The effect of the surface functionalization and the electrolyte concentration on the electrical conductance of silica nanochannels

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It is known that the conductance of nanochannels as a function of electrolyte concentration deviates from a linearly proportional relationship and approaches a value independent of the concentration as the electrolyte concentration is lowered. Most of the proposed models account for this behavior by considering a constant surface charge density and an ideal electrolyte solution. However, at low electrolyte concentrations, the ideal electrolyte approximation is no longer valid because the ions that result from the atmospheric carbon dioxide dissolution in water dominate the ionic concentration. In this paper, arrays of silica nanochannels were electrically characterized via conductance measurements. The conductance at low salt concentrations is modeled by a variable surface charge model that accounts for all ionic species in solution. This model was used to determine the variable surface charge of the bare silica nanochannels as well as of chemically modified nanochannels. The model correctly predicted the variation of the nanochannel conductance observed after silane (aminopropyldimethylethoxysilane) functionalization and single-strand DNA immobilization. Finally, pH modification of bulk KCl solutions was employed as an alternative method of changing the surface charge of silica nanochannels. Surface charge calculated from conductance measurements performed at different bulk pH values confirmed that the surface charge of the silica nanochannel walls is sensitive to the H^+ concentration.
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I. INTRODUCTION

With the advent of nanofabrication technology, we can now fabricate well controlled nanochannel structures.¹ Fluids within nanosized channels exhibit physical behaviors that are not observed in most microfluidic applications. Nanostructures, with a characteristic dimension in the range of 10–100 nm, have a very large surface area-to-volume ratio. Consequently, the fluid interaction with the walls is so dominant that can drastically affect the ionic composition of the nanoconfined fluid to the extent of near complete exclusion of co-ions.² This charge selectivity effect has been exploited to: sense^{3,4} and concentrate^{5,6} charged biomolecules such as DNA and proteins; produce fluidic equivalents of well-known electrical circuit elements such as diodes^{7–10} and transistors,^{9,11–14} which may find applications in control of pH, local concentration of ions, and separations processes in the future; and to enhance mechanical-to-electrical energy conversion.^{15,16} Surface chemistry is especially relevant in nanofluidic systems since the surface groups affect greatly the ionic composition of the nanoconfined solution. As a result, surface chemistry effects have been extensively studied in nanochannels. Streaming current^{15,17,18} and conductance measurements^{19,20} in a single nanochannel have been used to show that the release and uptake of protons from the walls have a profound effect on the ion transport in nanochannels. Other research

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groups have demonstrated active modulation of the electrostatic potential at the wall using gate electrodes in transistor-like nanofluidic structures.^{11–13} Application of a potential at the wall induces a shift in the chemical equilibrium between the wall and the filling solution, resulting in either a release or an uptake of protons from the walls,^{21–24} thereby changing the pH of the solution and influencing its transport properties.^{25,26} A significant amount of work has been dedicated to the study of non-equilibrium and nonlinear features of electrokinetic transport in nanochannels. Anomalous but important features of nano-electrokinetics have been described, namely: overlimiting current behavior,²⁷ rectification of ion transport,^{28,29} ionic concentration polarization,^{30,31} and vortex instability at the perm-selective interfaces.³²

Stein *et al.*³³ reported a constant electrical conductance, with respect to the bulk electrolyte concentration, at low salt concentrations in nanochannels. These authors presented an electrokinetic model of ion transport in nanochannels, parameterized only by the surface charge density. This model treated the surface charge on the nanochannel walls as being constant when in the presence of solutions with different salt concentrations and could predict fairly well the conductance of the nanochannels for the whole range of electrolyte concentration. Later, Schoch *et al.*^{34,35} proposed a less precise but much simpler constant surface charge model for the nanochannel conductance. This last model was then applied by Karnik *et al.*³ to study the effects of chemical surface reactions on nanochannels walls on nanochannel conductance. Through a study of the conductance of the nanochannel in the presence of electrolytes with low and high ionic concentrations, these authors could determine the absolute surface charge and size, respectively, of the molecules on the surface of the nanochannels. However, the above mentioned models could only be used to describe the conductance behavior at low ionic strength because in addition to the assumption of a constant surface charge the authors have also considered the KCl solutions as being ideal (i.e., without dissolved gases). In ideal KCl solutions, only K^+ and Cl^- ions are taken into account. However, it is known that for concentrations lower than $10^{-4.5}$ M KCl, ions (H^+ and HCO_3^-) from dissolved CO_2 significantly enhance the conductivity of KCl solutions.^{36,37} Therefore, in a silica nanochannel, the K^+ counterions are not the only counterions neutralizing the negative surface charge. At low salt concentrations, protons (H^+) also contribute to the electrical transport. In fact, K^+ contribution to the electrical conductivity of the solution decreases as the KCl concentration decreases towards 10^{-7} M (DI water), where the electrical double layer (EDL)²⁵ is formed only by H^+ .³⁸ Because H^+ have a uniquely high mobility, about five times higher than common salt ions in bulk solutions (K^+ and Cl^-), the constant charge models predict that the conductance in the nanochannels is not constant at low salt concentrations but that it decreases with KCl concentration (from DI water to 10^{-4} M KCl the conductance ought to decrease about five times), which goes against the experimental evidence.

In this paper, the model proposed by Schoch *et al.*^{34,35} is adapted to take into account the ionic species resulting from CO_2 dissolution in water. The resulting variable surface charge model developed was used to electrically characterize the walls of silica nanochannels from the experimentally measured electrical conductances. Label-free electrical detection of molecular films immobilized on the walls of nanochannels was also performed. A shift in the electrical conductance plateau, which according to the model is associated with surface charge modifications, was observed after functionalization of nanochannels with 3-aminopropyltrimethoxysilane (APDMES) and single stranded DNA (ssDNA). An additional method used to modify the surface charge of bare silica nanochannels was by changing the pH inside the nanochannel. The pH dependence of the surface charge qualitatively agreed with the equilibrium model of protons reacting with the charge determining silanol (SiOH) groups at silica surface.

II. THEORY

A. Electrical double layer in nanochannels

A solid surface when immersed in an aqueous solution becomes spontaneously charged. The surface charge present at the solid-liquid interface is created either by ionic adsorption to the surface or ionization of functional groups at the surface. For the interface region to remain neutral the surface charge is balanced by the redistribution of ions in the solution close to the

charged surface. The result is the formation of a charged fluidic layer with an excess of counter-ions and a deficit of co-ions known as electrical double layer,²⁵ next to the solid surface. The characteristic length of the EDL is called the Debye length λ_D and for a symmetric monovalent electrolyte it is given by³⁹

$$\lambda_D = \sqrt{\frac{\epsilon_0 \epsilon_r K_B T}{2000 (N_A e)^2 C}}, \quad (1)$$

where ϵ_0 is the permittivity of free space, ϵ_r is the dielectric constant of the electrolyte, K_B is the Boltzmann constant, T is the absolute temperature, e the elementary charge, N_A is the Avogadro number, and C is the bulk molar concentration. According to Eq. (1), the value of λ_D decreases as the bulk concentration of free ions increases. For typical electrolyte concentrations ($10^{-1} \text{ M} \geq C \geq 10^{-5} \text{ M}$), λ_D varies in the range of 1–100 nm. Outside the EDL, the bulk solution is neutral (i.e., has equal densities of cations and anions).

Nanochannels differ from microchannels in the ratio of the thickness of the EDLs relative to the channel height. Unlike the case with microfluidics, in nanofluidics the condition that $2\lambda_D$ is much smaller than the channel height does not necessarily apply. At high electrolyte concentrations (Fig. 1(a)), $2\lambda_D$ is smaller than the nanochannel height h (typically between 10 and 100 nm). However, when diluted ionic solutions are used, $2\lambda_D$ can easily become comparable to the nanochannel height h and consequently the diffusive part of the EDL from the top and bottom surfaces overlap (Fig. 1(b)). In the regime of low ionic concentrations, there is an excess of counterions inside the channel because the surface charge of the nanochannel attracts counterions for its neutralization, whereas co-ions are electrostatically repelled.

B. Electrical conductance of electrolytes in nanochannels

Application of a voltage gradient ΔV across the nanochannel generates a net ionic current I . As the electric field acts upon the ions making up the EDLs and the bulk solution, I will correspond to the sum of the current contribution from the EDL, I_{EDL} , and the bulk current contribution I_{bulk} (Fig. 2(a)):

$$I = I_{EDL} + I_{bulk}. \quad (2)$$

The conductance G of the nanochannel, considering Eq. (2), is given by the superposition of the EDL and bulk conductances

$$G \equiv \frac{I}{\Delta V} = \frac{I_{EDL} + I_{bulk}}{\Delta V} = G_{EDL} + G_{bulk}. \quad (3)$$

At high ionic concentrations, $2\lambda_D \ll h$. In this case, the ionic transport is dominated by the ions in the bulk solution (since $I_{EDL} \ll I_{bulk}$) and $G \approx G_{bulk}$. When G corresponds to the bulk

FIG. 1. Schematic of the EDLs in a nanochannel ($h \sim 50 \text{ nm}$) with negatively charged surfaces for different ionic concentration regimes. (a) At high ionic concentration, the EDLs (solid curved line) are thin when compared with the height h of the channel. (b) At low ionic concentration, the EDLs of top and bottom walls are thicker than h , and the EDLs overlap strongly (solid curved line) and the nanochannel is filled with a unipolar solution of counterions. Dashed lines represent the charge distribution of each EDL considering that the opposite wall was not present.

FIG. 2. Nanochannel ionic conductance G . (a) Equivalent electric circuit model used to describe G . G results from the sum of two components: EDLs conductance and bulk conductance. (b) Schematic behavior of G as a function of the electrolyte concentration. At high C , G is dominated by the bulk conductance (G increases with C and h) whereas at low C , G is dominated by the surface charge and saturates (assuming σ is approximately constant) at a value independent of C and h . The asymptotic limits of G (represented in solid lines) are extended to the regime where $\lambda_D \sim h$ (dashed and dotted line) to illustrate the enhancement of G at low C in a charged nanochannel (dashed line) relatively to a non-charged channel filled with an electrolyte solution keeping its bulk properties (dotted line).

component G_{bulk} , its value will depend on the nanochannel dimensions and electrolyte concentration C according to the following expression (neglecting electro-osmotic effects):^{34,35}

$$G \approx G_{bulk} = 10^3 (\mu_+ + \mu_-) C N_A e \frac{n w h}{L}, \quad (4)$$

where n is the number of nanochannels and μ is the ionic mobility (subscripts denote cation and anion).

At very low ionic concentrations, the situation is fundamentally altered since $2\lambda_D \gg h$. In this case the nanochannels are mainly filled with the counterions that neutralize the surface charge. Hence, counterion transport in EDLs dominates the current I created by ΔV (i.e., $I_{EDL} \gg I_{bulk}$) and $G \approx G_{EDL}$. To maintain the charge neutrality inside the nanochannel, an excess of counterions in the EDLs develops to compensate the net surface charge $Q_{surface}$, $Q_{surface} = Q_{solution}$. The surface charge governs the excess counterion concentration C_e inside the channel ($C_e \equiv C_{opp} - C_{eq}$, i.e., C_e the difference in concentration between counterions C_{opp} and co-ions C_{eq} inside the nanochannels) which is given by^{34,35}

$$Q_{surface} = Q_{solution} \iff 2 A |\sigma| = V e (1000 N_A C_e) \iff C_e = 10^{-3} \left(\frac{2 |\sigma|}{h N_A e} \right), \quad (5)$$

where σ is the surface charge density of the nanochannel. Assuming that the EDLs strongly overlap to the extent that the channel becomes a unipolar solution of counterions (i.e., $C_{opp} \gg C_{eq} \rightarrow C_e \approx C_{opp}$) and that the ion distribution is approximately uniform, then G_{EDL} at very low C can be calculated by^{34,35}

$$G \approx G_{EDL} = 10^3 \mu_{opp} C_e N_A e \frac{n w h}{L} = 2 \mu_{opp} |\sigma| \frac{n w}{L}, \quad (6)$$

where μ_{opp} is the ionic mobility of the counterions of opposite charge to the surface charge.

Equations (4) and (6) show the distinctive signatures of G for the two regimes of solutions of different ionic concentrations. At high ionic concentration, G depends on h and increases linearly with ionic concentration C whereas at low ionic concentrations G is governed by $|\sigma|$ and is independent of both C and h (Fig. 2(b)).

III. MATERIALS AND METHODS

A. General design considerations

To facilitate the fluid supply to the nanochannels, microfluidic channels have been used. Using microfluidic channels to access the nanofluidic channels allows a progressive increase in

channel size first from nanometer to micrometer scale and then to millimeter dimensions (Fig. 3(a)). The configuration used consisted of two polydimethylsiloxane (PDMS) access microchannels which are connected by silica nanochannels (Figs. 3(b) and 3(c)). The glass substrate and the silica capping layer of the nanochannels are optically transparent and electrically insulating. In addition, the nanochannel terminations are isolated from each other through a PDMS barrier for electrical measurements across the nanochannels. The array of parallel nanochannels is oriented perpendicularly to both microchannels (Fig. 3(c)). In each sample, an array of parallel nanochannels has been fabricated to lower the nanochannel impedance ($\ll 10^8 \Omega$) when working with low ionic strength solutions. Flow along the microchannels, which are perpendicular to the orientation of the nanochannels, allows a continuous supply of liquid to the nanochannels from both their extremities and prevents fouling at the nanochannel entrances. The electric contact through the solution across the nanochannels is provided by integrated electrodes located at the bottom of each microchannel and close to the nanochannels entrances to improve the sensitivity (this configuration minimizes the parasitic impedance of the electrolyte in the microchannels).

B. Nanofluidic device fabrication

The fabricated nanochannels have the following dimensions: height, h , between 30 nm and 50 nm; width, w , between $5 \mu\text{m}$ and $20 \mu\text{m}$; and length, L , of $40 \mu\text{m}$. Arrays of nanochannels were fabricated on glass substrates by surface micromachining using silica (SiO_2) as the nanochannel structural material and Al as the sacrificial material. The nanochannels fabrication process (Fig. 4) begins with the deposition of 50–100 nm of Al by DC magnetron sputtering on top of a cleaned Corning Eagle 2000 glass substrate. The Al is then patterned by optical lithography followed by wet etching to form the sacrificial layer. The thickness of the Al sacrificial layer was measured using a profilometer to obtain a precise measure of the final nanochannel height. Next, SiO_2 ($2 \mu\text{m}$ thick) was deposited by PECVD, patterned and etched by reactive ion etching. The Al sacrificial layer could then be accessed and selectively removed from beneath the SiO_2 capping layer by sample immersion in Al etchant solution at 40°C . Once the Al is removed, the devices are rinsed with DI water and dried. Integrated TiW electrodes were

FIG. 3. Nanofluidic device. (a) Photograph of the complete nanofluidic device. (b) 3-D schematic of the nanofluidic device showing the configuration of the two microchannels and of the electric contacts. (b) Optical micrograph showing the array of nanochannels bridging the two microchannels used to convey the solutions to the nanochannels. Electrodes positioned at both nanochannels terminations allowed the electrical contact across the nanochannels. (c) SEM micrograph of a nanochannel entrance. (d) SEM micrograph showing a cross-sectional view of a nanochannel.

FIG. 4. Nanofluidic device microfabrication. The microfabrication process presented has the following critical issues: the thickness and width of the Al sacrificial layer define the height and the width of the nanochannel, respectively; the RIE of the SiO₂ layer defines the length of the channel; PDMS microchannels convey the liquid to the hydrophilic SiO₂ nanochannel.

deposited by DC magnetron sputtering and defined by lift-off. Subsequently, the samples were diced.

PDMS microchannels with typical dimensions of $h = 5 \mu\text{m}$, $w = 150 \mu\text{m}$, and $L = 1 \text{ cm}$ are fabricated using the replica molding technique. In replica molding, the negative of the three-dimensional topography of a patterned solid surface (defined as mold) is transferred to a PDMS replica. An SU-8 mold is obtained by exposing the negative photoresist SU-8 to UV light through an Al/quartz shadow mask.

The next step consists of treating the processed samples and the PDMS microfluidic structures with UV/Ozone for surface activation. The oxidized surfaces are bonded together by bringing them into contact. During the bonding process, a precise alignment between the nanochannel array and the PDMS microchannels is achieved by placing a DI water droplet in between the two pieces and then performing the alignment under a microscope. The ensemble is left to dry overnight. The final device consists of: (i) two PDMS access microchannels; (ii) the PDMS microchannels are connected by the nanochannels which served as the only electrical and fluidic connection between them; and (iii) electrodes located near the nanochannel openings, at the bottom of the microchannels (Fig. 4).

C. Impedance spectroscopy

1. Impedance spectroscopy measurements

The measurements were performed with KCl solutions with molar concentrations ranging from 1M to 10^{-7} M (DI water), which correspond to λ_D varying from 0.3 nm to 200 nm, respectively. KCl solutions were prepared by serial dilutions of 1M stock solution with DI water ($18 \text{ M}\Omega \text{ cm}$). Before filling the device with the aqueous solutions, the microchannels were flushed with ethanol (EtOH). This procedure was required because the nanochannels could not be completely filled with aqueous solutions via capillary action from the microchannels (Fig. 5(b)). The incomplete filling indicates that the silica walls were not fully wetting. To circumvent this problem, EtOH was used to initially fill the microchannels. EtOH has a much

FIG. 5. (a) Empty nanochannels array. (b) Direct injection of aqueous solution into the microchannels. Many nanochannels do not get filled by the aqueous solution. (c) EtOH immediately fills all nanochannels by capillarity.

lower surface tension than water (21.55 mN m^{-1} for EtOH against 72 mN m^{-1} for water) and instantly fills the nanochannels by capillarity. Then, the microchannels were flushed with the desired KCl solution that diffused into the nanochannels and replaced the EtOH. All solutions were introduced into the microchannels with the help of a syringe pump.

The integrated electrodes making contact with the solution were connected to a Hewlett Packard 4284A precision LCR meter for impedance spectroscopy measurements. Impedance was acquired in the range of 20 Hz–2 MHz with a 50 mV peak-to-peak sinusoidal voltage (small voltages are required to avoid electrochemical reactions at the electrodes) and a DC bias voltage set to 0 V. Before the measurements, the LCR meter was calibrated to eliminate parasitic effects. This compensation was achieved by scanning and recording the open and the short-circuit impedance response of a glass substrate with open-circuit and short-circuit electrodes. A chip holder (not shown) made of poly-methyl-methacrylate (PMMA), with integrated spring-loaded contacts was used to allow fast and easy connections between the fluidic chip and the measurement unit. To shield the devices from electromagnetic interference, a homemade Faraday cage was used. Impedance measurements were carried out once the filled nanochannels reached equilibrium, defined as the point when nanochannel resistance value stabilizes (i.e., the relative deviation between two consecutive measurements was less than 1%). Sequential impedance measurements for different KCl concentrations were performed from lower to higher salt concentrations to avoid ionic contamination.

2. Resistance of nanochannels

Using the simple equivalent circuit shown in Fig. 6, it was possible to model the physical system consisting of the nanochannels, electrolyte solution, and measurement electrodes. The

FIG. 6. Schematic side view of the microchannels linked by nanochannels, planar electrodes, with their corresponding electrical circuit elements. The equivalent electric circuit models the EDL at the electrodes by a constant phase element with impedance Z_{CPE} ; the nanochannels resistance by R_{sol} ; the parasitic capacitance of the substrate by C_{stray} ; and the capacitance associated to the solution by C_{sol} . The AC voltage supply, represented by V , applied to the equivalent circuit, drives an AC current I .

interfacial impedance between the electrodes and solution Z_{CPE} is associated to the EDL charging on the electrodes and is modeled by a constant phase element (CPE). R_{sol} represents the resistance of the solution in the nanochannels, C_{sol} is the solution capacitance between the electrodes through the solution, and C_{stray} corresponds to the capacitance between the electrodes through the glass substrate (i.e., the capacitance measured when the device does not contain electrolyte solution). The resistances of the microchannels have been neglected because it is much lower than that of the nanochannels.

Figure 7 presents typical Nyquist plots of the system under investigation, recorded for different electrolyte concentrations. Looking at Fig. 7(b), different regions in the impedance spectrum can be distinguished: at the low frequency limit, the straight line is attributed to the EDL impedance Z_{CPE} (present at the electrode-electrolyte interface); as the frequency increases, the impedance value touches the horizontal axis and this point corresponds to R_{sol} (in Fig. 7(b) $R_{sol} \approx 300 \text{ k}\Omega$ for 0.1M KCl); a further increase in frequency shows an impedance characterized by a half-circle (a behavior typically associated to a parallel RC circuit), which corresponds to the solution resistance R_{sol} in parallel with the parasitic capacitance C_{prs} ($C_{prs} \equiv C_{sol} + C_{stray}$). In Fig. 7, the different impedance plots shown look different, both quantitatively and qualitatively. Indeed, Fig. 7(a) lacks the semi-circle and Fig. 7(c) lacks the straight line, both present in Fig. 7(b). The frequency range of the equipment used, from 20 Hz to 2 MHz, is responsible for the truncated curves. A larger frequency range including frequencies both sufficiently large

FIG. 7. Typical Nyquist plots obtained for the impedance of an array of nanochannels ($n = 168$; $h = 30 \text{ nm}$; $w = 10 \text{ }\mu\text{m}$; $L = 40 \text{ }\mu\text{m}$) filled with different KCl concentrations, from which R_{sol} is determined (corresponding to the non-zero intersection point of the impedance curves with the horizontal axis): (a) for 1 M KCl solution, $R_{sol} \approx 30 \text{ k}\Omega$; (b) for 0.1 M KCl solution, $R_{sol} \approx 300 \text{ k}\Omega$; and (c) for 10^{-5} M KCl, solution $R_{sol} \approx 12 \text{ M}\Omega$.

and sufficiently small to reach the asymptotic impedance limits (characterized by a semi-circle at high frequencies and straight line at very low frequencies) would allow the acquisition of the full impedance spectrum with the features shown in Fig. 7(b). In other words, for 1M KCl (Fig. 7(a)), one would need frequencies above the 2 MHz limit of our measurement setup to reach the regime of the semi-circular curve and for 10^{-5} M KCl (Fig. 7(c)), one would need to reach frequencies lower than 20 Hz to measure the linear part of the curve. Even for 0.1M KCl (Fig. 7(b)), ideally the frequency range should be larger to allow visualization of a complete semi circle (as in Fig. 7(c)). In fact, Fig. 7(b) lacks the asymptotic limit $\omega \rightarrow \infty$ in which the impedance tends to zero in a regime where the total parasitic capacitance C_{prs} dominates the measurements (the electric field oscillates so fast that the ions in solution can no longer follow).

A Nyquist type of visualization of the impedance data was used to allow the determination of the nanochannel resistance from the impedance measurements. From Fig. 7, R_{sol} can be readily determined for the three electrolyte concentrations, corresponding to the non-zero intersection point of the impedance curves with the horizontal axis Re Z.

D. Nanochannels surface charge modification

The nanochannel surface modification is schematically shown in Fig. 8. Silica nanochannels with hydroxyl groups at the surface were functionalized with silane 3-APDMES. The microchannels were filled with a 10% v/v solution of APDMES in EtOH and left to incubate overnight. During this period, the etoxy groups of APDMES react with the surface hydroxyl groups of silica and a covalent bond is formed. The resultant APDMES monolayer bears a positive net charge due to its amine termination. The non-reacted APDMES molecules were washed away by flushing EtOH for 10 min. Finally, to electrostatically immobilize the ssDNA on the silanized nanochannels, one of the microchannels was filled with a 5 μ M ssDNA solution and the other microchannel with phosphate buffer saline (PBS) 100mM (pH 7.4). Incubation took approximately 3 h (Fig. 9(a)) during which time the ssDNA in solution became electrostatically immobilized because of its opposite charge with respect to APDMES layer. Following immobilization, the excess of ssDNA was washed with DI water flow for 10 min.

IV. RESULTS AND DISCUSSION

A. Biomolecular immobilization in nanochannels

The resistance R_{sol} measured for different KCl concentrations (1 M to 10^{-7} M) was converted into conductance G using

$$G \equiv \frac{1}{R_{sol}}. \quad (7)$$

FIG. 8. Schematic of the interfacial structure of ssDNA immobilized, via electrostatic interactions, on an APDMES monolayer grafted to a glass or silica substrate.

FIG. 9. Fluorescent micrographs of nanofluidic device for different steps of ssDNA immobilization protocol. (a) Bottom and top microchannels filled with ssDNA solution and PBS, respectively. SsDNA entered the nanochannels by diffusion. (b) After washing the microchannels with DI water, the ssDNA remained immobilized inside the nanochannels. The ssDNA molecules are labeled with the fluorophore FITC for fluorescence microscopy visualization.

Figure 10 shows the nanochannel conductance values for several KCl concentrations C . The curves represent the conductance of bare SiO_2 nanochannels, APDMES functionalized SiO_2 nanochannels, and ssDNA immobilized on APDMES treated SiO_2 nanochannels. These chemical surface modifications were performed sequentially with the same device, with the following nanochannel array features: 168 nanochannels; $h = 30 \text{ nm}$; $w = 10 \text{ }\mu\text{m}$; $L = 40 \text{ }\mu\text{m}$. After each treatment, the nanofluidic device was filled with different KCl concentrations and the nanochannel resistance was measured for each concentration. Figure 10 also presents the conductance values expected for a hypothetical non-charged nanofluidic device. In this case, there would be no EDL and consequently the surface conductance would be zero and according to Eq. (3) $G = G_{\text{bulk}}$, for every C . The conductance of non-charged nanochannels was measured for real KCl solutions (Fig. 10, open hexagons) and calculated for ideal KCl solutions (Fig. 10, solid

FIG. 10. Measured nanochannel conductance before and after surface modification as a function of KCl concentration. Full squares are the bare nanochannel array conductance. Full circles and triangles indicate the device conductance after APDMES silanization and subsequent ssDNA immobilization, respectively. Solid line and open hexagons are different conductance models for a non-charged array of nanochannels. The difference between the curves is that the solid line assumes the ionic content of the KCl solutions to have only K^+ and Cl^- contributions, while the hexagons consider also the H^+ and HCO_3^- ions, resultant from the CO_2 dissolution in water. Dashed lines connecting the data points are given as guides to the eye.

line). For real KCl solutions, the non-charged nanochannels conductance was obtained by the formula

$$G = \gamma \frac{nwh}{L}, \quad (8)$$

where n is the number of nanochannels and γ the solution conductivity (determined experimentally with the aid of a conductometer). The solid line in Fig. 10 was obtained with Eq. (8) but the conductivity γ was theoretically calculated by^{5,34,35}

$$\gamma = 10^3 (\mu_{K^+} + \mu_{Cl^-}) C N_A e, \quad (9)$$

where μ_{K^+} and μ_{Cl^-} are the ionic mobility of K^+ and Cl^- ions, respectively. The conductance for the non-charged nanochannels obtained in these two different ways is close to measured values until the concentration of KCl solutions reaches a low level ($C < 10^{-4}$ M). The reason for the conductance increase with real KCl solutions at low C lies in the fact that the atmospheric CO_2 dissolves in the water and increases its ionic concentration in solution. The reference electrical conductivity of the DI water system was $0.055 \mu S cm^{-1}$, whereas the conductivity of the DI water measured immediately after being turbulently drawn from the purification system was $1 \mu S cm^{-1}$. This rapid increase of conductivity suggests that saturation of water with CO_2 occurs in a very short time (less than a second). Once ultra-pure DI water ($\gamma = 0.055 \mu S cm^{-1}$, $pH = 7$) comes into contact with air, the concentration of H^+ increases from 10^{-7} M ($[H^+] = [OH^-] = 10^{-7}$ M due to water self-dissociation) to 3.1×10^{-6} M. The latter value was determined considering the average pH of the solutions ($pH \sim 5.5$) and using $[H^+] = 10^{-pH} \times N_A$. Unlike H^+ ions, the concentration of OH^- decreases from 10^{-7} M to $10^{-8.5}$ M because of water self-dissociation, in such a way that $[H^+] \times [OH^-] = 10^{-14}$. Thus, for DI water in equilibrium with the atmosphere, the ionic composition can be estimated to be essentially $[H^+] = [HCO_3^-] = 3 \times 10^{-6}$ M (neglecting the presence of OH^-). Therefore, for $C < 10^{-4}$ M, the conductance is dominated by the ions present in DI water (in equilibrium with atmosphere); H^+ particularly affects the conductance in that range due to its very high mobility (because of its very small size), which is about 5 times higher than K^+ or Cl^- . When comparing the conductance data obtained for hypothetical non-charged nanochannels (Fig. 10, solid line and open hexagons) with the conductance obtained for real charged nanochannels (the remaining data in Fig. 10), one can see that the latter show a conductance offset (2–3 orders of magnitude at $C = 10^{-6}$ M) relative to the former at low C . The conductance plateau observed for the charged nanochannels at diluted solutions ($C \leq 10^{-4}$ M) is due to the overlap of the EDLs (since for $C \leq 10^{-4}$ M $\rightarrow 2\lambda_D \geq 60 nm > h$) inside the nanochannels. The EDL overlap causes conductance to be dominated by the excess of counterions inside the nanochannels (i.e., $G \approx G_{EDL}$) to equilibrate the surface charge. Conversely, for high ionic concentrations ($C \geq 10^{-2}$ M), all the data depicted in Fig. 10 coincide (for every case $G \approx G_{bulk}$), indicating that the surface charge effects do not dominate at high C and that the solution inside the nanochannels has bulk properties (indeed, $C \geq 10^{-2}$ M $\rightarrow 2\lambda_D \leq 6.2 nm < h$). The nanometric height $h = 30 nm$ of the nanochannels was confirmed using Eq. (4) together with the dimensions ($w = 10 \mu m$, $L = 40 \mu m$, and $n = 168$) of the lithographically defined channels and the conductance measured at high C .

The conductance results (Fig. 10) obtained for the nanofluidic device with SiO_2 nanochannels before and after the chemical surface modifications (APDMES silanization and ssDNA immobilization) differ essentially in the low ionic concentration regime ($C \leq 10^{-4}$ M KCl). This difference is characterized by a shift of the plateau conductance. It will be shown that such behavior suggests that the surface charge changed after each of the molecular surface immobilization steps. At high C (i.e., $C \geq 10^{-2}$ M KCl), no significant deviations are observed, which means (by Eq. (4)) that APTES and ssDNA films did not significantly decrease the effective height of the silica nanochannels. A quantitative analysis of the results presented in Fig. 10 will be provided next.

1. Bare silica nanochannels

The magnitude of the surface charge density was estimated for bare silica nanochannels at KCl concentrations varying from 10^{-7} M (DI water) up to 10^{-4} M. It is known that bare SiO_2 is negatively charged when in contact with aqueous solutions ($\text{pH} > 3$).⁴⁰ It is, therefore, expected that inside the bare SiO_2 nanochannels the counterions are positively charged (H^+ and K^+ being the only counterions expected to be present in solution) to maintain electroneutrality. When $C \leq 10^{-4}$ M, the EDLs inside nanochannels overlap (i.e., $2\lambda_D \geq h$), and hence the co-ion concentration can be neglected (assuming that the local concentration of each ion follows an equilibrium Boltzmann distribution and considering the expression of the potential between two parallel plates, we estimated the counterion to co-ion concentration ratio inside the nanochannels to be higher than 40 for $C \leq 10^{-4}$ M) and the excess counterion concentration C_e can be approximated as the sum of counterions inside the nanochannels

$$C_{\text{H}^+ \text{ nano}} + C_{\text{K}^+ \text{ nano}} \approx C_e. \quad (10)$$

The subscripts specify the type of ion and whether they are inside (nano) or outside the nanochannel in the microchannel (bulk). So, in the nanochannels, when $C \leq 10^{-4}$ M ($2\lambda_D \geq h$) both cations, K^+ and H^+ , contribute to the ion transport. When there is more than one type of counterions filling the nanochannel, Eq. (6) cannot be applied directly and has to be adapted accordingly. To determinate the counterion concentration ratio $C_{\text{H}^+ \text{ nano}}/C_{\text{K}^+ \text{ nano}}$, one can use the H^+ bulk conductance (G_{H^+}) to K^+ bulk conductance (G_{K^+}) ratio since

$$\frac{G_{\text{H}^+ \text{ bulk}}}{G_{\text{K}^+ \text{ bulk}}} = \frac{10^3 \mu_{\text{H}^+} C_{\text{H}^+} N_A e n w h / L}{10^3 \mu_{\text{K}^+} C_{\text{K}^+} N_A e n w h / L} \iff \frac{C_{\text{H}^+ \text{ bulk}}}{C_{\text{K}^+ \text{ bulk}}} = \left(\frac{G_{\text{H}^+ \text{ bulk}}}{G_{\text{K}^+ \text{ bulk}}} \right) \times \left(\frac{\mu_{\text{K}^+}}{\mu_{\text{H}^+}} \right) = \frac{C_{\text{H}^+ \text{ nano}}}{C_{\text{K}^+ \text{ nano}}}. \quad (11)$$

Note that in Eq. (11) the ratio $C_{\text{H}^+ \text{ bulk}}/C_{\text{K}^+ \text{ bulk}}$ is made equal to $C_{\text{H}^+ \text{ nano}}/C_{\text{K}^+ \text{ nano}}$ despite $C_{\text{H}^+ \text{ bulk}}$ not being necessarily equal to $C_{\text{H}^+ \text{ nano}}$ and $C_{\text{K}^+ \text{ bulk}}$ to $C_{\text{K}^+ \text{ nano}}$ (nanochannel and bulk ionic concentration difference is explained by the Donnan equilibrium theory (Sata, 2004 #1978)). The ratio of the two counterions concentrations should, however, remain approximately constant independent of whether it is referring to the nanochannels or microchannels, since apart from their relative availability in the bulk solution (given by the ratio $C_{\text{H}^+ \text{ bulk}}/C_{\text{K}^+ \text{ bulk}}$) there is no apparent reason for the nanochannels to show any preference for either of the counterions (H^+ or K^+) since they have the same valence and therefore shield equally the surface charge. Here, we neglect potential transient proton-cation exchange effects, described by Duan and Majumdar²⁶ and resulting from the H^+ ion having a higher diffusivity than the K^+ ion, by considering that sufficient time was allowed for equilibrium in the nanochannel to be reached (in our case, we experimentally observed that the conductance stabilized after a couple of hours for low electrolyte concentrations). To calculate $G_{\text{H}^+ \text{ bulk}}/G_{\text{K}^+ \text{ bulk}}$, the conductance data from the non-charged nanochannel (the conductance G_{exp} found using the conductivity of real KCl solutions and the conductance G_{theo} found using the conductivity of ideal KCl solutions non-contaminated by atmospheric CO_2) and the following equation were used

$$G_{\text{exp}} = \underbrace{(G_{\text{K}^+} + G_{\text{Cl}^-})}_{G_{\text{theo}}} + G_{\text{H}^+} + G_{\text{HCO}_3^-} \iff G_{\text{H}^+} + G_{\text{HCO}_3^-} = G_{\text{exp}} - G_{\text{theo}}. \quad (12)$$

To calculate G_{H^+} , one has to take into account that

$$\frac{G_{\text{H}^+}}{G_{\text{HCO}_3^-}} = \frac{\mu_{\text{H}^+}}{\mu_{\text{HCO}_3^-}} \iff G_{\text{HCO}_3^-} = \left(\frac{\mu_{\text{H}^+}}{\mu_{\text{HCO}_3^-}} \right) \times G_{\text{H}^+}. \quad (13)$$

Substituting Eq. (13) in Eq. (12) results in the conductance $G_{\text{H}^+ \text{ bulk}}$

$$G_{\text{H}^+} = \left(\frac{\mu_{\text{H}^+}}{\mu_{\text{H}^+} + \mu_{\text{HCO}_3^-}} \right) \times (G_{\text{exp}} - G_{\text{theo}}). \quad (14)$$

In order to calculate G_{H^+bulk}/G_{K^+bulk} , the conductance G_{K^+bulk} needs to be calculated as well. By recognizing that $G_{theo} \equiv G_{Cl^-} + G_{K^+}$ and $G_{Cl^-} \approx G_{K^+}$, it follows that

$$G_{K^+} = G_{theo}/2. \quad (15)$$

Substitution of Eqs. (14) and (15) in Eq. (11) gives the ratio of the counterions concentration inside nanochannels (defined hereafter as Ψ)

$$\Psi \equiv \frac{C_{H^+nano}}{C_{K^+nano}} = \left(\frac{G_{H^+bulk}}{G_{K^+bulk}} \right) \times \left(\frac{\mu_{K^+}}{\mu_{H^+}} \right) = 2 \times \left(\frac{\mu_{H^+}}{\mu_{H^+} + \mu_{HCO_3^+}} \right) \times \left(\frac{G_{exp} - G_{theo}}{G_{theo}} \right) \times \left(\frac{\mu_{K^+}}{\mu_{H^+}} \right). \quad (16)$$

Inserting Eq. (16) in Eq. (10) one arrives at

$$C_{K^+nano} = \left(\frac{1}{\Psi + 1} \right) \times C_e \quad (17)$$

and

$$C_{H^+nano} = \left(\frac{\Psi}{\Psi + 1} \right) \times C_e. \quad (18)$$

The total conductance G inside the negatively charged nanochannels, at low C , is due to the transport of both H^+ and K^+ counterions and given by

$$G = G_{H^+nano} + G_{K^+nano} = 10^3 N_A e \frac{nwh}{L} (\mu_{H^+} C_{H^+nano} + \mu_{K^+} C_{K^+nano}). \quad (19)$$

Equation (19) can be written as

$$\begin{aligned} G &= 10^3 N_A e \frac{wh}{L} C_e \left[\mu_{H^+} \left(\frac{\Psi}{\Psi + 1} \right) + \mu_{K^+} \left(\frac{1}{\Psi + 1} \right) \right] \\ &= \frac{2nwh|\sigma|}{L} \left(\mu_{H^+} \left(\frac{\Psi}{\Psi + 1} \right) + \mu_{K^+} \left(\frac{1}{\Psi + 1} \right) \right), \quad \text{if } \sigma < 0, \end{aligned} \quad (20)$$

considering Eqs. (17), (18), and (5). Equation (20) allows determination of $|\sigma|$ given a measured conductance G . Once $|\sigma|$ is known, the counterion concentration inside the nanochannels can be found using

$$C_{H^+nano} = \left(\frac{\Psi}{\Psi + 1} \right) \times C_e = \left(\frac{\Psi}{\Psi + 1} \right) \times 10^{-3} \times \frac{2|\sigma|}{hN_A e}, \quad \text{if } \sigma < 0 \quad (21)$$

and

$$C_{K^+nano} = \left(\frac{1}{\Psi + 1} \right) \times C_e = \left(\frac{1}{\Psi + 1} \right) \times 10^{-3} \times \frac{2|\sigma|}{hN_A e}, \quad \text{if } \sigma < 0. \quad (22)$$

Equations (21) and (22) allow calculation of C_{H^+nano} and C_{K^+nano} . The last two equations follow directly from substitution of Eq. (5) into Eqs. (17) and (18).

For bare SiO_2 nanochannels, at 10^{-7} M KCl (DI water) $\Psi \equiv C_{H^+nano}/C_{K^+nano} \approx 19$ (calculated by substitution of $G_{exp} \approx 10^{-10}$ S m⁻¹ and $G_{theo} \approx 2 \times 10^{-12}$ S m⁻¹ in Eq. (16)), from which it may be concluded that the nanochannels are filled mostly with H^+ ions. Equation (20) was then used to calculate $|\sigma| = 0.88$ mC m⁻² (parameters used: $G = 2.56 \times 10^{-8}$ S m⁻¹ and $\Psi = 19$) (Fig. 11 and Table I). Once $|\sigma|$ was computed, it could be employed to calculate C_{H^+nano} and C_{K^+nano} (Table I).

FIG. 11. Surface charge density σ vs. KCl concentration before and after the chemical surface modifications. Bare SiO_2 nanochannels (full squares) are negatively charged due to ionized silanol groups. Full triangles either pointing up or down represent the possible nanochannels surface charge densities after APDMES functionalization. In this case, positive or negative surface charges are possible. ssDNA immobilization results in a negatively charged surface, which is more negatively charged than the original bare SiO_2 surface. Dashed lines connecting the data points are given as guides to the eye. Data at 10^{-7} M KCl represent the conductance in DI water. For higher KCl concentrations, the conductance is independent of surface charge since $2\lambda_D < h$ (given by Eq. (4)).

At 10^{-5} M KCl, $G_{exp} = 7.8 \times 10^{-10} \text{ S m}^{-1}$ and $G_{theo} = 1.88 \times 10^{-10} \text{ S m}^{-1}$, hence the sought $\Psi \equiv C_{H^+ nano} / C_{K^+ nano} \approx 1.2$ (i.e., inside the nanochannels, the concentration of H^+ and K^+ is approximately the same). Substitution of this ratio in Eq. (20) yields $|\sigma| = 0.87 \text{ mC m}^{-2}$ (parameters used: $G = 1.69 \times 10^{-8} \text{ S m}^{-1}$ and $\Psi = 1.2$) (Fig. 11 and Table I). Table I also shows the concentration of the different counterions within the nanochannel, determined with the help of Eqs. (21) and (22).

Increasing the KCl concentration to 10^{-4} M, G_{exp} and G_{theo} change to $2.26 \times 10^{-9} \text{ S m}^{-1}$ and $1.88 \times 10^{-9} \text{ S m}^{-1}$, respectively. As a result, $\Psi \approx 0.07$ (Eq. (16)). Substitution of this ratio and $G = 2.00 \times 10^{-8} \text{ S m}^{-1}$ in Eq. (20) yields $|\sigma| = 2.51 \text{ mC m}^{-2}$ (Fig. 11 and Table I). The $C_{H^+ nano}$ and $C_{K^+ nano}$ found for bare silica nanochannels at $C \leq 10^{-4}$ M are shown in Table I.

2. APDMES silanized nanochannels

The downward sloping conductance plateau obtained after the grafting of positively charged APDMES to silica does not allow determination of the net surface polarity since the model can only provide information on the absolute value of the surface charge density $|\sigma|$. Positively charged APDMES could have either simply decreased the silica net negative surface charge or inverted the surface charge. In the first case, the surface of the nanochannels would still be negatively charged, and consequently the surface charge could be obtained, for $C \leq 10^{-4}$ M, in exactly the same way as described in the previous section for bare SiO_2 nanochannels, using Eq. (20). The calculated surface charge densities are shown in Fig. 11 and Table I. However, if the net surface charge is positive, the method from the previous section has to be slightly modified. One must consider that nanochannels after APDMES functionalization can bear a positive net charge. Taking this into consideration, the surface charge screening counterions must be negatively charged. Both counterions, Cl^- and HCO_3^- , can contribute to the nanochannel conductance. Consequently, the excess counterion concentration C_e is given by

TABLE I. Calculated values of the surface charge density σ , and of the counterion concentration inside the nanochannels, before and after each chemical surface modification (leftmost column), for different electrolyte concentrations (top row).

	C (M)			C (M)			C (M)		
	10^{-7}			10^{-5}			10^{-4}		
Surface	σ (mC m ⁻²)	$C_{H^+ nano}$ (M)	$C_{K^+ nano}$ (M)	σ (mC m ⁻²)	$C_{H^+ nano}$ (M)	$C_{K^+ nano}$ (M)	σ (mC m ⁻²)	$C_{H^+ nano}$ (M)	$C_{K^+ nano}$ (M)
Glass	-0.88	5.8×10^{-4}	3.0×10^{-5}	-0.87	3.3×10^{-4}	2.7×10^{-4}	-2.51	1.1×10^{-4}	1.6×10^{-3}
APDMES ($\sigma < 0$)	-0.18	1.2×10^{-4}	6.2×10^{-6}	-0.34	1.3×10^{-4}	1.1×10^{-4}	-0.97	4.4×10^{-5}	6.2×10^{-4}
ssDNA	-1.27	8.3×10^{-4}	4.4×10^{-5}	-1.53	5.8×10^{-4}	4.8×10^{-4}	-3.77	1.7×10^{-4}	2.4×10^{-3}
Surface	σ (mC m ⁻²)	$C_{HCO_3^- nano}$ (M)	$C_{Cl^- nano}$ (M)	σ (mC m ⁻²)	$C_{HCO_3^- nano}$ (M)	$C_{Cl^- nano}$ (M)	σ (mC m ⁻²)	$C_{HCO_3^- nano}$ (M)	$C_{Cl^- nano}$ (M)
APDMES ($\sigma > 0$)	+1.31	8.6×10^{-4}	4.5×10^{-5}	+1.30	4.9×10^{-4}	4.1×10^{-4}	+1.19	5.4×10^{-5}	7.7×10^{-4}

$$C_{HCO_3^-} + C_{Cl^-} \approx C_e. \quad (23)$$

Considering Eq. (23) and the ratio $\Phi \equiv C_{HCO_3^- nano}/C_{Cl^- nano}$ instead of Eq. (10) and the ratio Ψ (Eq. (16)), it is possible to derive, in an analogous way, an equation similar to Eq. (20) which relates G with $|\sigma|$, but valid for positively charged surfaces:

$$G = \frac{2w|\sigma|}{L} \times \left[\left(\frac{\Phi}{\Phi+1} \right) \mu_{HCO_3^-} + \left(\frac{1}{\Phi+1} \right) \mu_{Cl^-} \right], \quad \text{if } \sigma > 0. \quad (24)$$

Additionally, the concentration of each counterion inside positively charged nanochannels is given by

$$C_{HCO_3^- nano} = 10^{-3} \times \frac{2|\sigma|}{hN_A e} \times \left(\frac{\Phi}{\Phi+1} \right), \quad \text{if } \sigma > 0 \quad (25)$$

and

$$C_{Cl^- nano} = 10^{-3} \times \frac{2|\sigma|}{hN_A e} \times \left(\frac{1}{\Phi+1} \right), \quad \text{if } \sigma > 0. \quad (26)$$

At 10^{-7} M KCl (DI water), $\Phi \approx 19$ (i.e., Cl^- concentration is negligible in comparison with HCO_3^- ionic concentration) and consequently $|\sigma| \approx 1.31$ mC m⁻² (by Eq. (24)). At 10^{-5} M KCl, $\Phi \approx 1.2$, which means that the concentrations of HCO_3^- and Cl^- are similar. With $\Phi \approx 1.2$ in Eq. (24), one arrives at $|\sigma| = 1.31$ mC m⁻². Increasing the KCl concentration to 10^{-4} M results in $\Phi \approx 0.07$, and the excess counterions are formed mostly by Cl^- . Substitution of $\Phi \approx 0.07$ in Eq. (24) gives $|\sigma| = 1.19$ mC m⁻². The surface charge densities found for the APDMES silanized nanochannels, for different KCl concentrations, are shown in Fig. 11. Table I presents the counterion concentration and the surface charge density inside the channels

The conductance plateau shifts observed in Fig. 10 after each surface modification step indicates that the surface has been successfully modified. At 10^{-4} M, the conductance (coincides with the plateau conductance value), unlike for lower C , is independent of the surface polarity (as a result, it is given by a single equation). Indeed, at 10^{-4} M, if $\sigma > 0$, then (by Eq. (24) and $\Phi \approx 0$) G is well approximated by

$$G \approx \frac{2nw\mu_{Cl^-}|\sigma|}{L}, \quad \text{if } \sigma > 0. \quad (27)$$

Conversely, if $\sigma < 0$, then G (Eq. (20) with $\Psi \approx 0$) may be expressed as

$$G \approx \frac{2nw\mu_{K^+}|\sigma|}{L}, \quad \text{if } \sigma < 0. \quad (28)$$

Since $\mu_{Cl^-} \approx \mu_{K^+} \approx \mu_{Cl^-/K^+} \equiv (\mu_{Cl^-} + \mu_{K^+})/2 = (7.9 + 7.6) \times 10^{-8}/2 \text{ m}^2 \text{ s}^{-1} \text{ V}^{-1}$, G at 10^{-4} M KCl can be summarized by the single equation

$$G \approx \frac{2nw\mu_{Cl^-/K^+}|\sigma|}{L} \quad \forall \quad \sigma, \quad (29)$$

independent of the surface polarity. Equation (29) predicts that if the surface charge density changes (for example through adsorption of biomolecules), then a conductance shift at 10^{-4} M should be observed. Since G is approximately constant for $C \leq 10^{-4} \text{ M}$, then the conductance plateau shift observed after APDMES functionalization means that the APDMES molecules successfully reacted with the silica surface. Note, however, that direct comparisons of absolute surface charge density based only on the conductance value is valid only for $C = 10^{-4} \text{ M}$. For lower C , these comparisons are not necessarily valid since apart from the dependence of $|\sigma|$ on C , $|\sigma|$ also depends on the surface polarity. Indeed, in Fig. 10, the plateau conductance of bare SiO_2 nanochannels is higher than APDMES, yet calculations showed that for $C \leq 10^{-5} \text{ M}$ the $|\sigma|$ of APDMES functionalized channels can be higher than for bare silica nanochannels provided that the APDMES treated nanochannels bear a positive charge (Fig. 11 and Table I). One should bear in mind that, for nanochannels with the same channel geometry, having a higher plateau conductance does not necessarily mean that for a given C (surface charge comparisons between distinct surfaces have to refer to the same electrolyte concentration since the surface charge of a given surface varies with electrolyte concentration) the magnitude of the surface charge density is higher. A sufficient condition for that is to know that the surfaces have the same polarity (according to either Eq. (20) or Eq. (24), depending on the surface polarity). However, when that condition is not met and one is not sure about the polarity of the surface, one has to calculate all the possible $|\sigma|$, assuming either that $\sigma > 0$ or $\sigma < 0$ (as in the case presented above of APDMES functionalized silica nanochannels).

Previously, most papers^{33–35} suggested that the absolute value of the surface charge density $|\sigma|$ was constant and that different surfaces could be compared by simply looking at the conductance plateau. The model presented here, by considering the presence of H^+ and HCO_3^- from CO_2 dissolution in aqueous solutions, suggests two new features of the system: first, that $|\sigma|$ varies with C (the factors Ψ and Φ in Eqs. (20) and (24) are responsible for that dependence since they decrease with C); and second, that $|\sigma|$ varies with surface polarity (according Eqs. (20) and (24) depending on the surface polarity of the nanochannels the counterions will be different and therefore present different ionic mobilities).

3. ssDNA immobilization in nanochannels

The increase in the absolute value of conductance after ssDNA immobilization means that at 10^{-4} M the net surface charge of the nanochannels increased (Eq. (29)). Since ssDNA is negatively charged (because of the phosphate groups), it can only contribute to an increase of the net negative charge. Hence, one can conclude that after ssDNA immobilization, the walls must be negatively charged. The surface charge densities found (using Eq. (20) and the conductance data in Fig. 10) for the nanochannels with ssDNA immobilized on the surface, for different KCl concentrations, are depicted in Fig. 11. Table I presents the counterion concentration and the surface charge density inside the channels.

4. Nanochannel surface charge regulation mechanism

The features presented above are distinct from most of the models in the literature that model the surface charge as being constant with KCl concentration.^{33–35} Although these models

predict that at low KCl concentrations the nanochannels surface charge dominates the conductance, they neglect the existence of an excess of H^+ and HCO_3^- over the rest of ions for solutions with KCl concentrations lower than 10^{-4} M. If the real ionic composition of the solutions is considered, these models fail to explain how the nanochannel conductance for DI water (H^+ dominated transport) is approximately the same as the conductance for 10^{-4} M KCl (K^+ dominated transport). In fact, since H^+ ions have a mobility five times higher than K^+ ions, the constant charge models predict that for DI water the conductance should be about the five times higher than for 10^{-4} M KCl, which goes against the experimental evidence (Fig. 10).

According to the model proposed in this paper, the conductance of the silica nanochannels at $C = 10^{-4}$ M KCl is similar and not lower than the conductance measured for $C \leq 10^{-5}$ M KCl, despite the lower K^+ mobility, because the absolute value of the surface charge increases, which attracts more counterions to the nanochannels (thereby increasing the total counterion excess concentration C_e , see Table I).

The negative charge of SiO_2 nanochannels increases with KCl concentration, slowly up to 10^{-5} M and faster as it reaches 10^{-4} M (Fig. 11 and Table I). This behavior can be understood through the surface charge regulation mechanism in oxides. Oxides have surface groups that take up or release H^+ depending on the pH. In the case of silica, the surface charge is governed dynamically by a SiOH dissociation reaction

The values in Table I show that by increasing the bulk KCl concentration, the $C_{H^+ nano}$ decreases. According to the chemical equilibrium of Eq. (30), a decrease of H^+ concentration inside the nanochannels will shift the surface reaction towards the dissociation of SiOH and hence the negative surface charge will increase, which agrees with our results (Table I). A native pH shift of electrolyte solutions of varying ionic strengths as they enter an array of nanochannels has been reported by Bottenus *et al.*⁴¹ At low ionic strengths, these authors observed a mean pH shift of 1 pH unit whereas at high ionic strengths the mean pH shift was reduced to 0.1 pH units.

A net negative surface charge after APDMES silanization can only mean that the density of ionized silanol groups (SiO^-) exceeds the density of ionized amino groups ($R-NH_3^+$). Consequently, the surface charge density should still be dominated by the chemical equilibrium in Eq. (30). The results for a negatively charged silanized nanochannels (Table I) agree with Eq. (30). If, however, APDMES functionalization results in an overall positive surface charge, then an excess of ionized amino groups is present at the surface and the surface charge should in this case be determined by the chemical equilibrium given by

According to Fig. 11, the surface charge is not affected by variations in the KCl concentration. A possible explanation could be that the variation of the KCl bulk concentration does not produce a significant variation of $C_{H^+ nano}$ because the counterions, HCO_3^- and Cl^- , do not interfere with the equilibrium in Eq. (31) that regulates the aminated surface charge. This analysis shows that neither of the two chemical equilibriums (Eq. (30) and Eq. (31)) contradicts the two initial hypotheses regarding the polarity of the APDMES treated nanochannels. Therefore, no conclusion can be taken as to the real surface charge density of the surface. The surface charge polarity of silanized nanochannels could be probed, independently from the conductance measurements, using for example ion enrichment/depletion tests or streaming current measurements.

As in bare SiO_2 nanochannels, the nanochannels with immobilized ssDNA show an increase of the surface charge with KCl concentration (Fig. 11 and Table I). The increase in ssDNA charge is accompanied by a pH increase (i.e., decrease of $C_{H^+ nano}$) inside the nanochannels (Table I), which is in agreement with the literature.⁴⁰

Note that, in Table I, for a fixed electrolyte, a change of the surface charge due to biomolecular immobilization is accompanied by a change of the pH in nanochannels. Veenhuis *et al.*²⁴

demonstrated a similar pH shift effect in nanochannels using gate electrodes to change the wall potential and cause a release or uptake of protons from the surface groups to/from the solution.

The analyses performed here considered the presence of ions due to dissolution of CO_2 in water. These considerations lead to a surface charge that depends on the bulk KCl concentration. As shown in this section, such behavior is very likely to be due to the interaction of H^+ with the functional groups on the walls of the nanochannels (H^+ is the surface charge determining ion).

B. pH modification

The conductance was measured for two nanofluidic devices with bare SiO_2 nanochannels ($n=25$, $h=40\text{ nm}$, and $L=40\text{ }\mu\text{m}$) differing only in width ($w=10\text{ }\mu\text{m}$ and $w=30\text{ }\mu\text{m}$). Equations (4) and (6) predict that the conductance of the device with $30\text{ }\mu\text{m}$ wide nanochannels at both asymptotic regimes (high and low C) should be approximately three times the conductance of the device with $10\text{ }\mu\text{m}$ nanochannels. The data shown in Fig. 12 confirm the good agreement of the experimental results with the calculations (Eqs. (4) and (20)).

The device with $30\text{ }\mu\text{m}$ wide nanochannels was used to further validate that the surface charge of silica nanochannels is governed by pH dependent reactions. The experiment consisted of varying the amount of H^+ inside the nanochannels and observing the conductance variation resultant from the surface charge modification. This time the pH was varied inside nanochannels by varying the pH of the bulk solution and not by altering the bulk KCl concentration C (as in Sec. IV A 1, see Table I). For this purpose, a solution with a fixed KCl concentration of 10^{-4} M was chosen, for two reasons: first, at $C=10^{-4}\text{ M}$, the conductance is still in the surface charge governed conductance plateau region (Fig. 10); and second, pH adjustment can be performed without significantly altering the bulk K^+ concentration (since K^+ dominates the ionic transport at 10^{-4} M KCl). The pH of the solutions was varied between 3.6 and 8.9 by the addition of HCl and KOH. The KCl content was approximately $C_{\text{K}^+ \text{ bulk}} = C_{\text{Cl}^- \text{ bulk}} = 10^{-4}\text{ M}$ for the three solution but the H^+ concentration differed: $C_{\text{H}^+ \text{ bulk}} = 2.5 \times 10^{-4}\text{ M}$ for pH 3.6, $C_{\text{H}^+ \text{ bulk}} = 3.2 \times 10^{-6}\text{ M}$ for pH 5.5, and $C_{\text{H}^+ \text{ bulk}} = 1.3 \times 10^{-9}\text{ M}$ for pH 8.9. The three 10^{-4} M

FIG. 12. Conductance of two nanofluidic devices with bare SiO_2 nanochannels with different widths ($10\text{ }\mu\text{m}$ and $30\text{ }\mu\text{m}$). Circles represent the conductance data of a device consisting of 25 nanochannels ($n=25$), each one with $h=40\text{ nm}$, $w=30\text{ }\mu\text{m}$, and $L=40\text{ }\mu\text{m}$. Squares represent the conductance data of a device consisting of 25 nanochannels ($n=25$), each one with $h=40\text{ nm}$, $w=10\text{ }\mu\text{m}$, and $L=40\text{ }\mu\text{m}$. The calculated bulk values are shown as solid lines. The upward shift of the conductance associated to the device with $30\text{ }\mu\text{m}$ wide nanochannels with respect to the conductance of the device with $10\text{ }\mu\text{m}$ wide nanochannels is predicted by Eqs. (4) and (6).

KCl solutions with different pH values (3.6, 5.5, and 8.9) were injected sequentially into the devices and the nanochannels conductance was measured after each injection. A conductance increase was obtained with the increase of bulk pH (Fig. 13(a)).

Table II shows the surface charge density and the ionic concentration inside nanochannels calculated from the measured conductance for the different bulk pH values using Eq. (20) and Eqs. (21) and (22), respectively. An increase in the bulk pH value results in a simultaneous increase of negative surface charge and of the pH inside nanochannels (Fig. 13(b) and Table II). These results agree qualitatively with the chemical dissociation reactions governing the silica surface charge (Eq. (30)). An estimate for the equilibrium dissociation constant K_a of silanol groups and the maximum possible charge density (i.e., all silanol groups dissociated) σ_{max} can be calculated from the surface charge density of silica obtained for different bulk pH. K_a is defined as

$$K_a = \frac{[\text{SiO}^-][\text{H}^+]_{\text{local}}}{[\text{SiOH}]}, \quad (32)$$

where the surface concentrations of negatively dissociated groups $[\text{SiO}^-]$ and the neutral non-dissociated groups $[\text{SiOH}]$ are given in mol per surface area (not in mol per volume).

FIG. 13. Nanofluidic device ($n = 25$, $h = 40 \text{ nm}$, $w = 30 \mu\text{m}$, and $L = 40 \mu\text{m}$) filled with 10^{-4}M bulk KCl solutions of different pH. (a) Nyquist plot of the device for different bulk pH values. (b) Measured silica nanochannel conductance (open diamonds) when the pH inside nanochannels (pH_{nano}) is modified. From the conductance values, the surface charge (open circles) could be calculated. The surface charge density data between pH 3.4 and 4.1 were taken from Table I. The solid line represents a fit to the silica nanochannels charge density data using Eq. (33). Dashed and dashed-dotted lines connecting the data points are given as guides to the eye.

TABLE II. Properties of the nanofluidic system for 10^{-4} M KCl solutions with different pH.

pH_{bulk}	3.6	5.5	8.9
C_{H^+nano}/C_{K^+nano}	2.5	0.031	1.2×10^{-5}
σ (mC m $^{-2}$)	-0.87	-5.07	-7.78
C_{K^+nano} (M)	1.48×10^{-4}	2.5×10^{-3}	4.1×10^{-3}
C_{H^+nano} (M)	3.7×10^{-4} ($pH_{nano} = 3.43$)	7.79×10^{-5} ($pH_{nano} = 4.1$)	4.98×10^{-8} ($pH_{nano} = 7.3$)
ζ (mV)	-45	-109	-297

$[H^+]_{local}$ is the local proton concentration in the solution directly at the surface. This local concentration corresponds, in this case, to the proton concentration inside the nanochannels (i.e., $[H^+]_{local} = C_{H^+nano}$). Since the term $[SiO^-]/[SiOH]$ in Eq. (32) is equal to $\sigma/(\sigma - \sigma_{max})$, it follows that

$$\sigma = \frac{K_a \sigma_{max}}{K_a + [H^+]_{local}} = \frac{K_a \sigma_{max}}{K_a + 10^{-pH_{local}}}. \quad (33)$$

A fit of the curve based on Eq. (33) to the data in Fig. 13(b) results in $K_a = 10^{-4.11}$ M and $\sigma_{max} = -7.95$ mC m $^{-2}$. These values differ from the reported values of $K_a \approx 10^{-6}$ M and $\sigma_{max} \approx -1.28$ C m $^{-2}$,^{17,21} possibly either because of the relatively narrow pH_{nano} range used meant an insufficient number of points to obtain a reliable fit, or because of different properties of the inner walls of the nanochannel with respect to open silica surfaces.

The ζ potential of silica in Table II was determined through the following equation:⁴²

$$\zeta = \frac{K_a T}{e} \left[\ln \left(\frac{-\sigma}{-\sigma_{max} + \sigma} \right) + \ln \left(\frac{10^{-pH_{bulk}}}{K_a} \right) \right] - \frac{\sigma}{C_s}, \quad (34)$$

where C_s is the Stern's layer capacitance (2.9 C m $^{-2}$ for silica surface (van der Heyden,¹⁷ 2005 #1869)). For these calculation, the reference values of $K_a = 10^{-6}$ M and $\sigma_{max} = -1.28$ C m $^{-2}$ (Ref. 17) were used. The value of $\zeta = -109$ mV (at $pH_{bulk} = 5.5$) found for the nanochannels (Table II) differs slightly from the $\zeta \approx -80$ mV found for PDMS/glass microchannels filled with 10^{-4} M KCl solution (at $pH_{bulk} = 5.5$) by streaming current measurements in microchannels.^{37,43} A less negative ζ potential for the PDMS/glass microchannels can be linked to the PDMS used in the microchannel fabrication. UV/Ozone treated PDMS revealed higher contact angles than glass, which suggests a lower density of silanol ionizable groups that are responsible for the negative surface charge.

V. CONCLUSIONS

Arrays of 1-D silica nanochannels accessed by two PDMS microchannels with integrated electrodes at both nanochannel terminations have been successfully fabricated. Nanochannels conductance measurements were made with KCl solutions of different concentrations. To describe the conductance plateau observed at very low salt concentrations, a simple variable surface charge model, accounting for all the ions in solution including H^+ and HCO_3^- (from atmospheric CO_2 dissolution in water), was developed. This model was used not only to predict the variable surface charge of silica nanochannels but also to study the effect of chemical surface reactions on nanochannels walls. Electrical detection of APDMES functionalization and ssDNA immobilization, based on variations of the predicted surface charge, was achieved. Finally, to further validate the model, the surface charge calculated from conductance measurements performed in silica nanochannels filled with a fixed KCl concentration but different bulk pH was compared to the pH dependence of the silanol groups present on the surface of silica. The surface charge dependence with the pH obtained for silica nanochannels is consistent with the expected behavior for silanol groups.

Proposed applications of nananochannels, namely in label-free biosensing, require a detailed knowledge of the influence of the composition of the solution and that of the nanochannel surfaces. In this work, it was shown that both these factors need to be taken into account when measuring electrical conductance variations across nanochannels.

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