resins at their desired pH and then release the proteins by changing the pH *via* the gate voltage.

This paper attempts to address the native pH shift in nanochannels, which will be an important parameter in further experimentation where controlling the pH shift using a gate bias will be addressed. In order to monitor the pH shift using optical techniques in the visible and infrared (IR) range, Si IR waveguides²⁷ were fabricated into which a parallel array of nanochannels were integrated and sealed with an optically transparent Pyrex cover. This nanofluidic device enables the emission intensity from fluorescent molecules to be measured spectroscopically using a 488 nm Argon ion laser as an excitation source. In addition, multiple internal reflection Fourier transform infrared spectroscopy (MIR-FTIRS) is used to analytically probe IR vibrational modes of a pH indicator (*e.g.*, fluorescein) and measure the resultant pH shift. The two techniques are independently used to monitor and confirm the pH shift in nanochannels with pH sensitive dyes, 5-(and-6)-carboxySNARF®-1 and fluorescein, respectively.

Experimental results are verified with a numerical model showing the voltage profile, pH distribution, and mean pH value in the nanochannel. Solving for the pH distribution in three dimensions is ideally desired. However, to save computational time and avoid excessive finite element nodes, while capturing correct interfacial physics and chemistry, we limit ourselves to a two-dimensional model described in the next section. A two-dimensional analysis provides a basis for a quantitative comparison to experimentally observed results.

The pH distribution in the nanochannels without an external electric field is crucial to know in order to calculate the base pH before manipulating the ζ -potential and pH with an external electric field or gate bias. Here, the simulation of the pH distribution in a single nanochannel

without an external field will serve as the basis for future modeling work. Scanning electron microscope (SEM) images of the nanochannel array are shown in Fig. 2. Each nanochannel is approximately 100 nm wide and 400 nm deep. A 100 nm thick thermal SiO₂ phase functions as an insulator between the Si substrate and the nanochannels.

The model was developed using a top-view of a single nanochannel with a SiO₂ phase on each side of the nanochannel and two bulk reservoirs as shown in Fig. 3. This geometry was chosen because it best describes the transport-limiting dimension.

2 Theory

This paper investigates radial pH distributions across a nanochannel and mean pH shifts within a nanochannel array. A numerical simulation has been developed using Poisson's equation to describe the potential distribution. The potential distribution is coupled to a Nernst-Planck-based transport equation which is used to describe the ionic species distribution. The resulting non-linear partial differential equations are solved simultaneously using a commercial finite element solver (Comsol Inc., Palo Alto, CA, USA). Together, these two equations allow predictions of the mean pH in the nanochannels and the subsequent deviation from the bulk solution pH.

The potential distribution in the nanochannel is governed by Poisson's equation:²⁸

$$\nabla^2 \psi = - \frac{\rho_e}{\epsilon} \quad (1)$$

where ψ is the potential, ρ_e is the charge density, and ϵ is the dielectric permittivity of the medium, assumed to be constant. The charge density is a function of the molar concentrations of each analyte species, c_i , the characteristic charge of each species, z_i , and Faraday's constant, $F = 96485$ C/mol where

$$\rho_e = F \cdot \sum_i c_i \cdot z_i \quad (2)$$

Upon substitution of eqn (2) into eqn (1) we obtain a modified form of the Poisson-Boltzmann equation:¹⁰

$$\nabla^2 \psi = - \frac{F \cdot \sum_i c_i \cdot z_i}{\epsilon} \quad (3)$$

To describe the analyte distribution, consider a cross-section of a nanochannel with a fixed negative surface potential as shown in Fig. 1. Positive ions will be attracted towards the channel wall, and negative ions will be repelled from the wall. The equilibrium distribution of ions in the nanochannel is then governed by the Boltzmann Distribution:²⁸

$$c_i = c_i^b e^{\frac{-z_i \cdot F \cdot \psi}{R \cdot T}} \quad (4)$$

where c_i^b is the molar concentration of species i in the bulk buffer solution where the potential is assumed to be at ground, R is the universal gas constant [8.3145 J/(mol K)], and T is the temperature, taken to be 298 K.

Upon substitution of the Boltzmann Distribution into Poisson's Equation the non-linear Poisson-Boltzmann Equation is obtained:²⁸

$$\nabla^2 \psi = - \frac{F \bullet \sum_i c_i^b \bullet z_i e^{\frac{-z_i \bullet F \bullet \psi}{R \bullet T}}}{\epsilon} \quad (5)$$

For the linear case, where the Debye–Hückel approximation holds (i.e., $\psi \leq 20$ mV for a 1 : 1 electrolyte) eqn (5) reduces to:

$$\nabla^2 \psi = \kappa^2 \psi \quad (6)$$

where κ is known as the Debye–Hückel parameter and is defined as:

$$\kappa \equiv \sqrt{\frac{F^2 \bullet \sum_i z_i^2 \bullet c_i^b}{\epsilon \bullet R \bullet T}} \quad (7)$$

An analytical solution to the potential distribution for the linear case has been obtained for simple geometries.¹¹

In the case where the Debye–Hückel approximation is not valid we need to solve eqn (5) numerically. Here, however, instead of solving the Poisson-Boltzmann equation, which may not be general enough to describe the EDL overlap,²⁹ the modified Nernst-Planck equation written in terms of the chemical potential is used:²⁸

$$\nabla \bullet \left(-\frac{D_i}{R \bullet T} c_i \nabla \mu_i \right) = R_i \quad (8)$$

where μ_i is the chemical potential of species i , D_i is the diffusion coefficient of species i , and R_i is the rate of production of species i . The chemical potential for an electrostatic system is defined as:

$$\mu_i = \mu_i^0 + R \bullet T \bullet \ln(a_i) + z_i \bullet F \bullet \psi \quad (9)$$

where μ_i^0 is the chemical potential of a reference state which is not a function of position, and a_i is the activity of species i . The activity of species i is defined as:

$$a_i = \gamma_i \bullet c_i \quad (10)$$

where γ_i is the activity coefficient of species i . Upon substitution of the chemical potential and activity coefficient into eqn (8), the final form of the modified Nernst-Planck equation is obtained below and is equivalent to that obtained by Helfferich:³⁰

$$\nabla \bullet \left(-D_i \left[1 + \frac{c_i}{\gamma_i} \frac{\partial \gamma_i}{\partial c_i} \right] \nabla c_i - \frac{D_i \bullet z_i \bullet F}{R \bullet T} c_i \bullet \nabla \psi \right) = R_i \quad (11)$$

The modified Nernst-Planck equation contains a diffusive term in terms of the activity coefficient, an electrostatic term, and a reaction term. The activity coefficient is determined from the extended Debye-Hückel form²⁸ where the activity coefficient is a function of the ionic strength in terms of molality (I') where

$$I' = \frac{1}{2} \cdot \rho \cdot \sum_i z_i^2 \cdot c_i \quad (12)$$

and

$$\ln \gamma_i = \frac{-z_i^2 \cdot \alpha' \cdot \sqrt{I'}}{1 + B' \cdot a \cdot \sqrt{I'}} \quad (13)$$

ρ is the density of the solution, a is the mean diameter of ions or the Debye-Hückel effective radii,³¹ and α' and B' are Debye-Hückel parameters, defined as:

$$\alpha' = \frac{F^2 \cdot e \cdot \sqrt{2}}{8 \cdot \pi \cdot (\epsilon \cdot R \cdot T)^{3/2}} \quad (14)$$

$$B' = \frac{F}{\sqrt{\epsilon \cdot R \cdot T/2}} \quad (15)$$

The Debye-Hückel effective radii were obtained from Garrels and Christ³¹ and are shown in Table 1.

The diffusion coefficient of each species is related to the absolute mobility (u_i) by the Nernst-Einstein relation²⁸ where:

$$D_i = u_i \cdot R \cdot T. \quad (16)$$

Eqn (16) is rearranged for the absolute mobility and substituted into eqn (11) to give the final modified form of the Nernst-Planck equation used in the simulation:

$$\nabla \cdot \left(-D_i \left[1 + \frac{c_i}{\gamma_i} \frac{\partial \gamma_i}{\partial c_i} \right] \nabla c_i - u_i \cdot z_i \cdot F \cdot c_i \cdot \nabla \psi \right) = R_i \quad (17)$$

Eqn (1) and eqn (17) are coupled simultaneously through the potential and directly solved to determine the potential profile, charge distribution, and pH profile. Therefore, the simulation automatically takes into account how the ionic distribution affects the potential distribution, and subsequent ζ -potential. At low ionic strength, the ζ -potential will be high and the electrical double layer will protrude further into the nanochannel region and cause a radial pH gradient.

2.1 Ionic reactions

To accurately predict the pH distribution in the nanochannel, buffering reactions must be considered. The bulk pH of the phosphate buffered solutions used in this work is pH 7.4. Therefore, only the following ionic reactions need to be accounted for:

The other phosphate reactions that might occur are ignored because the bulk pH is 7.4 and only trace amounts of other phosphate species may be present. The equilibrium expressions for ion concentrations throughout the system can then be developed as follows:

$$\frac{c_{\text{HPO}_4^{2-}} \cdot c_{\text{H}_3\text{O}^+}}{c_{\text{H}_2\text{PO}_4^{1-}}} = K_{\text{Phos}} = \frac{k_{f,\text{Phos}}}{k_{r,\text{Phos}}} \quad (22)$$

$$c_{\text{H}_3\text{O}^+} \cdot c_{\text{OH}^-} = K_{\text{W}} = \frac{k_{f,\text{Water}}}{k_{r,\text{Water}}} \quad (23)$$

$$\frac{c_{\text{SNARF}^{2-}} \cdot c_{\text{H}_3\text{O}^+}}{c_{\text{SNARF}^{1-}}} = K_{\text{SNARF}} = \frac{k_{f,\text{SNARF}}}{k_{r,\text{SNARF}}} \quad (24)$$

$$\frac{c_{\text{Fluorescein}^{2-}} \cdot c_{\text{H}_3\text{O}^+}}{c_{\text{Fluorescein}^{1-}}} = K_{\text{Fluorescein}} = \frac{k_{f,\text{Fluorescein}}}{k_{r,\text{Fluorescein}}} \quad (25)$$

Ionic equilibrium reactions are nearly instantaneous; therefore, forward and reverse rate constants are used to solve the equilibrium relations throughout the nanochannel. Forward and reverse rate constants ($k_{f,i}$, $k_{r,i}$), diffusion coefficients, absolute mobilities, charge, mean diameter of ions, and initial concentrations used in the model are summarized in Table 1.

2.2 ζ -potential model development

The Nernst-Planck without electroneutrality and Poisson's equation application modes are built directly into Comsol Multiphysics V3.4 and are used to simulate the pH distribution in the nanochannels. In the ζ -potential based simulation, the ζ -potential and concentration of

each species, which follows a Boltzmann distribution, were specified as boundary conditions to simultaneously solve eqn (1) and (17). The ζ -potential is approximated for each ionic strength using experimental data in the literature for 0.7 μm silica particles in solutions of potassium chloride at pH 7.³² It is assumed that the ζ -potential is proportional to the logarithm of the molar counterion concentration.³³ The approximated ζ -potentials for each salt concentration are shown in Table 2. This ζ -potential is used as a boundary condition to solve the simulation. All of the boundaries labeled B1–B12 are shown in Fig. 3 and their associated boundary conditions for the ζ -potential based simulation are summarized in Table 3. Note that NA in Table 3 stands for boundaries that are not applicable in each simulation. The ζ -potential model, however, could not predict the pH shift seen experimentally. Further, the ζ -potential values determined from the model that could explain the pH shift experimentally were upwards of -250 mV and seemed unreasonably high for the ionic strength solutions tested here.

The failure of the ζ -potential based model to accurately describe the experimental results required a different explanation. As a result, a modified model was developed based on the observation that thermally grown dry SiO_2 exhibits characteristics (*e.g.*, increased porosity and irreversible adsorption of charged molecules²⁷) associated with lesser quality SiO_2 upon prolonged exposure to electrolyte solutions. This modified model presents one possible explanation for the observed experimental results and is described below.

2.3 Modified model development

In the modified model, there are two phases: a fully hydrolyzed SiO_2 phase on each side of the nanochannel (gray region in Fig. 3) that allows diffusion and adsorption of fluorescent dye molecules and a second bulk solution phase containing the actual nanochannel and two bulk reservoirs (white region in Fig. 3). Previously in the ζ -potential based model, it was assumed that the active surface silanol groups were only on the boundary between the SiO_2 phase and the nanochannel region and that diffusion into the SiO_2 phase was not possible. Here, in the modified model, it is assumed that the active silanol groups are dispersed throughout the SiO_2 phase and that this phase has a porosity or accessible volume where ions are allowed to diffuse into and out of this region. Charged dye molecules irreversibly adsorb to the surface of the SiO_2 phase²⁷ and here we are extrapolating this finding and relating it to a porosity in the SiO_2 phase. For the purposes here, a volumetric concentration of 100 mM active silanol groups is associated with the SiO_2 phase which is about half the ionic resin capacity associated with a protein ion exchange resin.¹⁷ The accessible volume of the SiO_2 phase could not be determined experimentally and, therefore, for analysis purposes an accessible volume coefficient of 0.5 is used. The accessible volume of the SiO_2 phase is accounted for in the model by coupling two Nernst-Planck physics, one for each phase, in Comsol and applying a concentration discontinuity and a flux concentration continuity condition at the boundary between the two phases of the nanochannel such that:

$$S_i = k_{AV} c_i \quad (26)$$

where c_i is the concentration of species i inside the nanochannel as described previously, k_{AV} is the accessible volume coefficient, and s_i is the concentration of species i in the SiO_2 phase. The potential is assumed to be continuous across this boundary.

In order to solve the PDEs (eqn (1) and (17)), the concentration of each species and a potential need to be referenced. We assumed that the potential in the bulk reservoir on the outer boundary is identically zero, and the concentration of each species assumes their bulk concentration value. All of the boundaries, labeled B1–B12, are shown in Fig. 3, and their associated boundary conditions are summarized in Table 4. The resulting distribution of ions

is equivalent to a Donnan equilibrium.^{12,30,34} In addition, co-ions are rarely completely excluded,³⁴ but rather only partially excluded from and counter-ions accumulate in the negatively charged SiO₂ phase.

2.4 Model assumptions and corrections

Experimentally, 5-(and-6)-carboxy SNARF®-1 and fluorescein are used as the reporter ion to determine the pH inside the nanochannels. The pH that the SNARF or fluorescein molecule reports to the detector in the experimental setup, which we will call $pH_{Reporter}$, however, will be different from the actual pH in the nanochannel. For instance, the SNARF and fluorescein molecule both carry a -1 or a -2 charge and will tend to concentrate in the center of the nanochannel because of the negatively charged wall;^{19,20} this will tend to decrease the magnitude of the effective pH shift that the SNARF or fluorescein molecule reports. Thus, if the SNARF or fluorescein molecule reports a pH shift of 1 unit to the detector, the actual pH shift inside the nanochannel will be higher due to the repulsion of the reporter ion away from the negatively charged wall where the shift is greater. As a result, $pH_{Reporter}$ will be biased toward the pH in the center of the channel where the pH shift is minimized because that is where the reporter ion will be most concentrated. Therefore, in order to determine the unbiased, actual pH in the nanochannel, a weighted function based on the reporter ion distribution is used to take this into account.

For simplicity, only the SNARF molecule will be described; however, the same analysis and technique applies for the fluorescein molecule. The total SNARF concentration, c_{SNARF}^T , is defined as:

$$c_{SNARF}^T = c_{SNARF}^{1-} + c_{SNARF}^{2-}. \quad (27)$$

In order to relate what the SNARF molecule is actually reporting to the detector to the actual pH inside the nanochannel, the location of the SNARF molecule and the local pH at that position need to be taken into account. This can be done by either averaging over the hydronium concentration or by averaging over the pH in the simulation. The latter method is chosen because the SNARF molecule reports the local pH to the detector rather than the local hydronium concentration. The following relation,

$$pH_{Model_SNARF} = \frac{\int_A pH \cdot c_{SNARF}^T dA}{\int_A c_{SNARF}^T dA} \quad (28)$$

where A is the cross sectional area of the channel, is used to take into account where the SNARF molecule is located in the channel and the local pH at that position. Therefore, pH_{Model_SNARF} describes the biased pH inside the nanochannel as a function of the SNARF molecule's position. pH_{Model_SNARF} is determined from the simulation and is different from what the SNARF molecule reports to the detector, $pH_{Reporter}$, for the reasons previously described. Also, from the simulation, the mean pH based on the hydronium concentration is calculated by integrating the pH of the hydronium ion distribution, $pH_{Model_Hydronium}$, over the entire domain and is independent of the location of the SNARF molecule. pH_{Model_SNARF} and $pH_{Model_Hydronium}$ are used as part of a weighting function to determine the unbiased, actual pH, pH , in the nanochannels such that

$$pH = pH_{Reporter} \cdot \frac{pH_{Model_Hydronium}}{pH_{Model_SNARF}} \quad (29)$$

This value of the pH gives us the actual, mean pH in the nanochannels corrected for the position of the reporter ion in the channel. Finally, the pH shift, pH_{Shift} , is calculated by:

$$pH_{Shift} = pH_{Bulk} - pH \quad (30)$$

where pH_{Bulk} is equal to 7.4. By simultaneously solving the Nernst-Planck equation(s), eqn (17), and Poisson's equation, eqn (1), in Comsol, and taking into account the distribution of the reporter ion in the nanochannel, the potential, charge distribution, pH profiles, and pH shift can all be obtained as a function of ionic strength.

3 Experimental

3.1 Fabrication

An integrated nanofluidic device is based on Si technology. The fabrication steps are described in detail in a previous publication.²⁷ Briefly, double-side-polished Si(100) is diced by 1 cm width × 5 cm length and used as a platform to prevent the scattering and loss of IR beam intensity during multiple internal reflection in the MIR-FTIRS analysis. To manipulate the gate for surface charge modulation of nanochannels a 3 mm-wide boron doped gate region is defined perpendicularly to the channel direction at the center of the wafer. The dopant diffusion was carried out for 60 min at 1050 °C in an O₂/N₂ environment, which results in the formation of a diffusion layer with a depth of 1–1.2 μm and a dopant level on the order of 10²⁰ cm⁻³. The boron-doped gate region was defined for the future purpose of manipulating local pH shifts by applying an external potential to the channel walls. An array of nanochannels is fabricated along the direction of IR propagation using interferometric lithography³⁵ and plasma etching. Fig. 2(a) shows a cross-sectional SEM image of the nanochannels after the etching process. Each channel is approximately 200 nm wide and 450 nm deep immediately following the etching. The nanochannel array occupies a total area of 3 mm wide × 16 mm long, which contains up to 8000 nanochannels. A thermal SiO₂ layer is grown up to 100 nm, reducing the channel width to 100 nm and the channel depth to 400 nm. Fig. 2(b) shows the nanochannel array covered with a thermally grown SiO₂ layer. This layer is used as an electrically insulating layer between the Si channel walls and the fluid. The nanochannels were sealed with a Pyrex slip cover by anodic bonding^{36,37} as shown in Fig. 2(c) at 380 °C and 1 kV was applied between the substrate and Pyrex cover. This process is conducted at 380 °C by pressing the Pyrex cover and the nanochannel substrate together, while -1 kV is applied to the Pyrex cover, and the substrate is grounded. As a final step, the longitudinal ends of the substrate are beveled at 45° and polished to use this device as an analytical tool for MIR-FTIRS.

3.2 Materials

The structure of the pH sensitive dye 5-(and-6)-carboxy SNARF®-1 (Invitrogen, Carlsbad, CA, USA), is shown in Fig. 4(a). At low pH the SNARF molecule takes on its protonated form and at high pH the deprotonated form. Stock dye solutions of 1 mg/mL were prepared using nanopure water from a Barnstead Thermolyne Nanopure Infinity UV/UF system (Dubuque, IA, USA). Phosphoric acid, sulfuric acid, hydrofluoric acid, potassium hydroxide, fluorescein, and potassium chloride were purchased from Sigma-Aldrich (St. Louis, MO, USA). All solutions were filtered through a 50 kDa centrifugal filter unit

(UFV5BQK25, Millipore, Billerica, MA) prior to injection into the nanochannel array to prevent fouling of the nanochannels.

In the MIR-FTIR analysis, fluorescein was the fluorescent reporter molecule used, and Fig. 4(b) shows how the molecular structure of fluorescein progressively varies as a function of pH from low pH to high pH. In addition, deuterated water (D_2O , 99.9 atom % D, Sigma-Aldrich) instead of H_2O was used to avoid overlapping between the vibrational modes of H_2O ($3000\text{--}3400\text{ cm}^{-1}$ and 1640 cm^{-1}) and those of fluorescein ($3000\text{--}2800\text{ cm}^{-1}$ and $1600\text{--}1580\text{ cm}^{-1}$). The stretching vibrational modes of D_2O and HDO were $2800\text{--}2200\text{ cm}^{-1}$. The scissor modes of D_2O and HDO were assigned to 1210 and 1450 cm^{-1} , respectively.^{38,39} Various buffers were used to monitor IR spectra of fluorescein in different pH buffer solutions ranging from pH 3 to 8. Chloroacetic acid ($pK_a = 2.83$) buffer for pH 3, acetate buffer ($pK_a = 4.76$) for pH 4–5, pyridine buffer ($pK_a = 5.23$) for pH 6, phosphate buffer ($pK_a 7.21$) for pH 7, and tris-glycine buffer ($pK_a = 8.02$) for pH 8 were used, respectively. The pH for each buffer was adjusted with HCl and NaOH, and the ionic strength for all buffer solution was approximately 1–2 mM.

3.3 Experimental setup

SNARF solutions were excited with a 2 W 488 nm argon ion laser (Coherent Innova-90, Evergreen Laser Corporation, Durham, CT, USA). The complete experimental setup is shown in Fig. 5(a). The laser light was transmitted through a Pyrex cover piece anodically bonded to the nanochip, and the evanescent wave excites the solution in the nanochannels. The measured emission spectrum was collected by an optical fiber mounted vertically above the nanochannel array and filtered through a HQ500 long-pass filter (Chroma Technology Corp, Rockingham, VT, USA) to remove the excitation source. The filtered emission spectrum was then detected with an EPP2000-CXR_s UV-VIS spectrophotometer (StellarNet Inc, Tampa Bay, FL, USA). Spectra Wiz software (StellarNet Inc, Tampa Bay, FL, USA) measured the ratio of emission intensity at 580 nm and 640 nm, respectively. This ratio was converted to a pH using eqn (31) in Section 4.1. The pH dependence of the emission of carboxy SNARF@-1 was first measured in bulk solutions using the experimental setup described above. The SNARF molecule was diluted to 0.01 mg/mL in different pH solutions ranging from pH 5–10 in 0.75 mM phosphoric acid (H_3PO_4) and 0.75 mM potassium chloride (KCl) to produce pH dependent calibration curves for each experiment. All solutions were titrated with 1 mM potassium hydroxide (KOH) to the desired pH. A calibration curve was produced by depositing 1 μ L of each SNARF solution into a divot on a glass slide, covering the divot with a Pyrex glass piece, and recording the emission spectrum.

SNARF solutions were allowed to flow into nanochannels with an anodically bonded top window. Nanochannels were easily filled by capillary action (see Fig. 6). The SNARF in the nanochannels was excited and the ratio of 580 nm to 640 nm was recorded by the spectrophotometer. The mean pH in the nanochannel array was then determined from the calibration curve as a function of ionic strength.

A conceptual diagram of the experimental setup for the MIR-FTIRS analysis is shown in Fig. 5(b). A nanofluidic IR waveguide was mounted on top of a metal housing that encases IR optics. Reflective IR optics direct the IR beam onto one of the beveled edges. The IR beam that enters the Si MIR crystal makes approximately 35 top reflections before the beam exits the opposite end. The IR signal leaving the second beveled edge was collected by a HgCdTe detector. Due to the multiple reflections, the Si MIR crystal was opaque to IR below 1500 cm^{-1} . To monitor the pH shift, a buffered solution was injected into the nanochannels, the system was equilibrated for approximately 20 minutes, and the IR background spectrum was recorded. The channels were then cleaned in DI water and dried.

A buffered solution of fluorescein dye molecules in various pH solutions was injected into one of the two solution wells to fill the nanochannels by capillary action, and a series of sample IR spectra were taken to monitor the characteristic vibrational modes of fluorescein dye molecules that were sensitive to the pH shift. In the nanochannel system, the pH shift was determined by filling the array with 0.5 mg/mL fluorescein in pH 7 phosphate buffer (total concentration of 0.47 mM) and calculating the intensity ratio of 1580 to 1600 cm^{-1} and converting this ratio to a pH. The conversion step is described in Section 4.5. Potassium chloride was added to change the ionic strength. A total of three ionic strengths ~3, 5, and 300 mM were analyzed.

4 Results and discussion

4.1 Ratiometric analysis

5-(and-6)-carboxy SNARF®-1 has multiple charge states characterized by several ionizable functional groups. Fig. 4(a) shows two relevant charge states, protonated and deprotonated forms for pH values in the pH 5–10 range. Initially, SNARF solutions are excited externally from the nanochannels in a Pyrex covered divot as described in the previous section to better understand the pH emission behavior. Fig. 7 shows five emission spectra at five pH values at the same ionic strength used in the calibration data. There is clearly an isosbestic point at approximately 610 nm. The isosbestic point indicates the formation of two distinct species at high and low pH.⁴⁰

This data reveals significant pH-dependent changes in the SNARF's emission spectrum. At high pH (pH 9.0) only a single peak is observed at ~640 nm, whereas at low pH (pH 6.0) a different single peak is observed at ~580 nm. At intermediate pH values, two peaks, at both 640 nm and 580 nm, are observed, respectively. The pH is determined from the ratio ($R = I_{580\text{nm}}/I_{640\text{nm}}$) of these two peaks, *i.e.* ratiometric analysis.

Quantitatively, the mean pH of the solution can be determined from the following modified Henderson-Hasselbalch relationship:⁴¹

$$pH_{\text{Reporter}} = pK_a + C \log \left(\frac{R - R_{\min}}{R_{\max} - R} \right) + \log \left(\frac{I(A)}{I(B)} \right) \quad (31)$$

where $R_{\min}$ and $R_{\max}$ are the minimum and maximum limiting values of R at low and high pH in Fig. 8. $I(A)$ and $I(B)$ are the emission intensities measured at 640 nm for pH 5 (acidic) and pH 10 (basic) values. The pK_a is the intercept in the range pH 7–8 and C is the slope of

the curve in the range pH 7–8 when $pH_{\text{Reporter}} - \log \left(\frac{I(A)}{I(B)} \right)$ is plotted versus $\log \left(\frac{R - R_{\min}}{R_{\max} - R} \right)$. These parameter values are shown in Table 5 and are close to values obtained in the literature.^{40,42} The calibration curve for bulk pH solutions of ~3 mM ionic strength is shown in Fig. 8.

The SNARF solution in the nanochannels is excited and the emission spectrum and R is recorded. The experimental pH of the SNARF solution, pH_{Reporter} , is then calculated by substituting R back into eqn (31) and using the parameters from the calibration curve.

4.2 ζ -potential based results

An experimental comparison to the ζ -potential model is shown in Fig. 9. For most ionic strength solutions, the experimentally seen pH shift is much greater than the pH shift predicted by the model. The simulation predicts a maximum pH shift of approximately 0.22

pH units at low ionic strengths whereas a pH shift of approximately 0.78 pH units is observed experimentally using a spectrophotometer and a pH shift of 0.85 pH units is seen after correcting the raw experimental data using eqn (27–30). Therefore, the ζ -potential based simulations with ζ -potentials determined from the literature^{32,33} could not explain the pH shift seen experimentally. This may be due to possible errors in calibration data or to the exclusion of a porous hydrolyzed SiO₂ phase that contains a fixed charged density. The calibration data and subsequent ratiometric analysis may not correlate well with the ratiometric data seen in the nanochannels. For example, if the ratiometric analysis in a chip yielded slightly different results than ratiometric analysis in a Pyrex covered divot, then calibration data could be incorrect. This hypothesis could easily be tested in a fabricated chip that included both nanochannels and microchannels. The microchannel emission spectrum would then serve as the calibration data instead of an external source used here. The next generation of chips will include both microchannel and nanochannel features to more accurately generate the calibration curve.

In addition, the ζ -potential model does not include a fully hydrolyzed SiO₂ phase which may be necessary to account for the experimental deviation. Thus, a hypothesized system that allows the transport of ions between the channel electrolyte and a porous phase that includes a charge density due to active silanol groups in the porous SiO₂ phase gives better agreement with the experimental results. Evidence in the literature supports the hypothesis of a porous SiO₂ phase.^{15,43} The simulation results for the modified channel are discussed below.

4.3 Modified simulation results

The predicted potential distribution across the nanochannel is shown in Fig. 10(a) and demonstrates how the potential starts at a highly negative value, stays approximately constant in the SiO₂ phase, and drops towards 0 potential quickly as it approaches the center of the channel at $x = 0$. The Debye-Length or EDL thickness is given from the inverse of eqn (7) and for ionic strengths ranging from ~3 mM to 150 mM is 7.8 nm to 1.1 nm, so the protrusion of the EDL into the nanochannel is very small for these particular ionic strengths, and it is not surprising to see the potential drop off at such a fast rate.

A trend can be seen from the potential profile [Fig. 10(a)] where the absolute value of the ζ -potential increases with decreasing ionic strength. If the ζ -potential is approximately 1 nm from the inside boundary separating the SiO₂ phase from the electrolyte phase, the ζ -potential ranges from -140 mV at low ionic strength to -3.5 mV at high ionic strength. At low ionic strength, the reported ζ -potential from the model seems rather high for a SiO₂ system, but there have been reports of ζ -potentials as high as -150 mV in silica particles at low KCl concentrations and high pH values.³² ζ -potentials of approximately -100 mV have been reported in silica capillaries at concentrations of 1 mM KNO₃.⁴⁴ The ionic distributions and pH follow a similar pattern where the pH is low inside the SiO₂ phase and quickly drops off to the bulk pH value in the center of the channel. An example of the pH distribution is shown in Fig. 10(b–c). In the SiO₂ region the pH is approximately 6 and in the bulk reservoir the pH is approximately 7.4. The mean pH in the nanochannel is then calculated as described in the previous section.

4.4 Experimental comparison

A comparison between the modified simulation and experimental data is shown in Fig. 11. Note that the experimental data has been corrected to account for the case where the SNARF molecule is located in the nanochannel and the local pH in the nanochannel using eqn (27–30) in the simulation. The raw experimental data from the spectrophotometer is shown in Fig. 9 with no such correction. The experimental results indicated a slightly higher pH shift

than the model predicted. To alleviate this discrepancy, SNARF binding is included in the modified simulation to explain the deviation between the simulation and the experimental results. Although a Donnan equilibrium exists between the nanochannel region and the SiO₂ phase where SNARF is partially excluded, a small amount of SNARF penetrates the SiO₂ phase. There is no direct evidence that SNARF binds to the SiO₂ phase, but this provides one alternative explanation for the discrepancy between the simulation and experimental results which is explained further below.

In the -1 charge state the SNARF molecule has a positive electrostatic region and a negative electrostatic region as shown in Fig. 4(a). These charged regions may have the ability to bind to neutral surfaces of the porous SiO₂ phase. A Langmuir isotherm¹¹ is used to model possible SNARF binding:

$$\theta = \frac{K \bullet c_{\text{SNARF}^{1-}}^B}{K \bullet c_{\text{SNARF}^{1-}}^B + 1} \quad (32)$$

where K is the adsorption isotherm constant and θ is the fraction of surface sites occupied. K is an adjustable parameter used to fit the experimental data to the model. The adjustable parameter that best fit the experimental data was 0.05. This parameter was not found by a least-squares fit, but rather a value that reasonably fits the experimental data. There were two reasons for not using a least-squares fit approach (1) the simulation software did not readily include this type of statistical analysis and (2) the parameter needs to be measured independently through an external experiment and not estimated as a multi-parameter fit. As a result, the authors are simply stating a possible hypothesis to the aforementioned discrepancy between the model and the experimental results. The K value resulted in a small amount of SNARF binding, less than 1% of the total number of charged sites in the SiO₂ phase which the authors concluded is a reasonable physical assumption.

A comparison between the model and the experimental data with SNARF binding is also shown in Fig. 11. Again the curve in Fig. 11 has been corrected to account for the SNARF location as described previously. Further, there is literature that confirms that charged molecules, in particular, Rhodamine B may adsorb to SiO₂ surfaces.^{18,27} Rhodamine B [Fig. (4c)] at pH 8.3 is a neutral molecule with a similar ring structure to the SNARF molecule that consists of both an electrostatic positive region and an electrostatic negative region which supports the assumption that SNARF adsorption is entirely possible.

4.5 Independent MIR-FTIR analysis

Fluorescein is a fluorescent dye molecule^{45,46} with a high quantum yield. The absorption and emission wavelength of fluorescein are 494 and 521 nm, respectively. The dye molecule has also been used as a pH indicator that shows a variation in quantum yield in different pH buffer solutions.^{45,47} This responsiveness is further enhanced because the chemical structure of fluorescein molecules strongly depends on the pH as shown in Fig. 4(b). Thus, the change in chemical structure and the corresponding change in IR vibrational modes in MIR-FTIRS can be used to probe the pH shift. The pK_a values of fluorescein that demarcate the valence states of cation, neutral, anion, and dianion, are 2.08, 4.31, and 6.43 [Fig. 4(b)], respectively.

Fig. 12 shows representative IR spectra of fluorescein in pH 4 and pH 8 buffer solutions. Although various skeletal vibrational modes of the xanthene moiety of fluorescein appear in the range, 1200 to 1800 cm⁻¹,⁴⁶ the vibrational modes below 1500 cm⁻¹ are not observable with Si nanofluidic MIR waveguides. However, the MIR-FTIR analysis shows that asymmetric stretching vibration of COO⁻ at 1580–1585 cm⁻¹ and xanthene skeletal C–C

stretch containing a conjugated carboxyl band at 1596–1600 cm^{-1} yield strong intensities.⁴⁶ These two vibrational modes are used to probe the pH shift in nanochannels. At pH 4 (solid line in Fig. 12), the asymmetric stretch of COO^- and xanthene skeletal C–C stretch display pronounced IR absorption. In contrast, at pH 8 (dashed line in Fig. 12), the absorption intensity of the xanthene skeletal C–C stretch decreases, whereas the asymmetric COO^- stretching vibration is slightly shifted to 1580 cm^{-1} . These changes are due to protonation and deprotonation of the carboxyl group and the OH^- group of xanthene ring according to different pH values of buffer solutions. Fluorescein at pH 4 is in the neutral state [Fig. 4(b)]. However, as the pH increases, fluorescein becomes a dianion where the carboxyl group and the OH^- group of the xanthene group are deprotonated. Thus, the intensity ratio of 1580 to 1600 cm^{-1} absorption peaks increases as pH increases from pH 3 to 8. In Fig. 13, the intensity ratio of 1580 to 1600 cm^{-1} peaks as a function of pH from 3 to 8 is shown. Note that the observed intensity ratio is less reliable below pH = 3. A linear fit to the experimental data from pH 4 to 8 is used as a calibration curve that relates IR intensity to a pH shift in the nanochannels.

The pH shift of the fluorescein in the MIR-FTIR analysis is then calculated from the raw data using techniques equivalent to those described by the SNARF non-binding model. The fluorescein molecule is negatively charged at pH 7 and, unlike the SNARF molecule, an electrostatic positive region does not exist on fluorescein so binding is not likely in the fluorescein case. The results are shown in Fig. 11 and again the magnitude of the pH shift decreases with increasing salt concentrations. The fluorescein molecule shows a pH shift of 1.22 pH units at low ionic strength and a pH shift of 0.03 pH units at high ionic strengths.

5 Conclusions

We have experimentally measured a native pH shift of electrolyte solutions of varying ionic strengths as they enter an array of nanochannels. Experimental pH shifts using the fluorescent pH indicators SNARF and fluorescein were compared to and corrected for by a numerical simulation resulting in an unbiased pH distribution across the nanochannel. In a simulation based on having a fixed ζ -potential at a nonporous surface, the model correctly predicted the trend seen experimentally; however, the experimental results were much higher than the model predictions over most of the ionic strengths evaluated. A modified model with a fully hydrolyzed porous SiO_2 phase also predicted the trend seen experimentally and was slightly higher than the model predictions. In comparison, when SNARF was allowed to bind to the interior of the porous SiO_2 phase, the simulation agreed *quantitatively* with experimental results. This suggests that charged molecules can enter and adsorb to the interior of the SiO_2 phase. Future work will attempt to test this hypothesis and validate the existence of a porous SiO_2 phase.

A pH shift of approximately 1 pH unit was seen at low ionic strengths (~3 mM). Even lower ionic strengths could have been studied; however, the tradeoff was lower buffering capacity if the phosphate concentration was decreased, and a reduced fluorescent signal if the SNARF or fluorescein concentration was decreased.

A modified model using fluorescein also accurately predicted the trend seen experimentally; also, the experimental results agreed with the model predictions for most data points analyzed. There is a discrepancy between the model and the data at low ionic strength solutions that has not been identified. However, experiment and theory confirm that a rather large pH shift occurs in nanochannel arrays at low ionic strengths and that this shift declines monotonically in higher ionic strength solutions.

Future work will seek to control the pH shift by applying multiple external gate voltages to the nanochannel array in a manner-analogous to a fluidic-FET. External voltages should allow local control of the ζ -potential, the silanol charge concentration, and thus the local pH. Multiple electrodes with different external voltages applied to each electrode may allow the formation of a stable, adjustable pH gradient where IEF separations could be achieved without the need for synthetic carrier ampholytes.

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Fig. 1. Structure of the EDL and the potential distribution as a function of distance from the channel wall. In the Stern layer, immobilized solvated positive ions are attracted to the negative surface charge from the dissociated silanol groups. In the diffuse layer, which is similar to the bulk except electroneutrality does not exist, there is an influx of positive solvated ions compared to negative solvated ions. Together, the Stern layer and diffuse layer make up the EDL which has a length defined by the Debye length, k^{-1} , and also a potential difference, ψ , across the region.

Fig. 2.

Cross-sectional SEM images of nanochannels. (a) Cross-sectional images of nanochannels after plasma etching. The Si substrate is demarcated by the arrows. (b) Cross-sectional images after thermal oxidation showing the 100 nm thick SiO₂ phase on sidewalls and bottom of the nanochannel. (c) Cross-sectional images after anodically bonding Pyrex coverslip to nanochannel array. The Pyrex coverslip is shown by the region above the dotted line and the Si/SiO₂ region below the dotted line.

Fig. 3.

Schematic of domain solved using Comsol v3.4. The domain includes two bulk reservoirs where the concentrations of species are equal to their bulk value, the nanochannel region, and a phase of thermal SiO₂ on each side of the nanochannel. B1–B12 represents the boundaries. Each SiO₂ phase and the nanochannel region have a thickness of 100 nm.

Fig. 4.

(a) Chemical structure of protonated and deprotonated 5-(and-6)-carboxy SNARF®-1 drawn in ChemDraw Ultra 10.0. Note: In the protonated form there is an electrostatic positive region and an electrostatic negative region. (b) Chemical structures of fluorescein based on different pH. Fluorescein is a cation at $\text{pH} < 2.08$, neutral at $2.08 < \text{pH} < 4.31$, an anion at $4.31 < \text{pH} < 6.43$, and a dianion at $\text{pH} > 6.43$. (c) Chemical structure of Rhodamine B which will be discussed in the Results section. Note: The similarities between the Rhodamine B and SNARF molecule.

Fig. 5.

(a) Experimental setup. An argon ion laser transmits a 488 nm beam into the nanofluidic device. SNARF solution in the nanochannel array is excited and the emission spectrum is collected by an optical fiber coupled to a UV/VIS spectrophotometer. (b) Experimental setup to monitor pH shift in nanochannels with fluorescein, using MIR-FTIRS.

Fig. 6.

Nanochannel array filling by capillary action. At $t = 0$ s, the nanochannel is being filled with buffered solution. At $t = 15$ s, the fluid has traveled approximately half way through the nanochannel array and at $t = 30$ s, the fluid has completely filled the nanochannel array. Note: The boron gate electrode on the top-center part of the chip which could be used for pH modulation.

Fig. 7.

An example of fluorescence emission spectrum of 5-(and-6)-carboxy SNARF®-1 measured in solutions of various pH used for the calibration curve in Fig. 9. Based on the intensity ratio (R) of 580 nm to 640 nm, the mean pH can be determined from eqn (31). An isosbestic point is observed at approximately 610 nm indicating the formation of two different species above and below 610 nm.

Fig. 8.

Calibration curve of the pH dependence of 5-(and-6)-carboxy SNARF®-1. The ratio (R) of fluorescence intensities at 580 nm and 640 nm was measured in a divot in a glass slide covered with a Pyrex coverslip for different pH values at the same ionic strength. The calibration curve was then used to determine the pH in the nanochannels.

Fig. 9.

A comparison between experimentally observed pH shift and that predicted by a ζ -potential based model. The (■) data points are the raw experimental data obtained indirectly from the spectrophotometer and the (●) data points are the experimental data that have been corrected to account for where the SNARF molecule is located in the channel and the local pH at that position. The model undershoots the experimental data by approximately a factor of two to four for most ionic strength solutions.

Fig. 10.

(a) Cross-sectional plot of the potential profile across the nanochannel. $x = 0$ is the center of the channel. As the ionic strength decreases, the potential increases in the SiO_2 phase and eventually decays to 0 at the center of the nanochannel. The overshoot that is seen is most likely due to numerical noise in the simulation. (b) Numerical simulation of the pH profile with an ionic strength of ~3 mM. The pH profile strongly shows the EDL in the nanochannel. In the SiO_2 phase the pH is low because hydronium ions are attracted to the active silanol groups. As you move away from the SiO_2 phase the pH rapidly drops off to its bulk value of pH 7.4. (c) Cross-sectional plot of the pH profile across the nanochannel. As the ionic strength decreases, the pH approaches its bulk value.

Fig. 11.

Experimental to modified simulation comparison of the pH shift. Both sets of experimental data have been corrected to account for where the SNARF molecule is located in the channel and the local pH at that position. The raw experimental data from the spectrophotometer can be found in Fig. 9. Without SNARF binding the experimental results indicate a pH shift slightly higher than what the model predicts. With SNARF binding and a $K = 0.05$ parameter from the Langmuir isotherm, the experimental results match the model. The MIR-FTIR analysis and fluorescein data (3 data pts) is also shown here and agrees with the model predictions except at low ionic strength.

Fig. 12.
MIR-FTIR analysis of fluorescein in pH 4 and pH 8 buffer solution in nanochannels.

Fig. 13.

The calibration curve that is plotted by the intensity of 1580 cm⁻¹ to 1600 cm⁻¹ in different pH buffer solutions from pH 4 to 8.

Table 1

List of simulation parameters^a

Species	$D_i \times 10^{-10} \text{ m}^2/\text{s}$	$\mu_i \times 10^{-13} \text{ m}^2/\text{s}$	z_i	$c_i^B \text{ (mM)}$	$a \text{ (nm)}$
K^+	20.3	8.2	1	—	30
Cl^-	19.6	7.9	-1	—	30
H_3O^+	93.1	37.6	1	4.05e-5	90
OH^-	52.7	21.3	-1	2.5e-4	35
H_2PO_4^-	8.8	3.5	-1	0.29 [■] and 0.29*	40
HPO_4^{2-}	8.8	3.5	-2	0.46 [■] and 0.18*	40
SNARF^{1-}	2.0	0.8	-1	0.10	45
SNARF^{2-}	2.0	0.8	-2	0.13	45
Fluorescein ¹⁻	5.0	2.0	-1	0.28	40
Fluorescein ²⁻	5.0	2.0	-2	1.05	40

Rate Constants (L/(mols))	Value
k_f, Phos	1.0e5
k_r, Phos	1.6e9
k_f, Water	1.0e5
k_r, Water	1.0e13
k_f, SNARF	1.0e5
k_r, SNARF	2.0e9
$k_f, \text{fluorescein}$	1.0e5
$k_r, \text{fluorescein}$	2.0e8

^aNote: ■ represents the phosphate concentration in the SNARF system and * represents the phosphate concentration in the fluorescein system.

Table 2

Calculated ζ -potentials based on the work by Matijevic³² for each salt concentration. These ζ -potentials are used in the boundary conditions to solve the ζ -potential based simulation

KCl concentration (mM)	Calculated ζ -potential (mV)
0.75	-100
3	-85
7.5	-70
30	-55
150	-35

Table 3

Boundary conditions for both Nernst-Planck and Poisson's equation application modes (Comsol v3.4) in the ζ -potential based simulation^a

Boundary	c_i	ψ
B1,B5	NA	NA
B2,B4,B6,B8	NA	NA
B3,B7	NA	NA
B9,B11	Insulation/Symmetry	Insulation/Symmetry
B10,B12	$c_i^B \exp\left(\frac{-z_i F \zeta}{R \cdot T}\right)$	ζ

^aNote NA signifies not applicable in the domain.

Table 4

Boundary conditions for both Nernst-Planck and Poisson's application modes (Comsol v3.3) in the modified simulation^a

Boundary	c_i	s_i	ψ
B1,B5	c_i^B	NA	0
B2,B4,B6,B8	Insulation/symmetry	NA	Insulation/symmetry
B3,B7	NA	Insulation/symmetry	Insulation/symmetry
B9,B11	Continuity	NA	Continuity
B10,B12	s_i/k_{av}	Insulation/symmetry	Continuity

^aNote NA signifies not applicable in the domain.

Table 5

List of parameter values obtained from eqn (31)

Parameter	Value from calibration curve	Values from literature	
		Fu <i>et al.</i> ⁴⁰	Brasselet <i>et al.</i> ⁴²
R_{min}	0.37	0.14	0.15
R_{max}	2.36	1.23	1.50
C	-1.21	-1.30	-1.10
pK_a	7.30	7.50	7.60
$I(A)/I(B)$	0.50	0.83	0.40