Refinement of current monitoring methodology for electroosmotic flow assessment under low ionic strength conditions

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Current monitoring is a well-established technique for the characterization of electroosmotic (EO) flow in microfluidic devices. This method relies on monitoring the time response of the electric current when a test buffer solution is displaced by an auxiliary solution using EO flow. In this scheme, each solution has a different ionic concentration (and electric conductivity). The difference in the ionic concentration of the two solutions defines the dynamic time response of the electric current and, hence, the current signal to be measured: larger concentration differences result in larger measurable signals. A small concentration difference is needed, however, to avoid dispersion at the interface between the two solutions, which can result in undesired pressure-driven flow that conflicts with the EO flow. Additional challenges arise as the conductivity of the test solution decreases, leading to a reduced electric current signal that may be masked by noise during the measuring process, making for a difficult estimation of an accurate EO mobility. This contribution presents a new scheme for current monitoring that employs multiple channels arranged in parallel, producing an increase in the signal-to-noise ratio of the electric current to be measured and increasing the estimation accuracy. The use of this parallel approach is particularly useful in the estimation of the EO mobility in systems where low conductivity mediums are required, such as insulator based dielectrophoresis devices. Published by AIP Publishing. [<http://dx.doi.org/10.1063/1.4953183>]

I. INTRODUCTION

Microfluidics is the field of science dedicated to the design and application of systems where small volumes of liquid (sample and reagents) are handled. Many of the materials employed in the fabrication of microfluidic devices acquire surface electric charges upon contact with an electrolyte solution. These surface charges influence the distribution of nearby ions present in the fluid, leading to the development of an electrical double layer (EDL).¹ Under the influence of an externally applied electric potential, the mobile ions within the EDL acquire momentum, which is transmitted to adjacent layers of fluid through the action of viscosity, generating an electroosmotic (EO) fluid flow.¹ This EO flow is commonly employed to pump fluid and particles through microfluidic devices, with the added advantage of producing low sample dispersion due to its plug-like velocity profile. Moreover, EO flow can be easily manipulated by varying the applied electric potential and by adding EO flow suppressants.²⁻⁴

Current monitoring is a common, simple, and low cost technique used for characterizing the average EO velocity in microfluidic devices.⁵ This method relies on monitoring changes in the electric current through a microchannel when a test solution is displaced by an auxiliary solution, which has a slightly different ionic concentration. Current monitoring has been successfully employed to estimate the zeta potential of microfluidic devices made from glass^{5,6} and

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polymers.^{5,7–9} In the traditional approach, a single straight channel with a known length is employed. A schematic representation of a traditional experimental setup for this approach is shown in Figure 1(a), and a description of the methodology is included below.

Briefly, the channel and reservoirs R_1 and R_2 are filled with a test solution with an ionic concentration C_T . A direct current (DC) electric potential is then applied across the channel length generating a stable current signal ($t < t_1$ in Fig. 1(b)), since the electric resistance of the channel is constant. This stable value (I_B) is the total electric current flowing through the channel before the test solution is displaced. Next, reservoir R_1 is manually emptied and an auxiliary solution with an ionic concentration C_A is added to reservoir R_1 . The ionic concentration difference between the two solutions is $\Delta C = C_T - C_A$. As the auxiliary solution displaces the test solution inside the microchannel, there is a progressive decrease (if $C_T > C_A$) in the electric current ($t_1 < t < t_2$ in Fig. 1(b)), caused by a change in the electrical resistance across the channel. When the auxiliary solution reaches the end of the channel, the current reaches a new steady value ($t_2 < t < t_3$ in Fig. 1(b)), since the electric resistance of the channel is again constant. This stable value (I_A) is the total electric current flowing through the channel when it is filled with the auxiliary solution. In this traditional approach, the average EO velocity can be estimated dividing the channel length by the time required for the auxiliary solution to completely displace the test solution.

The ionic concentrations of the test and auxiliary solutions define the electric current plateaus *before* (I_B) and *after* (I_A) the displacement process, while their concentration difference (ΔC) defines the time response of the electric current during the displacement process. If the concentration difference is small (usually $\Delta C \leq 0.10 C_T$),¹⁰ the time response of the electric current is intrinsically linear, as shown in Figure 1(b). However, if the interface between the two solutions has a large mismatch in ionic concentration, internal pressure is generated at the

FIG. 1. (a) Schematic illustration of a traditional experimental setup for current monitoring measurements employing an individual plain channel. An electric potential is applied between reservoirs R_1 and R_2 to generate electroosmotic fluid flow. (b) Behavior of the electric current across the channel as function of time before (I_B), during, and after (I_A) the test solution is displaced by the auxiliary solution.

interface.¹⁰ This can ultimately result in undesired pressure-driven flow advection and dispersion at the interface, causing the electric current to shift from a linear to a non-linear behavior.

The described traditional approach for carrying out current monitoring measurements requires constant manual interaction with the experimental setup,⁵ which can affect the accuracy in the estimation of the EO velocity. For instance, the complete removal of the solution at reservoir R_I is crucial; otherwise, a large mixing region may form, complicating the determination of the exact time required for the auxiliary solution to completely displace the test solution.⁵ Therefore, several experiments are usually performed before a successful displacement, and an accurate assessment, can be achieved.⁵ To overcome the uncertainty in determining the time needed for the displacement process to occur, Sze *et al.*⁸ proposed to estimate the average EO velocity by using the slope of the electric current response during the displacement process ($t_1 < t < t_2$ in Fig. 1(b)). For a small ionic concentration difference (ΔC), the electric current variation is intrinsically linear, despite any curved behavior observed at the beginning or ending segments.⁸ This approach has the advantage of being more accurate, since it relies on several current-time data pairs and is less affected by experimental error. By using this approach, Sze *et al.*⁸ reported zeta potentials for glass and polydimethylsiloxane (PDMS) devices which were reproducible within $\pm 6\%$ deviations. In a more recent report, Almutairi *et al.*⁵ improved the slope-based methodology by also considering the surface conductance of the channel, which is a significant contribution to the overall electric current in channels with high surface-to-volume ratios.¹¹

Additional practical difficulties arise when the ionic concentration of the test solution (C_T) is low, which is the case of low conductivity media systems such as those employed in insulator-based dielectrophoretic (iDEP) systems.^{12,13} In this case, the signal-to-noise ratio (SNR) decreases and the electric current signal to be measured may be concealed by noise, making the measurement inaccurate. Considering that a small ionic concentration difference (ΔC) is needed, employing low conductivity solutions also leads to a small difference between the stable currents before and after the displacement process (I_B and I_A in Fig. 1(b), respectively). These effects pose an increasing challenge in the accurate estimation of the current-time slope during the solution displacement process.

Along with a challenging decrease in the signal-to-noise ratio, shifting to low conductivity solutions also increases the EO velocity.¹⁴ This is the opposite effect of the EDL shielding¹⁴ observed at high conductivities and leads to a faster displacement process and less current-time data pairs for the accurate estimation of the current-time slope. The present contribution describes a system where several channels are used in parallel (Fig. 2(a)) to increase the signal-to-noise ratio and to enhance the estimation of the slope of the current-time response. Although the measurement process can be enhanced with the acquisition of a more sensitive ammeter or by filtering the data to remove a portion of the noise, the approach presented here constitutes a practical solution that does not require any additional equipment. The proposed approach is validated through careful experimentation, and a detailed discussion of the advantages of using this generalized parallel method when working with low conductivity media systems is included. The findings from this study allow for an improved accuracy in the estimation of the average EO mobility employing current monitoring methodology and extending its use to systems with low conductivity media.

II. THEORETICAL BACKGROUND AND MATHEMATICAL MODEL

A. Electroosmosis

Electroosmosis is the motion of an electrolyte fluid induced by an electric field $\vec{E}$ that acts on the ions within the EDL. The ions movement exerts a drag force to the fluid, producing an effective slip velocity outside the EDL. The velocity of the fluid varies spatially from its value at the solid surface (channel wall) to that at the bulk of the solution (outside the EDL). A general description of this spatial variation is given by the Navier slip model^{15,16}

FIG. 2. (a) Experimental setup of a three-parallel channel system for current monitoring measurements, mounted on a 10-cm diameter glass wafer. The platinum wire electrodes, custom-made reservoirs, and displacement channels are shown with arrows. (b) For parallel channel experiments, a single electrode was split into three positive and three negative electrodes to interact with the power supply.

$$v_{wall} = b \frac{v_{eo}}{\lambda_D}, \quad (1)$$

where v_{wall} and v_{eo} are the fluid velocities at the wall and bulk solution, respectively, the later corresponding to the EO velocity, b is the slip length, and λ_D is the Debye length. The Debye length gives a rough measure of the characteristic length over which the overpotential at the wall decays into that of the bulk¹⁵

$$\lambda_D = \sqrt{\frac{\epsilon_m RT}{2F^2 I_C}}, \quad (2)$$

where ϵ_m and T are the real permittivity and absolute temperature of the suspending medium, respectively, R and F are the universal gas and Faraday constants, respectively, and I_C is the ionic strength of the bulk solution (expressed in terms of C_T). There is a direct relation between λ_D and the bulk EO velocity in the channel, as shown in Equation (1): thinner EDL (small λ_D) produces lower EO velocities, while thicker EDL (large λ_D) produces higher EO velocities.

In the limit of a thin EDL, the resulting EO velocity is given by the Helmholtz-Smoluchowski equation¹

$$v_{eo} = \mu_{eo} E_x = -\frac{\epsilon_m \zeta_w}{\eta} E_x, \quad (3)$$

where μ_{eo} denotes the EO mobility, which can be defined in terms of the real permittivity and viscosity of the suspending medium, ϵ_m and η , respectively; ζ_w is the zeta potential of the

microchannel wall and E_x is the x component of the local electric field. The microchannels employed in this study, made from polydimethylsiloxane (PDMS), have a negative wall zeta potential;¹³ thus, the fluid motion is towards the negative electrode (R_2 in Fig. 1(a)).

B. Current-time analysis: The slope method

In steady EO flow, the total electric current flowing through a channel filled with the test solution (I_B) can be described considering contributions from the bulk solution and from the channel surface⁵

$$I_B = A_C E_x \sigma_B^{bulk} + P E_x \sigma_B^{surface}, \quad (4)$$

where A_C and P are the cross-sectional area and perimeter of the channel, respectively, σ_B^{bulk} is the conductivity of the bulk test solution, and $\sigma_B^{surface}$ is the channel surface conductance. The convection current has been neglected, since it is several orders of magnitude smaller than the currents presented in Equation (4).¹⁷

If the time response of the electric current is linear, the EO velocity can be directly calculated from the slope (m) of the current-time relationship⁵

$$v_{eo} = \frac{m \cdot L}{(I_B - I_A)}, \quad (5)$$

where I_B and I_A are the current plateaus before and after the displacement process, respectively, and L is the channel length. As proposed by Almutairi *et al.*,⁵ I_B and I_A are measured directly from the current-time data (see Fig. 1(b)), since the current plateau values convey the experimental conductance of the bulk solution and channel walls (as shown in Eq. (4)). Combining Equations (3) and (5) yields expressions for μ_{eo} and ζ_w

$$\mu_{eo} = -\frac{m \cdot L}{E_x \cdot (I_B - I_A)}, \quad (6)$$

$$\zeta_w = -\frac{m \cdot L \cdot \eta}{E_x \cdot \epsilon_m \cdot (I_B - I_A)}. \quad (7)$$

III. MATERIALS AND METHODS

A. Microdevices

Microchannels were made from PDMS (Dow Corning, Midland, MI) and fabricated using standard soft-lithography techniques.¹⁸ The mold for the microchannels was defined in a SU-8 3050 photoresist (MicroChem, Newton, MA) coated on a silicon wafer (Silicon Inc., Boise, ID) with a diameter of 10 cm. To fabricate the channels, PDMS was cast onto the mold to produce a film with an approximate thickness of 3 mm. The PDMS film containing the microchannels was then activated using a plasma corona wand (Electro Technic Products, Chicago, IL) for 60 s to promote sealing. A glass substrate coated with a 25 μm thick layer of PDMS was also activated using the plasma corona wand and bonded to the PDMS film containing the microchannels. An exposure time of 60 s yields a reproducible EO flow, as observed from previous reports.^{13,19–21} This process produced microchannels where all the interior surfaces were PDMS and had the same zeta potential. Each channel was 6 cm long, 1 mm wide, and 35 μm deep and was soaked in deionized (DI) water for two days in order for the zeta potential of the channel to reach an equilibrium state.⁵

B. Equipment and software

A high voltage sequencer (Model HVS6000D; LabSmith, Livermore, CA) was used to apply DC electric potentials by means of platinum wire electrodes. The voltage sequencer was manipulated with the software *Sequence* provided by the manufacturer, which also incorporates a module for monitoring the electric current in the system as a function of time.

C. Suspending mediums

Seven KCl electrolyte solutions, with ionic concentrations of 0.1, 0.2, 0.3, 1.0, 2.0, 5.0, and 10.0 mM, were used as test solutions for the current monitoring experiments. The three solutions with the lower ionic concentration (0.1, 0.2, and 0.3 mM KCl) had conductivities of 17, 27, and 41 $\mu\text{S}/\text{cm}$, respectively. These low conductivity values are common in iDEP studies.^{12,19,22} The pH values of these three solutions were 6.01, 5.92, and 5.88, respectively. The 1.0, 2.0, 5.0, and 10.0 mM KCl solutions had conductivities of 122, 234, 568, and 1126 $\mu\text{S}/\text{cm}$, respectively, and pH values of 5.87, 5.95, 5.88, and 5.86, respectively. Although some of these conductivities are not suitable in standard iDEP settings, they provide a reference to compare the performance of individual and parallel channel systems in a broad conductivity range. The auxiliary solutions were 90% dilutions ($\Delta C = 0.10 C_T$) of each test solution.

D. Experimental procedure

Each experiment with a parallel channel system (Fig. 2(a)) started by filling up all channels with a given test solution. In order to decrease the generation of pressure driven flow, large cylindrical liquid reservoirs with an height of 40.3 mm and a diameter of 8.2 mm (total volume = 1.5 ml) were embedded at the inlet and outlet port of the channels (Fig. 2(a)). Water electrolysis reactions take place on electrode surfaces in contact with aqueous solutions when DC potentials are applied. The products from these reactions can significantly alter pH.²³ The height of these reservoirs ensures a separation distance of ~ 3.5 cm between the electrode tip and the channel inlet. This large spacing between the electrode and the channel inlet, in combination with a large liquid reservoir volume, hinders electrolysis products from entering the channel, preventing the formation of large pH gradients inside the channel. The test solution was introduced to each channel by pressure driven flow, which was easy to manipulate given the large custom-made reservoirs. Platinum wire electrodes were then placed at the top of the channels reservoirs (next to the walls,²⁴ see Fig. 2(a)), and a DC electric potential of 1000 V was simultaneously applied across the R_1 and R_2 reservoirs of each channel by employing the high voltage supply. The initial stable current signal (I_B) of the parallel system was recorded for each experiment employing the high voltage supply. Next, the electric field was removed, each R_1 reservoir was emptied, and the corresponding auxiliary solution was added to the same reservoirs. An electric potential of the same magnitude was applied to all channels, and the time response of the electric current during the displacement process was recorded. The experiment ended after the electric current reached the second plateau (I_A). The experiments with individual channels followed the same experimental procedure.

E. Fluorescence measurements

Fluorescence microscopy measurements were performed to assess pH variations inside the channel volume. These measurements were quantified as the fluorescence emission ratio of the pH-sensitive dye fluorescein isothiocyanate (FITC) Isomer I (Sigma-Aldrich, St. Luis, MO) and the pH-insensitive dye tetramethylrhodamine (TRITC) (Sigma-Aldrich, St. Luis, MO). This two-dye strategy offers a better accuracy than standard fluorescence measurements by referencing the fluorescence intensity of the pH-dependent FITC dye to that of the pH-independent TRITC dye.²³ The fluorescence emission ratio (R_k) was measured at the pixel resolution

$$R_k = \frac{I_k^{FITC}}{I_k^{TRITC}}, \quad (8)$$

where I_k indicates the fluorescence intensity of either the FITC or TRITC dyes at the k -th pixel of the image under analysis. The pH of the fluid within the channel volume was determined using the average fluorescence emission ratio, by means of a calibration curve. The quantification of the average fluorescence emission ratio was performed using Fiji image processing package (a distribution of ImageJ focused on biological-image analysis)²⁵ and R (software environment for statistical computing and graphics).²⁶

IV. RESULTS AND DISCUSSION

A. Applying current monitoring to low conductivity systems

As depicted by Equation (5), the electroosmotic velocity is inversely proportional to the difference between the plateau currents before and after the displacement process ($I_B - I_A$). This difference, in turn, is directly proportional to the changes in conductivity of the bulk solution ($\sigma_B^{bulk} - \sigma_A^{bulk}$) and surface conductance ($\sigma_B^{surface} - \sigma_A^{surface}$)

$$I_B - I_A = A_C E_x (\sigma_B^{bulk} - \sigma_A^{bulk}) + P E_x (\sigma_B^{surface} - \sigma_A^{surface}). \quad (9)$$

The requirement of a small ionic concentration difference (ΔC) poses a practical difficulty on the accurate assessment of the EO velocity when low conductivity mediums are employed. Figures 3(a)–3(d) show the behavior of the electric current measured for the 0.1, 0.3, 2.0, and 10.0 mM KCl solutions, respectively, in systems with only one channel. Several observations can be drawn from Figures 3(a)–3(d). First, a lower ionic concentration of the test solution reduces the overall difference between the magnitudes of the two electric current plateaus ($I_B - I_A$). The current plateaus for the 10.0 mM KCl solution (Figure 3(d) and Table I) are $I_B = 117.9 \mu A$ and $I_A = 107.4 \mu A$, resulting in a difference of $10.5 \mu A$. When the ionic concentration of the test solution is decreased to 2.0 mM (Figure 3(c) and Table I), these values decrease to $I_B = 21.4 \mu A$ and $I_A = 18.5 \mu A$, thus, $I_B - I_A = 2.9 \mu A$. At the lowest ionic concentration (0.1 mM) of the test solution (Figure 3(a) and Table I) current plateaus of $I_B = 1.7 \mu A$ and $I_A = 1.4 \mu A$ were measured, thus,

FIG. 3. Electric current measurement obtained during the displacement of KCl test solutions by their respective auxiliary solutions. (a)–(d) Individual channel systems and (e)–(h) three-parallel channel systems. Four different ionic strengths were employed: 0.1, 0.3, 2.0, and 10.0 mM KCl. The behavior of the electric currents before (I_B), during, and after (I_A) the displacement processes is shown. The signal-to-noise ratio (SNR) for each electric current signal was calculated as the ratio of the current signal mean to its standard deviation²⁷ during the first current plateau (I_B). A higher SNR value means lower noise present compared to the electrical current. The applied electric potential was 1000 V.

TABLE I. First (I_B) and second (I_A) experimental current plateaus, current plateaus differences ($I_B - I_A$), and signal-to-noise ratios (SNR) of experimental signals when KCl test solutions with ionic concentrations of 0.1, 2.0, and 10.0 mM are displaced by their respective auxiliary solutions in individual channel and three-parallel channel systems.

System type	Ionic strength (mM)	I_B (μA)	I_A (μA)	$I_B - I_A$ (μA)	SNR
Individual channel	0.1	1.7	1.4	0.3	19.7
	2.0	21.4	18.5	2.9	153.6
	10.0	117.9	107.4	10.5	1687.1
Three-parallel channel	0.1	5.3	4.6	0.7	72.1
	2.0	63.9	57.4	6.5	764.3
	10.0	314.3	285.6	28.7	4845.5

$I_B - I_A = 0.3 \mu\text{A}$. This confirms that the magnitude of the electric current signal decreases along with the ionic concentration of the test solution. Figures 3(a)–3(d) also depict the magnitude of the signal-to-noise ratio (SNR), calculated as the ratio of the current signal mean to its standard deviation²⁷ during the first current plateau (I_B). Along with a reduction in the magnitude of the electric current signal, the quality of the signal also decreases, as observed by the decreasing SNR with ionic concentration. When the ionic concentration is decreased by a factor of 100 (from 10.0 to 0.1 mM), the SNR roughly decreases in the same proportion (~ 86), showing that the noise remains approximately the same. This reduction in the signal-to-noise ratio translates to a lower accuracy in the estimation of the current-time slope when a system with one channel is employed.

While several algorithms have the potential to discern the underlying noise in the electric current signal, they usually require numerous current-time data pairs to provide accurate estimations.²⁸ Figures 3(a)–3(d) show, however, that decreasing the ionic concentration of the test solutions results in a shorter displacement time (see **green** regions in Figs. 3(a)–3(d)). The displacement time decreases from 129 s to 56 s for the 10.0 and 0.1 mM KCl solutions, respectively. This faster displacement process is related to the shielding effect of the EDL.¹⁴ At high ionic strengths, the excess of counter-ions in the test solution shields the surface charge and compresses the EDL (smaller Debye lengths), reducing the zeta potential of the surface and the overall EO velocity; which in turn increases the time required to displace the test solution. At low ionic strengths, the lack of counter-ions decreases the shielding effect producing a thicker EDL (a larger Debye length), leading to an increase in the EO velocity and a faster displacement process. A shorter displacement time produces fewer current-time data pairs to effectively estimate the slope, leading to inaccurate estimations when coupled with a small signal-to-noise ratio. Other sources of variation, such as the formation of a mixing region due to an incomplete removal of the solution at reservoir R_1 , can also increase the inaccuracy of the estimation.⁵ The uncertainty in the estimation of the displacement process, where the “true” electric current signal can be concealed by noise, might be one of the main reasons to perform several experiments before a successful estimation can be achieved.

B. Signal amplification by employing parallel channels

The combined effect of a reduction in the current plateaus difference, a decrease in the signal-to-noise ratio, and a faster displacement can lead to inaccurate estimations of the current-time slope and, hence, of the EO velocity. From these three conditions, the faster displacement cannot be avoided, since it is an intrinsic phenomenon of the EDL when employing solutions with low ionic strengths (Eq. (1)). Although the use of pressure-driven flow to accelerate/decelerate the overall fluid flow velocity has been previously suggested,²⁹ this can lead to non-reproducible results caused by mixing effects at the interface between the test and auxiliary solutions.

The reduction in the current plateaus difference and the decrease of the signal-to-noise ratio are direct consequences of having a small ionic concentration difference (ΔC) between the test

and auxiliary solutions, which is necessary in current monitoring measurements. An increase in the plateaus differences and in the signal-to-noise ratio can be achieved by increasing the applied electric potential (Eq. (6)). However, increasing the applied voltage also leads to the disadvantages of an even faster EO velocity with fewer current-time data pairs and an increase in Joule heating.^{30,31} Increasing the cross-sectional area of the channel, which decreases its electrical resistance, is also an alternative to increase the electric current that flows through the channel. However, it also reduces the hydrodynamic resistance of the channel and may lead to undesired pressure-driven flow.¹⁰ In addition, there is a maximum limit in the channel width set by its aspect ratio, above which the channel suffers from roof collapse. This roof collapse effect is caused by adhesion between the top layer of the channel and the substrate.³² We have observed this effect in aspect ratios (width to height) as low as 125:1.

A simple approach to keep constant the hydrodynamic resistance of the system, while increasing its electric current flow, is the use of parallel channels. In the case where n channels are used in parallel, the electric current flowing through the entire system (I_{total}) is the sum of the individual electric currents flowing through each channel

$$I_{total} = I_{channel,1} + I_{channel,2} + \cdots + I_{channel,n} = \sum_{j=1}^n I_{channel,j}. \quad (10)$$

According to Equation (4), the electric current flowing through each channel is a function of the bulk and surface electric currents. Since the cross-sectional area (A_C), perimeter (P), and local electric field (E_x) are the same for all channels, then

$$I_{total} = \sum_{j=1}^n A_C E_x \sigma_B^{bulk} + \sum_{j=1}^n P E_x \sigma_B^{surface} = n (A_C E_x \sigma_B^{bulk} + P E_x \sigma_B^{surface}). \quad (11)$$

Notice in Equation (11) that the bulk test solution conductivity and the surface conductance are also the same for all channels. Equation (11) implies that the total electric current flowing through the system can be easily scaled-up by the addition of parallel channels. To further demonstrate this, a set of experiments with up to six channels was performed employing 0.1 mM KCl as the test solution. Figure 4(a) shows the magnitude of the electric current during the first plateau as a function of the number of parallel channels; a well-defined linear behavior is observed ($R^2=0.991$), as confirmed by the fitting to a linear model using a least-squares approach.²⁸ The data fitting process revealed a slope of 1.118 for this linear model, indicating

FIG. 4. Effect of the number of parallel channels in current monitoring measurements. (a) Electrical current during the first plateau (I_B) and (b) signal-to-noise ratio (SNR) as a function of the number of parallel channels in the system. The best linear fit for each case, according to the least-squares approach, is shown as an equation and as a red, dotted line. The test solution was a 0.10 mM KCl solution.

that the current adequately scales by a factor of $\sim n$, as suggested by Equation (11). Moreover, Figure 4(b) introduces the enhancement in the SNR by the addition of parallel channels. A similar fitting process demonstrated that the SNR increases almost 14 times with the addition of each parallel channel.

Figures 3(e)–3(h) show the measurements of the electric current for the 0.1, 0.3, 2.0, and 10.0 mM KCl solutions, respectively, when a three-parallel channel system ($n=3$) was employed. The use of parallel channels increases the overall difference between the electric current plateaus ($I_B - I_A$), leading to more accurate estimations of the current-time slope. The current plateaus for the 0.1 mM KCl solution shift from $I_B = 1.7 \mu\text{A}$ and $I_A = 1.4 \mu\text{A}$, for a difference of $I_B - I_A = 0.3 \mu\text{A}$ when an individual channel system is employed (Fig. 3(a) and Table I), to $I_B = 5.3 \mu\text{A}$, $I_A = 4.6 \mu\text{A}$, and $I_B - I_A = 0.7 \mu\text{A}$ when a three-parallel channel system is used (Fig. 3(e) and Table I), an increment of 130%.

Figures 3(e)–3(h) also show the results for the SNR obtained with the three-parallel channel systems. The decrease in noise can be noticed when comparing to the results obtained with individual channel systems (Figs. 3(a)–3(d)). Table I lists the SNR values obtained for all solutions, illustrating the significant scaling-up in SNR when three-parallel channel systems are employed. These differences in the SNR magnitudes clearly demonstrate that higher quality and cleaner signals are obtained by employing three-parallel channel systems, when compared to individual channel systems.

In addition to the gains in the electric current plateaus difference and in the SNR, there is a more defined transition between the plateaus and the displacement process when three-parallel channel systems are used, as observed in Figure 3. Notice in Figure 3(a), for instance, how hard is to visually discern (in the individual channel system) where the initial current plateau (I_B) finishes and the displacement process begins, and where the displacement process finishes to give place to the second current plateau (I_A). Alternatively, by using a three-parallel channel system, it is much easier to visually discern each one of the three stages of the process. This observation indicates that it will be easier for any algorithm to select the subset of data points that belong to the linear current-time transition, and this will be reflected on a more accurate estimation. It is important to note that the location of the displacement time (green regions in Fig. 3) is not affected by the addition of parallel channels.

C. Estimation of the EO mobility and zeta potential in parallel channels

Figure 5(a) presents the estimated λ_D , μ_{eo} , and ζ_w values for all test solutions when systems with individual channel (red line, Fig. 5(a)) and three-parallel channel systems (blue line, Fig. 5(a)) are employed. The larger differences between the current plateaus and the higher SNR values achieved with the use of three-parallel channel systems provide more accurate estimations, as observed on their lower variability (compare the size of error bars for the individual channel and three-parallel channel systems in Fig. 5(a)). Three distinct behaviors, depending on the concentration of the solutions employed, were observed. First, a similar estimation accuracy was reached in both systems for the higher ionic strength solutions (5.0 and 10.0 mM), where the maximum difference in the ζ_w values was below 2 mV. Hypothesis testing confirms the lack of significant differences in the estimation accuracy of the single and parallel channel systems ($p=0.49$ and $p=0.74$ for the 5.0 and 10.0 mM solutions, respectively, two sided t -test, $N=3$ observations each) for ionic concentrations above 5.0 mM. This indicates that the higher variability observed for the individual channel system does not compromise an accurate estimation of μ_{eo} and ζ_w . Second, the difference in the estimated values obtained with both systems increased for the intermediate ionic strength solutions (1.0 and 2.0 mM), where the difference in the ζ_w values ranged from 3 to 8 mV (Fig. 5(a)). Even so, hypothesis testing shows no significant differences in the estimation accuracy of the single and parallel channel systems ($p=0.55$ and $p=0.08$ for the 1.0 and 2.0 mM solutions, respectively, two sided t -test, $N=3$ observations each). Third, and more importantly, the difference in the estimations increases for the low ionic strength solutions (0.1, 0.2, and 0.3 mM), where discrepancies in ζ_w ranging from 8 to 12 mV were observed. Although these differences are non-statistically significant ($p=0.32$, $p=0.24$,

FIG. 5. (a) Behavior of the zeta potential (left axis) and EO mobilities (right axis) values in PDMS channels as functions of the ionic concentration (bottom axis) and Debye length (top axis). These values were estimated using individual channel (in red) and three-parallel channel systems (in blue). For these experiments, the test solutions were displaced by the auxiliary solutions. (b) Standard deviation values of the zeta potential as function of the ionic concentration for the individual channel (in red) and three-parallel channel systems (in blue).

and $p = 0.11$ for the 0.1, 0.2, and 0.3 mM solutions, respectively, two sided t -test, $N = 3$ observations each), the variability obtained with individual channel systems increases with the decreasing ionic concentration (Fig. 5(b)). In contrast, the variability obtained with three-parallel channel systems does not show a defined pattern with respect to ionic concentration (Fig. 5(b)).

In order to extend the analysis of the estimation precision for both systems with respect to ionic strength, the ζ_w values were clustered in two groups (low and high conductivity) and compared to their predicted Debye lengths. According to Equation (1), the EO velocity is roughly proportional to the Debye length.^{15,16} Since the EO velocity is directly proportional to the zeta potential (Eq. (3)), then the magnitude of ζ_w should be directly proportional to λ_D for the conditions studied here. Figure 5(a) shows the μ_{eo} and ζ_w values for all the test solutions as function of λ_D (Eq. (2)), when systems with individual channels (red line, Fig. 5(a)) and three-parallel channels (blue line, Fig. 5(a)) are employed. The ζ_w values were fitted to linear models of the form

$$\zeta_w = A_0 + A_1 \lambda_D, \quad (12)$$

using a least-squares approach.²⁸ The fitting process of the high conductivity solutions ($C_T \geq 1.0$ mM) showed that both the individual channel ($A_0 = -23.51 \pm 3.01$ and $A_1 = -3.28 \pm 0.50$) and three-parallel channel ($A_0 = -22.83 \pm 1.93$ and $A_1 = -3.95 \pm 0.34$) systems arrived to approximately the same model. All parameters were statistically significant at $\alpha = 0.01$, and no significant differences were found among the two models ($p > 0.27$). However, the coefficient of determination, which is a comparison of the residuals with the total variability (the goodness of fit),²⁸ was considerably higher in the tree-parallel channel system ($R^2 = 0.91$) compared to that of the individual channel system ($R^2 = 0.77$).

Similarly, when only the low conductivity solutions ($C_T < 1.0$ mM) were considered, the fitted parameters of both systems seemed to be in qualitative agreement ($A_0 = -46.69 \pm 17.04$ and $A_1 = -2.14 \pm 0.69$ for the individual channel system; $A_0 = -40.63 \pm 5.45$ and $A_1 = -1.96 \pm 0.23$ for the three-parallel channel system). Even if no significant differences were found among the two models ($p > 0.74$), only the parameters for the three-parallel channel system were statistically significant at $\alpha = 0.01$. Further, while the R^2 coefficient of the three-parallel channel system was not altered ($R^2 = 0.91$), it decreased for the individual channel system ($R^2 = 0.59$). The decrease in the R^2 is directly related to a decrease in the SNR. This observation indicates that only the ζ_w

values obtained with the three-parallel channel system can be accurately described as linear functions of λ_D . This analysis makes evident how the measurements performed with individual channel systems lose accuracy when low conductivity solutions are analyzed, illustrating the importance of using parallel channel systems when working with low ionic strength conditions. The lower prediction variability (caused by larger differences in the current plateaus and higher SNR values) clearly demonstrates how the use of parallel channel systems can improve the estimation accuracy by simply scaling-up the current signal to be measured. These results indicate that parallel channel systems allow for a more precise estimation of the current-time slope during the displacement process when low conductivity solutions ($C_T < 1.00$ mM) are employed.

D. Deviations from the linear behavior caused by pH variations in the channel

Electrochemical reactions occur at the electrodes during the application of the electric potentials.²⁴ This electrolysis effect generates hydrogen and hydroxide ions in the solution volume surrounding the electrodes, and these ions are then transported inside the channel by means of EO flow and electrophoretic movement. The migration of these ions generates drastic pH variations inside the channel.²³ Several strategies have been proposed to minimize pH changes in the channel volume, including the use of low conductivity solutions,^{33–35} frequent media replenishment,^{33,36,37} large spacing between the electrodes and the channel inlets,^{33,38} and increasing reservoir size.³³ The first strategy (low conductivity solutions) arises naturally in this study, while frequent media replenishment is inherent for each experiment of the current monitoring technique adopted here.⁸ The large custom-made reservoirs used in this study incorporate the last two strategies in an attempt to compensate the employed unbuffered solutions. Since pH is one of the main parameters that affects EOF, variations in pH inside the channels were assessed experimentally, as described next.

Fluorescence measurements employing FITC (pH-sensitive dye) and TRITC (pH-insensitive dye) were used to assess pH changes in the channel volume.²³ All channels were initially filled with the 0.1 mM KCl test solution containing both FITC and TRITC dyes. The average fluorescence intensity for each dye was measured over the channel volume and the intensity ratio R_k (Eq. (8)) was estimated. Prior to applying the electric potential, the pH inside the channel was estimated to be 5.98 ± 0.10 , which is in agreement with the measured pH of the test solution. Next, an electric potential of 1000 V was applied across the channels for 45 s. Notice that 45 s exceeds the time needed to get a stable electric current value (I_B). The pH inside the channel was measured again as 5.88 ± 0.21 after the electrical treatment (Table II), indicating only slight pH changes in the channel volume despite the use of the unbuffered solution. The test solution was then removed from the R_1 reservoirs, the auxiliary solution was added, and an electric potential of 1000 V was applied through the channel for 4 min. This time exceeds the time needed for the displacement process and for reaching the second current plateau (I_A). The pH after this second electrical treatment was measured to be 5.73 ± 0.21 (Table II). Considering that the channel is now full of the auxiliary solution (0.09 mM KCl, pH = 5.94), then it can be established that only slight pH changes were caused by the overall electrical treatment.

In order to further assess the influence of the large custom-made reservoirs employed in this study, two additional set of experiments were performed employing devices with much smaller reservoirs: (i) 10 μ l pipette tips as reservoirs and (ii) with no added reservoirs (i.e., 2 mm holes punched in the PDMS device were used as reservoirs). After 45 s of applying

TABLE II. Experimental pH after applying 1000 V for 45 s (to emulate the first current plateau) and after applying 1000 V for 4 min (to emulate the displacement process and the second current plateau).

Reservoirs type	pH after applying 1000 V for 45 s	pH after 1000 V for 4 min
No added reservoirs	5.32 ± 0.34	4.92 ± 0.19
10 μ l pipette tips	5.86 ± 0.04	5.39 ± 0.07
Large custom-made reservoirs	5.88 ± 0.21	5.73 ± 0.21

1000 V, the pH of the channels with pipette tips dropped to 5.86 ± 0.04 , while it dropped to 5.32 ± 0.34 for the case of the channels with no reservoirs. This indicates that using reservoirs, even with volumes as low as $10 \mu\text{l}$, could adequately prevent significant pH changes caused by electrolysis during the stabilization of the first current plateau (I_B). However, after removing/adding the solutions and applying 1000 V for 4 min, the pH measured inside the channels with pipette tips dropped to 5.39 ± 0.07 , while it dropped to 4.92 ± 0.19 for the channel with no added reservoirs. These results demonstrate the importance of using external reservoirs for current monitoring experiments. Interestingly, the current-time behavior for the experiments with no reservoirs did not show a well-defined linear behavior (a monotonically increasing current over time was observed), preventing an adequate estimation of the zeta potential.

E. Aging effects on zeta potential of PDMS surfaces

In order to assess any aging effects on the capacity of PDMS to produce a stable EO flow, current monitoring measurements were performed over a time period of 10 days using three-parallel channel systems. During this time period, several experiments were conducted under two distinct scenarios: (i) when the test solution was displaced by the auxiliary solution ($I_B \rightarrow I_A$, **blue** line, Fig. 6), and (ii) when the auxiliary solution was displaced by the test solution ($I_A \rightarrow I_B$, **red** line, Fig. 6). The test and auxiliary solutions corresponded to 0.10 mM KCl and 0.09 mM KCl, respectively. The magnitude of ζ_w when $I_B \rightarrow I_A$ (**blue** line) initially shows a decrease from -101 mV at day three to -80 mV at day five, followed by a stable period between -80 and -82 mV during days five to seven. This observation seems to indicate that it takes close to five days for the zeta potential to reach a stable value under the conditions studied here. At day 10, the magnitude of ζ_w slightly increases to -86 mV . The progressive decay in the magnitude of the zeta potential from days 5 to 10 might be caused by a long-term aging effect.^{39,40} However, only slight variations in the electric current signals were found within the studied time period, and the observed fluctuations did not affect the estimation of the current-time slope, as shown by the small and constant variability in all measurements. The magnitude of ζ_w when $I_A \rightarrow I_B$ (**red** line) followed a similar behavior, with a progressive decrease from -95 mV at day three to -69 mV at day six. After day six, the magnitude of ζ_w slowly increased to a final value of -81 mV at day ten. Interestingly, the ζ_w magnitude estimated when the auxiliary solution was displaced by the test solution ($I_A \rightarrow I_B$, **red** line) was found to be smaller than when the test solution was displaced ($I_B \rightarrow I_A$, **blue** line) in all experiments. This hysteresis behavior is addressed in Sec. IV F.

FIG. 6. Aging effect of the zeta potential of PDMS channels measured at days 3, 4, 5, 6, 7, and 10. The measurements were made employing a three-parallel channel system when a test solution was displaced by its auxiliary solution ($I_B \rightarrow I_A$, **blue** line) and when the auxiliary solution was displaced by the test solution ($I_A \rightarrow I_B$, **red** line). The test solution was a 0.10 mM KCl solution, while the auxiliary solution was a 0.09 mM KCl solution.

F. Hysteresis in the zeta potential behavior of PDMS surfaces

Although no significant patterns were found in the behavior of ζ_w over time (see Section IV E), an interesting effect that is flow direction-dependent was observed. The time required for the test solution to displace the auxiliary solution ($I_A \rightarrow I_B$, red line, Fig. 6) was longer than the time required for the auxiliary solution to displace the test solution ($I_B \rightarrow I_A$, blue line, Fig. 6). A similar hysteresis behavior in the estimation of ζ_w values was previously reported by Lim and Lam⁴¹ during the displacement of a 1.00 mM KCl solution by 0.20, 0.50, 0.70, and 0.95 mM KCl solutions and vice versa. The authors reported that these variations in displacement time (and hence, in the zeta potential) were higher at greater ΔC values ($\Delta C = C_T - C_A$). They reported time differences in the order of $\sim 1\%$ when a 30% dilution was employed for the auxiliary solution ($C_A = 0.7 C_T$) in PDMS devices and concluded that the effect is caused by an unbalance of the ionic profiles near the EDL.⁴¹ In the present work, hysteresis differences in the zeta potential as large as 12 mV were observed (Fig. 6). Although a rigorous interpretation of this hysteresis cannot be provided, since temperature was not controlled among experiments, its presence did not significantly affect the estimation of the ζ_w and μ_{eo} values since, regardless of the displacement order, a well-defined linear behavior was observed in the current-time relationship.

V. CONCLUSIONS

This contribution demonstrates the use of a parallel channel system to scale-up the measured electric current signal in current monitoring assessments, to reliably determine the electroosmotic velocity and zeta potential values. This approach is particularly beneficial for systems that require low conductivity electrolytes. The use of parallel channels was shown to significantly increase the signal-to-noise ratio of the electric current signal by a factor of n , where n corresponds to the number of parallel channels in the system. This increase in the magnitude of the signal resulted in a higher accuracy in the estimation of electroosmotic mobilities and zeta potential values when the slope-based methodology was employed. Experiments were run with systems that contained three channels arranged in parallel and also systems with a single individual channel. By comparing the results obtained with three-parallel channel systems vs. individual channel systems, it was observed that the parallel channel system produced results that are more consistent and have lower variability over a range of ionic strengths of the test solutions. The results were fitted to theory in terms of the thickness of the electrical double layer (EDL) and the expected electroosmotic velocity as function of the potential drop across the EDL. This fitting demonstrated that the predictions obtained with the parallel channel system have a better agreement with theory than those obtained with the standard individual channel system. This evident gain in accuracy becomes essential when low ionic strength solutions are employed, as is the case with direct current insulator-based dielectrophoresis (DC-iDEP) studies, where high current must be avoided to prevent electrolysis and Joule heating effects. The use of parallel channel systems enhances accuracy by allowing to discern small changes in the electric current, which would be otherwise concealed by noise in the traditional individual channel systems. Furthermore, aging effects on these measurements were assessed by measuring the electric current vs. time behavior for several channels for a period of 10 days, where slight effects were found due to PDMS aging. Hysteresis effects as a function of the solution displacement order were as high as 12 mV for the determination of the zeta potential. However, the current-time behavior was linear in all experiments, demonstrating that the presence of hysteresis does not significantly affect the accuracy of the estimation of the channel's zeta potential. In addition, the use of large custom-made reservoirs was shown to prevent significant pH drops within the channel volume caused by the electrical treatment. The present study clearly demonstrates that current monitoring can be successfully extended to systems that employ low ionic strength electrolytes by utilizing systems with parallel channels, which increases the signal-to-noise ratio and produces measurements with higher accuracy.

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