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Impact of leakage current and electrolysis on FET flow control and pH changes in nanofluidic channels†

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Abstract

We have fabricated multiple-internal-reflection Si infrared waveguides integrated with an array of nanochannels sealed with an optically transparent top cover. The channel walls consist of a thin layer of SiO₂ for electrical insulation, and gate electrodes surround the channel sidewalls and bottom to manipulate their surface charge and ζ -potential in a fluidic field effect transistor (FET) configuration. This nanofluidic device is used to probe the transport of charged molecules (Alexa 488) and to measure the pH shift in nanochannels in response to an electrical potential applied to the gate. During gate biasing for FET operation, laser-scanning confocal fluorescence microscopy (LS-CFM) is used to visualize the flow of fluorescent dye molecules (Alexa 488), and multiple internal reflection-Fourier transform infrared spectroscopy (MIR-FTIRS) is used to probe the characteristic vibrational modes of fluorescein pH indicator and measure the pH shift. The electroosmotic flow of Alexa 488 is accelerated in response to a negative gate bias, whereas its flow direction is reversed in response to a positive gate bias. We also measure that the pH of buffered electrolyte solutions shifts by as much as a pH unit upon applying the gate bias. With prolonged application of gate bias, however, we observe that the initial response in flow speed and direction as well as pH shift becomes reversed. We attribute these anomalous flow and pH shift characteristics to a leakage current that flows from the Si gate through the thermally grown SiO₂ to the electrolyte solution.

Introduction

In order to characterize and understand protein function and regulation, proteins must be first systemically separated and detected. The most common technique for protein separations is gel electrophoresis.^{1,2} Today, 1-D and 2-D polyacrylamide gel electrophoresis (PAGE) setup is commercially available and widely used as a standard technique. Despite its wide usage, however, the PAGE technique has its own limitations, such as large amount of required sample, low reproducibility, breakdown under high electric field, and low dynamic range. An emerging solution to overcome these limitations is to use micro/nanofluidic lab-on-a-chip (LOC) devices fabricated from conventional semiconductor manufacturing steps. Due to their reduced size, these devices require lower sample amount, lower electrical field, shorter analysis time, and higher throughput than the PAGE technique.^{3,4–6} Thus, the LOC devices provide a potential for improved bioseparation, detection, and chemical analysis.^{7,8}

†Electronic supplementary information (ESI) available: pH-dependent fluorescein molecular structure.

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One distinct advantage of using micro/nanofluidic devices is flow control of molecules, using electroosmosis (EO), when a longitudinal electrical potential (V_{EO}) is applied along the channels. The speed and direction of electroosmotic flow can be further controlled by modulating the electrical double layer (EDL) at the interface of electrolyte solution and channel wall surface. For instance, Ghowsi and Gale proposed field effect electroosmosis, where the flow in a microscale capillary is controlled by an electrical potential applied to the external surface of the capillary.^{9,10} Later, van den Berg *et al.* demonstrated field-effect-transistor (FET) flow control in a microfluidic device with a perpendicular potential of ± 50 V applied to the channel wall.⁸

The FET control scheme works in the following manner. Upon contacting an electrolyte solution, the surface of dielectric channel wall, such as SiO_2 , assumes a negative surface charge according to its dissociation constant (pK_a).^{11–13} Typically, the SiO_2 surface is negatively charged in aqueous solutions, since hydroxyl groups (Si-OH) on the SiO_2 surface are deprotonated to produce Si-O^- when the solution pH is above ~ 3.7 . The polarity and magnitude of this surface charge and therefore the ζ -potential is then adjusted by an electric potential (V_G) applied to the channel walls. The modulation of ζ -potential in this manner allows manipulation of flow speed and direction of electroosmosis. The flow speed accelerates when a negative potential ($V_G < 0$) is applied to the channel wall to lower the ζ -potential. Conversely, the flow speed decelerates, or the flow direction is reversed when a positive potential ($V_G > 0$) is applied to the channel wall to increase the ζ -potential. This flow control is analogous to the current control by the gate in conventional metal oxide semiconductor field effect transistors (MOSFETs). The fluidic devices that use this control scheme are hence termed fluidic FETs.^{8–10,14}

The extent of FET flow control is more pronounced in nanochannels than in microchannels.^{6,15,16} The transport-limiting dimension in nanochannels is on the order of Debye screen length (λ_D) in the electrolyte solution. Thus, the electrical double layer (EDL) can occupy a substantial portion of the channel cross-section and can have a significant overlap at low ionic strengths.^{17–19} EDL overlap and ζ -potential control subsequently manifest as more pronounced flow manipulation as well as transverse migration of charged molecules, depending on their valence state.^{20,21} This controllability on flow and transverse migration in turn enhance the difference in longitudinal mobility and separation of biomolecules. Recognizing these advantages, various studies have been conducted to demonstrate FET flow control in nanochannels. These studies have focused on electrokinetic molecular control,¹⁷ flow characteristics based on ionic strength^{15,17} and channel dimension,^{5,22} and fabrication techniques.^{16,19}

Herein, we report the fabrication of an integrated nanofluidic device that serves as an analytical tool and as a separation medium not only for charged dye molecules, but potentially for proteins. The device is fabricated using conventional Si-based integrated circuit manufacturing technology. This device allows the use of laser-scanning confocal fluorescence microscopy (LS-CFM) and multiple internal reflection Fourier transform infrared spectroscopy (MIR-FTIRS). In our previous publication,²³ these techniques were used to probe wall-molecule interactions and their impact on ζ -potential. In this study, we have used these techniques to monitor the FET flow control of fluorescent dye molecules as well as the shift in solution pH in response to the transverse gate potential. In addition, we have probed the effect of small, but measurable leakage current through the thermally grown gate SiO_2 during FET flow control, which leads to anomalous flow characteristics over time. The extent of electrolysis and subsequent pH shift are also characterized.

Fabrication

The fabrication steps are described in detail in our previous publication.²⁴ In brief, Fig. 1 shows a schematic diagram of the nanofluidic device for monitoring electrokinetic transport of fluorescent dyes and pH changes in nanochannels under FET flow control. Double-side-polished Si(100) wafers ($1\text{ cm } w \times 5\text{ cm } l$) are used as substrates to prevent the scattering and loss of IR beam intensity during multiple internal reflection in our MIR-FTIRS analysis system. A 3 mm wide boron doped gate region is defined perpendicularly to the channel direction at the center of the wafer. The dopant diffusion is carried out for 60 min at $1050\text{ }^{\circ}\text{C}$ in an O_2/N_2 environment, which results in the formation of a diffusion layer with a depth of $1\sim 1.2\text{ }\mu\text{m}$ and a dopant level on the order of $1 \times 10^{20}\text{ cm}^{-3}$. The heavily doped gate is used to control the surface charge on nanochannel walls more efficiently by reducing the contact resistance.²⁴ An array of nanochannels is fabricated along the direction of IR propagation, using interferometric lithography (IL)²⁵ and plasma etching. Fig. 1(a) shows a cross-sectional scanning electron microscope (SEM) image of the nanochannels after the etching process. The SEM images [Fig. 1(a)–(c)] are taken at different stages of fabrication, and they can be viewed as magnified cut-away, cross-sections of the nanochannels pointed by the diverging arrows in Fig. 1(d). The SEM view angle is along the right-to-left channel orientation in Fig. 1(d). Each channel is approximately 200 nm wide and 450 nm deep immediately following the etching. The nanochannel array occupies a total area of $3\text{ mm } w \times 16\text{ mm } l$, which contains up to 8000 nanochannels. A thermal SiO_2 layer is grown up to 100 nm, reducing the channel width to 100 nm and the channel depth to 400 nm. Fig. 1(b) shows the nanochannel array covered with a thermally grown SiO_2 layer. This layer is used as an electrically insulating layer between the Si channel walls and the fluid. The nanochannels are sealed with a Pyrex cover by anodic bonding²⁶ as shown in Fig. 1(c). This process is conducted at $380\text{ }^{\circ}\text{C}$ by pressing the Pyrex cover and the nanochannel substrate together, while -1 kV is applied to the Pyrex cover, and the substrate is grounded. The optical transparency of Pyrex slip cover allows monitoring of flow speed and wall-adsorption of fluorescent molecules in the channels with LS-CFM (Zeiss Axioskop with an LSM5 scanning head). 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 and to probe the signature vibrational modes of molecules flowing through the nanochannels. The changes in observable vibrational modes can provide diffusion rate, flow speed, and wall adsorption/desorption of molecules, along with a pH shift in the nanochannels.²⁴

Experimental

Fig. 1(d) shows schematic diagrams of our experimental setup for LS-CFM and MIR-FTIRS. For FET flow control, we first introduce a buffer solution into the nanochannels by capillary force. Platinum (Pt) wires, immersed in two solution wells on opposite ends of the channels, are used as electrodes. To induce an electroosmotic flow along the channels, a positive potential ($V_{\text{EO}} > 0$) is applied to the inlet, and the outlet is grounded, generating a longitudinal electrical field ($\vec{E}$). After the electroosmotic flow is induced, a potential (V_{G}) is applied to the highly doped gate to modulate the surface charge on channel walls and conduct FET flow control. We note that V_{EO} and V_{G} share a common ground to maintain the same reference potential. During the FET control, the flow of fluorescent dye molecules in the nanochannels is monitored by LS-CFM.

Alexa 488 maleimide ($\text{MW} = 479$, $\text{C}_{30}\text{H}_{25}\text{N}_4\text{NaO}_{12}\text{S}_2$) is used to visualize our FET flow control with LS-CFM. The excitation and emission wavelengths of Alexa 488 are 488 and 519 nm, respectively. Fluorescence intensity of Alexa 488 is very strong and stable in a relatively large pH range ($\text{pH} = 4$ to 9).²⁷ In our FET flow control experiments, Alexa 488 is dissolved in a pH 4 buffer. The reason for choosing pH 4 is that the isoelectric point (pK_{a}) of

SiO₂ channel walls is near 3.7, where the net charge on the surface is zero.¹¹ Above the isoelectric point, the surface charge becomes increasingly negative, as surface hydroxyl groups (SiOH) become deprotonated. Conversely, the surface charge gradually turns off or can become further protonated as [SiOH₂]⁺ as pH decreases below pK_a.^{11–13} Therefore, the surface charge control and its impact on FET flow control are relatively more pronounced near the isoelectric point. Ghowsi *et al.* and later Schasfoort *et al.* have demonstrated that the ζ -potential control and therefore EO flow control are most pronounced near the isoelectric point.^{8–10}

For the MIR-FTIRS technique [Fig. 1(d)], a nanofluidic IR waveguide is mounted on top of a metal housing with IR optics. The 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 from the channel bottom before the beam exits the opposite end. The IR signal leaving the second beveled edge is collected by an HgCdTe detector. Due to these multiple reflections, the Si MIR crystal is opaque to IR below 1500 cm⁻¹. To monitor the pH shift, we first inject a buffer solution with a desired pH value (pH = 2 to 8) into the nanochannels, wait approximately 20 min for the system to reach equilibrium when no noticeable change in IR spectrum is observed, and take an IR background spectrum with 2 cm⁻¹ resolution averaged over 100 scans. Taking the background only with the buffer solution minimizes interference from absorption bands of water, when sample spectra are taken with fluorescein solution. The channels are then cleaned in DI water and dried on a hot plate. A buffer solution of fluorescein (C₂₀H₁₂O₅) dye molecules with a known pH is then injected into one of the two solution wells to fill the nanochannels, and a series of sample IR spectra with the same resolution and averaging are taken to monitor the characteristic vibrational modes of fluorescein dye molecules that are sensitive to the pH shift.

Fluorescein (Sigma-Aldrich) is used as a pH indicator in our MIR-FTIR analysis. Fluorescein is a commonly used fluorescent dye molecule,^{28,29} whose quantum yield is strongly affected by solution pH. The absorption and emission wavelengths of fluorescein are 494 and 521 nm, respectively. The variation in quantum yield for fluorescein is often monitored with FTIR spectroscopy to relate a pH shift to the molecule's structural change.²⁸ Depending on the pH of buffer solutions, fluorescein can become a cation (pK_a < 2.08), a neutral molecule (pK_a = 2.08 ~ 4.31), an anion (pK_a = 4.31 ~ 6.43) or a dianion (pK_a > 6.43) by protonation or deprotonation of carboxyl group and OH group on the molecule. Thus, we are able to monitor the pH in nanochannels by analyzing the molecular structure of fluorescein using MIR-FTIRS.

Deuterated water (D₂O, 99.9 atom % D, Sigma-Aldrich) instead of H₂O is used to avoid overlapping between the vibrational modes of H₂O [ν_s (OH) at 3400 ~ 3000 cm⁻¹ and δ_s (HOH) at 1640 cm⁻¹]^{30,31} and those of fluorescein [ν_s (CHx) and ν_{as} (CHx) at 3000 ~ 2800 cm⁻¹ and ν_{as} (COO⁻) and ν_s (C–C) at 1600 ~ 1580 cm⁻¹].^{28,29} Various buffers are used to monitor IR spectra of fluorescein in different pH buffer solutions from pH 2 to 8. We have used chloroacetic acid (pK_a = 2.83) buffer for pH 2 to 3, acetate buffer (pK_a = 4.76) for pH 4 ~ 5, pyridine buffer (pK_a = 5.23) for pH 6, phosphate buffer (pK_a = 7.2) for pH 7, and tris-glycine buffer (pK_a = 8.02) for pH 8. The pH for each buffer is adjusted with HCl or NaOH, and the ionic strength of all buffer solutions is approximately adjusted to 1~2 mM. We expect the electroosmosis to be much more pronounced than the electrophoresis within this range of ionic concentration. At this ionic strength, λ_D ($= 1/\kappa$) is approximately 10 nm, where κ is given by

$$\kappa^2 = \frac{e^2}{\epsilon \epsilon_0 k_B T} \sum_i z_i^2 n_i \quad (1)$$

where e , ϵ , ϵ_0 , k_B , T , z_i , and n_i represent unit charge, dielectric permittivity, vacuum dielectric constant, Boltzmann's constant, temperature, valence charge, and charge density, respectively.

Results and discussion

Using LS-CFM, the FET flow control is monitored with Alexa 488 maleimide (1 mg mL⁻¹) dissolved in a pH 4 buffer solution. After filling the nanochannels only with the buffer solution, Alexa 488 is injected into the inlet well. To induce an EO flow in the nanochannels, we apply $V_{EO} = +6$ V to the right-side well in Fig. 1–3 where Alexa 488 is contained, while the opposite well on the left side is grounded. Fig. 2(a)–(b) show that the electroosmotic flow of Alexa 488 moves from the right side ($V_{EO} = +6$) to the left side (ground) at a rate of $3.2 \mu\text{m s}^{-1}$. Reference points are provided for ease of visualizing moving fronts and their displacement over time. Note that within the field of view, approximately 5000 channels are present, extending horizontally in Fig. 2 and 3. Since the channel width (~ 100 nm) is below the diffraction limit, adjacent nanochannels are not optically distinguishable. We note that the moving front is not vertically straight across the channels in Fig. 2(a)–(b), reflecting the circular shape of the solution wells. That is, the dye molecules are introduced into the channels near the middle row at an advanced position than they are introduced to the channels near the outer edge. For even introduction of molecules, we are currently exploring a new fabrication technique to drill square holes into Pyrex slip covers. As the FET flow control is successively tested with positive and negative V_G , the non-uniformity in the moving front becomes more pronounced (Fig. 2). We suspect that channel-to-channel dimensional non-uniformity both in transverse and longitudinal directions leads to a non-uniform, irregular moving front, as the dye molecules traverse farther into the channels, and the flow direction is repeatedly manipulated. Thus, the dimensional uniformity amongst nanochannels will have to improve, if one desires efficient isolation and elution of separated molecules.

To control the surface charge of channel walls, a DC potential (V_G) is applied to the gate, while maintaining a constant longitudinal electrical field ($\vec{E}$) with V_{EO} . The flow velocity of Alexa 488 is increased to $25 \mu\text{m s}^{-1}$, when a negative bias ($V_G = -30$ V) is applied to the gate to lower the ζ -potential. Fig. 2(c)–(d) show that Alexa 488 moves from right to left at an accelerated pace. The enhanced flow velocity upon applying the negative V_G is an order of magnitude greater than the EO flow velocity ($3.2 \mu\text{m s}^{-1}$) induced only by the longitudinal electrical field without V_G . Conversely, Alexa 488 rapidly reverses its flow in Fig. 2(e)–(f) when $V_G = +30$ V is applied to the gate to raise the ζ -potential. The flow response to the gate bias is immediate and repeatable in our FET flow control experiments. The observed flow response is also independent of the position of dye molecules with respect to the gate position. That is, the flow response is identical, independent of whether the dye molecules are fore or aft of the gate region.

With prolonged application of the gate bias, however, we observe unusual flow characteristics. Fig. 3 shows a time series of confocal images of Alexa 488 upon inducing an EO flow with $V_{EO} = +6$ V and subsequently applying a positive gate bias ($V_G = +30$ V). After inducing the reverse flow with the positive V_G , we observe that the speed and direction of EO flow are not maintained constantly over time. Fig. 3(a) shows the final movie frame of the EO flow moving from right to left at $3.2 \mu\text{m s}^{-1}$. Reference points are provided for

ease of visualizing moving fronts and their displacement over time. Upon applying the gate bias ($V_G = +30$ V), Alexa 488 reverses its flow moving from left to right [Fig. 3(b)–(c)] up to 60 s. However, the reversed flow of Alexa 488 moving to the right starts to reverse its flow again and move to the left in Fig. 3(d)–(f), as the positive gate bias is maintained over 1 min. We observe that this double-reverse flow occurs also during FET flow control with a negative gate bias ($V_G = -30$ V). However, an observable difference is that the double-reverse flow occurs over a much shorter time period (12 to 15 s) with the negative V_G . We attribute the double-reverse flow phenomenon to a leakage current, which flows from the Si gate through the thermally grown SiO_2 to the electrolyte solution, and the difference in the rate of change to asymmetry in leakage current, where negative V_G causes much larger leakage current than positive V_G (Fig. 4).

Fig. 4 shows the leakage current density (J_{leak}) through the gate SiO_2 . The leakage current is measured from the gate voltage source equipped with a current readout and simultaneously verified with a current meter connected between the left well and the ground in Fig. 1(d), while the gate bias is swept from $V_G = -30$ V to $+30$ V. The leakage current density is on the order of nA cm^{-2} for V_G within the range of -6 to $+8$ V as shown in the inset of Fig. 4. However, J_{leak} increases in magnitude up to $-1.4 \mu\text{A cm}^{-2}$ for V_G below -6 V and up to $0.05 \mu\text{A cm}^{-2}$ for V_G above $+10$ V. Note the asymmetry in J_{leak} , where J_{leak} is significantly larger in magnitude with a negative gate bias than with a positive gate bias. That is, the SiO_2 walls are not as leaky with a positive gate bias up to $V_G = +20$ V, whereas $V_G < -10$ V leads to a significant leakage current. In order to identify the mechanism by which the leakage current flows from gate to solution and *vice versa* asymmetrically, we have tested a variety of different buffers (*e.g.*, tris-glycine, propionate, and NaOH) at varying pH and observe that the I–V characteristics (Fig. 4) are identical and independent of the buffer solution. Thus, the exact charge transport mechanism is yet to be determined. However, this leakage current causes water electrolysis in the nanochannels, generating hydronium ions (H_3O^+) or hydroxyl ions (OH^-) from the surface of nanochannels according to the polarity of the gate bias.²⁸

The following reactions show electrolysis as well as other side reactions that occur at the anode and the cathode.

Upon applying a positive gate bias ($V_G > 0$ V), the SiO_2 surface bordering the heavily doped Si gate serves as an anode, where O_2 and H_3O^+ ions are generated [eqn (2)–(3)]. The H^+ ions produced from the anode, in turn, reach equilibrium with H_2O via eqn (4). Conversely, when a negative bias is applied to the gate, the SiO_2 wall surrounding the gate serves as a cathode, and OH^- ions are generated by decomposition of H_2O according to eqn (5) and (6). Note also that the side reaction in eqn (7) entails that the cathode can deplete H^+ ions that are generated from the anode and have diffused to the cathode, while producing hydrogen gas (H_2). This latter reaction would result in a greater absolute magnitude for the rate of increase in pH with a negative gate bias than the absolute magnitude for the rate of decrease in pH with a positive gate bias of equal magnitude.

The pH shift in nanochannels in response to the FET surface charge control and leakage current is monitored using fluorescein as a pH indicator. To demonstrate, Fig. 5 shows representative IR spectra of fluorescein in pH 4 and pH 8 buffer solutions. Fluorescein has skeletal vibrational modes of xanthene moiety in the range of from 1200 to 1800 cm^{-1} .²⁹ However, the observable range of wavenumbers in our nanofluidic waveguide is limited to above 1500 cm^{-1} for the reasons described in the experimental section. Due to the fact that Si MIR crystal is opaque below 1500 cm^{-1} , we monitor the asymmetric stretching vibrational mode of COO^- [$\nu_{\text{as}}(\text{COO}^-)$] at 1580~1585 cm^{-1} and the xanthene skeletal C–C stretch [$\nu_{\text{s}}(\text{C}–\text{C})$] containing a conjugated carboxyl band at 1596~1600 cm^{-1} to probe pH changes.²⁹ The IR spectra of pH 4 and pH 8 buffer solutions show different characteristics in Fig. 5. The IR spectrum of fluorescein in neutral state at pH 4 shows that both intensities of $\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{C}–\text{C})$ are strong. At pH 8, however, the absorbance intensity of $\nu_{\text{s}}(\text{C}–\text{C})$ relatively decreases in comparison to the absorbance of $\nu_{\text{as}}(\text{COO}^-)$. Note that the peak position of $\nu_{\text{as}}(\text{COO}^-)$ is slightly shifted to 1580 cm^{-1} with increasing pH (pH 4 to 8). These pH-dependent differences in IR spectra are due to the protonation/deprotonation of fluorescein.²⁹ The neutral fluorescein at low pH becomes a dianion due to the protonation of carboxyl and OH groups of xanthene ring. Thus, fluorescein becomes significantly less symmetric. In contrast, fluorescein has a highly symmetric structure consisting of a xanthene moiety with two identical oxygens by the deprotonation at high pH values.

The absorbance intensity of $\nu_{\text{s}}(\text{C}–\text{C})$ and $\nu_{\text{as}}(\text{COO}^-)$ is monitored in pH 2 to 8 buffer solutions in nanochannels, using MIR-FTIR spectra, and the intensity ratio of $\nu_{\text{s}}(\text{C}–\text{C})/\nu_{\text{as}}(\text{COO}^-)$ as a function of pH is plotted in Fig. 6. This graph provides a calibration curve to estimate the pH in nanochannels based on the peak ratio. We have independently verified the calibration curve by laser absorbance spectroscopy, using SNARF as a pH indicator.³² This independent verification and the native pH shift of buffer solution upon entering nanochannels were discussed in our previous publication.³² Since the magnitude of this native pH shift depends on the initial value of the buffer solution and is not a constant shift, we will use the bulk solution pH as a representative indicator of the initial pH in the nanochannels. Using this calibration curve, the pH shift in nanochannels is experimentally monitored using MIR-FTIRS as a function of polarity and magnitude of the gate bias (V_G). The pH shift is an indirect indicator of the level of electrolysis and therefore the level of H_3O^+ or OH^- production caused by electrolysis. Based on our observations, we deduce that the total pH change ($\Delta\text{pH}_{\text{total}}$) in nanochannels consists of two main contributions (1) the initial protonation/deprotonation of SiOH groups on SiO_2 walls upon gate biasing ($\Delta\text{pH}_{\text{surf}}$) and (2) the generation of OH^- or H_3O^+ ions by water electrolysis ($\Delta\text{pH}_{\text{elect}}$) with prolonged gate biasing:

$$\Delta\text{pH}_{\text{total}} = \Delta\text{pH}_{\text{surf}} + \Delta\text{pH}_{\text{elect}}. \quad (8)$$

In order to delineate $\Delta\text{pH}_{\text{surf}}$ from $\Delta\text{pH}_{\text{elect}}$, a series of IR absorbance spectra [Fig. 7(a)] are taken in real time for a pH 4 acetate buffer solution containing fluorescein, while the gate bias is modulated. At pH 4, fluorescein is a neutral molecule. The solution of neutral fluorescein is first introduced into nanochannels by capillary force until the channels are completely filled with the solution. The solution is further added into inlet and outlet wells to completely fill the solution wells. An IR background spectrum is subsequently taken in the absence of any applied electrical potential. In order to isolate the impact of gate biasing from electroosmosis, two Pt electrodes inserted into the inlet and outlet wells are grounded. Sample IR absorbance spectra are then collected every 90 sec, while a DC potential is applied to the gate varying from $V_G = \pm 10$ to 20 V. The sample IR Spectra from 1500 to 1700 cm^{-1} are plotted in Fig. 7(a) as a function of time. We observe that the intensity ratio of $\nu_s(\text{C}-\text{C})/\nu_{\text{as}}(\text{COO}^-)$ increases or decreases, depending on the polarity and magnitude of the gate bias. The observed intensity ratio is then converted to its corresponding pH value, using our pH calibration curve in Fig. 6. The resulting pH shift is plotted in Fig. 7(b).

Upon applying a positive gate bias ($V_G = +10$ V), we first observe that the pH in nanochannels increases from pH 4.5 to 5.3 for 10 min (Period I) in Fig. 7(b). The positive gate bias ($V_G = +10$ V) induces positive charges on SiO_2 walls, possibly by protonation of SiOH groups on channel walls.^{11–13} The positively charged walls in turn attract negatively charged ions, including OH^- . The accumulation of OH^- near the walls then increases pH. Thus, the pH increase qualitatively coincides with the accumulation of negative charges and the reversed flow direction during FET control [Fig. 2(e)–(f) and Fig. 3(a)–(c)]. Since the gate bias used here ($V_G = +10$ V) to monitor the pH shift is less than that ($V_G = +30$ V) used to observe the double-reverse flow, the initial pH increase occurs over a longer period (10 min) than 60 s. Following the initial increase in pH, the pH starts to decrease after 10 min with prolonged gate biasing at $V_G = +10$ V. This decrease in pH is likely due to the production of H_3O^+ ions by water electrolysis [eqn (2)–(3)]. In fact, the rate of decrease in pH is more pronounced as V_G is increased from +10 to +20 V [Period II in Fig. 7(b)]. Based on Fig. 4, this increase in V_G causes J_{leak} to increase from 0.7 to 7 nA cm^{-2} . The increased leakage current density produces H_3O^+ ions at a faster rate and subsequently results in the increased rate of pH decrease.

The pH in nanochannels is continuously monitored upon grounding the gate and then switching to a negative gate bias ($V_G = -10$ to -20 V). Period III in Fig. 7(b) represents a 10 min period during which V_G is set to zero. Upon grounding the gate, the pH reaches a steady state pH value (pH ~ 3) slightly greater than the pH value when a positive gate bias is applied. We observe that the rapid transient fluctuation in pH during Period III before reaching the steady state is random and varies from experiment to experiment.

When a negative gate bias ($V_G = -10$ V) is applied during Period IV, the pH decreases for 3 min. We attribute this decrease in pH to the accumulation of positively charged ions near the channel walls, including H_3O^+ , due to the deprotonation of SiOH groups on SiO_2 walls. This pH decrease qualitatively coincides with the accumulation of positive charges and the accelerated electroosmotic flow during FET control [Fig. 2(c)–(d)]. With prolonged negative gate biasing ($V_G = -10$ V), however, the pH starts to increase counteracting the initial decrease. This increase in pH is due to the production of OH^- ions from the channel walls by water electrolysis [eqn (5)–(6)]. The rate of increase in pH becomes more pronounced as V_G is increased in magnitude from -10 to -20 V during Period V. The absolute magnitude for this rate of increase is approximately a factor of two greater than the rate of decrease in

pH with a positive gate bias of equal magnitude. The depletion of H^+ at the negatively biased gate electrode (cathode) described in eqn (7), in addition to the asymmetrically larger leakage current with a negative gate bias (Fig. 4), is the suspect cause of this pronounced rate of change in pH with a negative gate bias.

The observed pH shift in nanochannels can be compared to a prediction calculated from the measured leakage current and the generated amount of H_3O^+ or OH^- . Assuming that the leakage current is used entirely for electrolysis, the generated amount of H_3O^+ or OH^- is capable of decreasing or increasing the pH by an order of magnitude. We assume that the rate of production of H_3O^+ or OH^- is proportional to the experimentally measured leakage current ($I_{leak} = Q/t$). For instance, $I_{leak} = 0.536 \mu A$ is measured when $V_G = +30$ V. This I_{leak} generates 5.53×10^{-12} moles of H_3O^+ over 1 s. To decrease the pH from 4 to 3, 9.0×10^{-4} moles of H_3O^+ L^{-1} are required. Considering the total volume ($700 \mu m^3$) for 8000 parallel channels, therefore, the molar amount of H_3O^+ needed is 5.04×10^{-12} moles of H_3O^+ . Thus, one unit of pH decrease from 4 to 3 would take approximately 10 s by applying $V_G = +30$. Compared to the calculations, however, the actual pH shift is observed over a longer period [see Fig. 7(b)] close to 10 min. This apparent delay in pH shift is likely due to diffusion and recombination of H_3O^+ and OH^- ions through nanochannels. That is, while the gate electrode is serving as either an anode or a cathode, the grounded electrodes in end wells serve as a cathode or an anode, respectively. The neutralization would effectively reduce the observed rate of change in pH caused by the leakage current. In addition, the buffer capacity of the solution, including the amount of buffer solutions in the inlet and outlet reservoirs, must slow down the change in pH. In order to understand the pH shift over time in a more systematic way, we have previously developed a model to describe a native pH shift of buffer solutions upon entering nanochannels,³² and we are currently developing a model describing the molecular flow in nanochannels, which takes into account charged molecules' wall adsorption;³³ electrolysis from leakage current and from the electrodes that drive the electroosmosis; pH shift due to surface charge manipulation and from the electrolysis; and H_3O^+ and OH^- diffusion and reaction.

The experimentally observed pH shift in response to the polarity and magnitude of the gate bias consistently reflects the sign and magnitude of the leakage current. In addition to the pH shift, Fig. 8 pictorially describes how the leakage current may ultimately induce the double-reverse flow during FET flow control. Since the initial buffer solution pH is 4, the SiO_2 channel walls are negatively charged, and positively charged counterions are accumulated near the channel walls. Fig. 8(a) shows that upon turning on the electric field pointing to the left, these positively charged ions (e.g., H_3O^+ and other positive buffer ions) near the wall move from right (anode) to left (cathode), inducing an EO flow. To reverse the direction of the EO flow, a positive bias ($V_G > 0$ V) is applied to the gate to raise the ζ -potential. Positively charged ions are repelled from the walls, whereas negatively charged ions (e.g., OH^- and other negative buffer ions) are attracted to the walls as shown in Fig. 8(b). Thus, the EO flow is reversed. This reversal in the direction of EO flow is induced solely by the surface charge control and is shown in Fig. 2(e)–(f) and 3(a)–(c). With a continuing application of the positive gate bias ($V_G > 0$ V), however, the leakage current through the gate SiO_2 causes water electrolysis and generates H_3O^+ ions near the surface of the channel walls [Fig. 8(c)]. These positive H_3O^+ ions populate the solution–wall interface by displacing the negative ions that have previously accumulated at the interface. The population of H_3O^+ then leads to the double-reverse flow. A similar phenomenon occurs with negative V_G that produces OH^- near the gate region. The only difference is the accelerated pace of double-reverse flow due to the asymmetry in the magnitude of leakage current depending on V_G polarity.

Conclusions

We have fabricated an integrated nanofluidic FET device that enables monitoring the flow of fluorescent dye molecules using LS-CFM and probing the pH shift in buffer solutions using MIR-FTIRS. The surface charge of channel walls and therefore ζ -potential are modulated by the gate potential during FET flow control. The modulation of ζ -potential, with concomitant protonation or deprotonation of SiOH groups on SiO₂ walls, governs the direction of electroosmotic flow and the pH in nanochannels. We observe that the flow of Alexa 488 is accelerated in response to a negative gate bias ($V_G < 0$), whereas its flow direction is reversed in response to a positive gate bias ($V_G > 0$). Depending on the sign and magnitude of gate bias, a pH shift close to a whole unit is also observed, using fluorescein as a pH indicator. The molecular structure and characteristic vibrational modes of fluorescein strongly depend on the buffer solution pH, and we use its IR absorbance to monitor the solution pH in the nanochannels. The observed pH shift strongly suggests that isoelectric focusing of charged molecules is possible with multiple gates placed along the channels to create a longitudinal pH gradient. With prolonged application of gate bias, however, anomalous flow characteristics and pH shift are manifest, where the initial flow and pH response is reversed. We attribute this reversal to a leakage current that flows from the Si gate through the thermally grown SiO₂ to the electrolyte solution. This leakage current causes water electrolysis to generate H₃O⁺ or OH⁻ ions that populate the region near the channel walls surrounded by the gate. The generation and accumulation of these H₃O⁺ or OH⁻ ions then reverse the initial flow direction and pH shift set by the gate bias. In order to effectively exploit the pH shift by surface charge control, the leakage current therefore must be properly managed. In the future, we will investigate alternative materials (*e.g.*, Si₃N₄ and Al₂O₃) to replace SiO₂, thus eliminating or minimizing the leakage current to a negligible level.

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Fig. 1.

Cross-sectional SEM images of nanochannels and experimental setup to perform LF-CFM and MIR-FTIRS. (a) Nanochannels after plasma etching. (b) Thermally grown oxide (SiO₂)-covered nanochannels. The thickness of oxide layer, channel width, and channel depth are 100 nm, 100 nm, and 400 nm relatively. (c) Nanochannels sealed with a Pyrex cover slip using anodic bonding. (d) LS-CFM setup to monitor Alexa 488 during FET flow control. A positive bias ($V_{EO} > 0$) is applied to right hand side well, and the opposite side well is grounded to induce electroosmotic flow. The gate bias (V_G) is applied to the gate to modulate the surface charge of nanochannel walls. MIR-FTIR analysis is to probe pH changes in nanochannels. A nanofluidic IR waveguide is mounted on top of a metal housing with IR optics. Inlet and outlet well are grounded after filling fluorescein in nanochannels. Absorbance IR spectra of fluorescein are collected as a function of time, while varying the magnitude and polarity of gate bias.

Fig. 2. LS-CFM images of Alexa 488 in a pH 4 buffer solution under electroosmotic flow with and without the gate bias. (a)–(b) Electroosmotic flow of Alexa 488 from right to left at $3.2 \mu\text{m s}^{-1}$ upon applying $V_{EO} = +6 \text{ V}$ to the well in which Alexa 488 is contained, while the opposite well is grounded. (c)–(d) Alexa 488 flow accelerates up to $25 \mu\text{m s}^{-1}$ upon applying a negative gate bias ($V_G = -30 \text{ V}$). (e)–(f) The flow direction of Alexa 488 is reversed and flows at $-30 \mu\text{m s}^{-1}$ upon applying a positive gate bias ($V_G = +30 \text{ V}$).

Fig. 3. LS-CFM images of double-reverse flow of Alexa Fluor 488 maleimide in a pH 4 buffer solution during the FET flow control with positive gate bias ($V_G = +30 V_G$). (a) The initial EO flow moving from right to left at $3.2 \mu\text{m s}^{-1}$. (b)–(c) Alexa 488 reverses its flow moving from left to right upon positive gate bias ($+30 V_G$) for 60 s. (d)–(f) The reversed flow of Alexa 488 moving to the right starts to reverse its flow again and move to the left, as the positive gate bias is maintained over 1 min.

Fig. 4. Leakage current density that is measured with our nanofluidic device applying the gate biasing from -30 to $+30$ V_G .

Fig. 5.

Absorbance IR spectra of fluorescein in (a) pH 4 buffer solution and (b) pH 8 buffer solution in nanochannels. The IR spectrum of fluorescein in neutral state at pH 4 shows that both absorbance intensities of $\nu_{\text{as}}(\text{COO}^-)$ at 1580 cm^{-1} and $\nu_{\text{s}}(\text{C}-\text{C})$ at 1600 cm^{-1} are strong. At pH 8, the absorbance intensity of $\nu_{\text{s}}(\text{C}-\text{C})$ relatively decreases in comparison to the absorbance of $\nu_{\text{as}}(\text{COO}^-)$.

Fig. 6. pH calibration curve that is plotted by the intensity ratio of $\nu_{as}(\text{COO}^-)/\nu_s(\text{C-C})$ in correspondence to the pH of buffer solutions from 2 to 8.

Fig. 7.

(a) A series of IR absorbance spectra as a function of time as well as the amplitude and polarity of gate bias, showing the variation of absorbance intensities of $\nu_{\text{as}}(\text{COO}^-)$ and $\nu_{\text{s}}(\text{C}-\text{C})$. The gate bias (V_G) is applied from ± 10 to 20 V. Each IR spectrum is taken every 3 min.

(b) The observed intensity ratio and its corresponding pH value are plotted, using our pH calibration curve (Fig. 6).

Fig. 8.

The pictorial description of double-reverse flow induced by water electrolysis during the FET flow control. (a) Upon turning on the electric field pointing to the left, positively charged ions (*e.g.*, H_3O^+ and other positive buffer ions) near the wall move from right (anode) to left (cathode), inducing an EO flow. (b) A positive bias ($V_G > 0$ V) is applied to the gate to raise the ζ -potential to reverse the direction of the EO flow. Positively charged ions are repelled from the walls, whereas negatively charged ions (*e.g.*, OH^- and other negative buffer ions) are attracted to the walls. (c) With a continuing application of the positive gate bias ($V_G > 0$ V), the leakage current through the gate SiO_2 causes water electrolysis and generates H_3O^+ ions near the surface of the channel walls. These H_3O^+ ions in turn induce double-reverse flow.