Abnormal Ionic-Current Rectification Caused by Reversed Electroosmotic Flow under Viscosity Gradients across Thin Nanopores

Yinghua Qiu,*†, Zuzanna S. Siwy,‡ and Meni Wanunu*†

1Department of Physics, Northeastern University, Boston, Massachusetts 02115, United States
2Department of Physics and Astronomy, University of California, Irvine, California 92697, United States

Supporting Information

ABSTRACT: Single nanopores have attracted much scientific interest because of their versatile applications. The majority of experiments have been performed with nanopores being in contact with the same electrolyte on both sides of the membrane, although solution gradients across semipermeable membranes are omnipresent in natural systems. In this manuscript, we studied ionic and fluidic movement through thin nanopores under viscosity gradients both experimentally and using simulations. Ionic-current rectification was observed under these conditions because solutions with different conductivities filled across the pore under different biases caused by electroosmotic flow. We found that a pore filled with high-viscosity solutions exhibited a current increase with applied voltage in a steeper slope beyond a threshold voltage, which abnormally reduced the current-rectification ratio. Through simulations, we found that reversed electroosmotic flow, which filled the pore with aqueous solutions of lower viscosities, was responsible for this behavior. The reversed electroosmotic flow could be explained by slower depletion of co-ions than of counterions along the pore. By increasing the surface charge density of pore surfaces, current-rectification ratio could reach the value of the viscosity gradient across thin nanopores. Our findings shed light on fundamental aspects to be considered when performing experiments with viscosity gradients across nanopores and nanofluidic channels.

Because of their confined space and surface charges, nanopores provide a versatile tool to tune ionic and fluidic transport, which is applicable to object sensing, fluid-pump design, energy-conversion systems, and ionic transistors. In a typical experimental setup, nanopores connect two conductivity cells filled with electrolytes, so that ion, fluid, and analyte transport can only occur through the nanopores.

The majority of reported nanopore experiments have been performed in symmetric electrolyte conditions, and few measurements under gradients across the membranes, such as concentration gradients, pH gradients, and viscosity gradients, have been reported. In nature, concentration gradients exist widely, including different salt concentrations across ion channels in cell membranes and at the junction points between rivers and seas. The investigations of concentration gradient could provide detailed physical information for various applications, such as ionic gating and energy conversion. For example, with an ion-selective membrane, energy conversion can be achieved through an entropy difference across the membrane. A pH gradient, as a kind of concentration gradient of proton or hydroxide ions, can affect the surface charge density along the pore. Such gradients can be used to control fluid flow in the pore by modulating the surface charge density under different biases. Because of the electroosmotic flow, the solution on the entrance side of the pore can be dragged into the pore. The surface charge density can be controlled by the pH of the solution in the pore.

Viscosity gradients appear usually with a kind of mass gradient across the pore, such as with glycerol or dimethyl sulfoxide, which can control the viscosity and conductivity of the solution. Viscosity gradients also exist widely in nature, such as across cell membranes as a result of the crowded nature of inner cell environment or in underground porous media, like aquifers. With the advantage of the high sensitivity of micro- and nanopores, viscosity gradients across nanopores have been investigated before with potential applications in the detection of solution viscosity, for slowing down the speed of particles in resistive-pulse detection, and in ionic transistors. Some groups have detected fluid flow under viscosity gradients with long micropores. Experiments show that the generated electroosmotic flow fills the pore with the solution from the entrance side, which can result in potential-dependent electrical resistance in the system.
(i.e., current rectification in current—voltage (IV) curves). The current rectification caused by viscosity gradients is different from traditional ionic-current rectification, which is caused by enrichment or depletion of ions under different biases in pores with asymmetric geometries or asymmetric charge distributions.

The effect of gradients across long pores can be understood by considering the two regions that differ in viscosity or ionic concentrations, without explicitly considering the liquid—liquid miscible region because of its small length scale. Therefore, the current rectification, caused by the voltage-dependent electrical resistance of the system, has a ratio equal to the ratio of the solution conductivities. However, when the pore length approaches the nanoscale, the liquid—liquid miscible region cannot be ignored in the nanopore because it might occupy a large fraction of the pore volume, a condition that has been seldom considered in the literature.

In this work, experiments and simulations of ionic and fluidic transport through silicon nitride (SiN) nanopores as thin as 50 nm had been conducted under viscosity gradients established between water and glycerol solutions. In order to minimize the influence of the pore shape on ionic transport, lower ratios of the Debye layer thickness over the pore diameter were considered through a combination of nanopores with ~20 nm in diameter and electrolyte solutions with higher concentrations than 0.5 M. Under viscosity gradients, abnormal current rectification could be found. With increasing voltage, the nanopore rectified more obviously until it reached a threshold, at which point there was a steeper current increase with voltage, and the current-rectification ratio was abnormally reduced. From numerical simulations, we found that the voltage-dependent ionic-current rectification resulted from the reversed electroosmotic flow caused by excess of co-ions along the pore axis. This finding is closely related to the ionic and fluidic movement in the pore under viscosity gradients, which has potential applications in the design of ionic circuits and electroosmotic flow pumps, as well as in the detection of solution viscosities. When abnormal current rectification occurs, the fluid near the pore walls and in the center of the pore move in opposite directions, which could lead to mixing of the fluids from both sides of the pore in the confined space. In this case, the nanopore could be used as a nanoreactor to investigate dynamic interactions between the analytes from both sides of the pore and probe chemical reactions and products.

### EXPERIMENT AND SIMULATION METHODS

**Experimental Methods.** The scheme of the experimental setup is shown in Figure 1a. Single nanopores ~20 nm in diameter were drilled using a transmission electron microscope (JEOL 2010F) in 50 nm thick freestanding SiN membranes. The SiN membranes (20–50 μm square windows) were fabricated on a 5 × 5 mm² silicon chip using a previously described process.33 Brieﬂy, low-pressure chemical-vapor deposition was used to deposit a SiN layer on a 500 μm thick silicon wafer with <100> orientation. Prior to the SiN deposition, in order to reduce the electrical-capacitance noise, a SiO₂ barrier layer 2.5 μm in thickness was formed through thermal oxidation. Before ionic-current measurements, all drilled nanopores were cleaned with hot piranha solution (1:1 H₂SO₄/H₂O₂) for 15 min, followed by hot deionized water for 10 min, and then they were dried under a vacuum. A custom polytetrafluoroethylene (PTFE) conductivity cell was used to accommodate the SiN chips with nanopores. Quick-curing silicone elastomer was used to seal the gap between the chip and the cell, as well as to reduce the capacitive noise. Solutions were prepared using 99.0% KCl (Fisher Scientiﬁc) in deionized (DI) water (purified using a Millipore system), as well as mixed deionized water and glycerol solutions (Amresco). Solution conductivities (Table S1) were measured with a Fisher Scientiﬁc Accumet Basic AB30 conductivity meter at room temperature. Current—voltage curves were acquired with an Axopatch 200B ampliﬁer and a Digidata 1200 ( Molecular Devices, Inc.) and recorded using custom LabVIEW software. The recorded data were digitized and recorded at a sampling frequency of 250 kHz after application of a low-pass Bessel filter of 10 kHz. Two homemade Ag/AgCl electrodes were used to apply voltages and measure currents. The ground electrode was put in the chamber ﬁlled with aqueous solution, and the working electrode was placed on the other side of the pore in contact with the water/glycerol mixture. Voltage values were changed between −3 and +3 V with a 0.1 V step. KCl solutions (0.5, 0.75, and 1.0 M) adjusted to pH 10 with 10 mM Tris base were used. KCl solutions in water/glycerol mixtures were prepared with 20, 40, and 60% glycerol in weight ratios to consider the cases with high viscosities. During the experiments, the chambers were cleaned with DI water twice after each detection and rinsed twice before the next detection with the solution to be used. For each case, detection was conducted 5 min after the solution was ﬁlled, and the current—voltage curve was obtained by averaging at least six runs. Au nanoparticles (10 nm in core size) with polyethylene glycol (PEG)₉₀₀₀ modifications were purchased from Sigma-Aldrich. The particle size was detected as being 33.8 nm in diameter with a NanoBrook 90Plus PALS Particle Size Analyzer (Brookhaven Instruments Corporation). In the detection, 1 μL of as-obtained particle solution was added to 70 μL of KCl solution.

**Simulation Methods.** Simulations of ionic transport and fluid flow under viscosity gradients were conducted by solving coupled Poisson–Nernst–Planck (PNP) and Navier–Stokes (NS) equations with COMSOL Multiphysics 5.2. The scheme of the simulation system is shown in Figure 1b. All boundary conditions were listed in Table S2. In this work, the length of the cylindrical pore varied from 20 to 1000 nm, and its diameter ranged between 5 and 200 nm. The surface-charge density of the pores was chosen to be −0.01 C/m² to simulate the case of SiN membranes in pH 10 solutions. For the inner pore surface, a 0.1 nm mesh size was used to take into account the size of the nanopore.
account the effect of electrical double layers and ensure the convergence of the systems. The mesh of 0.5 nm was chosen for the charged boundaries of the reservoirs to lower the memory cost during simulations. KCl, KF, LiCl, and NaCl were considered in the simulations. Diffusion coefficients of the ions in water used in this work were assumed to be equal to the bulk values as show in Table S3. The salt concentrations was set from 0.5 to 1 M. Voltages applied in each case were from −3 to 3 V with a 0.2 V step.

In the system, viscosity gradients were considered by adding glycerol molecules to the system. The dielectric constants of pure water and pure glycerol were set as 80 and 42.5, respectively. Glycerol molecules were simulated as neutral particles and put in one reservoir. The physical properties of the mixed solutions were calculated with the mass ratio of the glycerol according to the equations given below. The density, dielectric constant of the solution, and viscosity were calculated using eqs 1–3, respectively. The dependence of the diffusion coefficient of the glycerol molecule on its concentration was considered with eq 5. The diffusion coefficients of ions in mixed solutions were calculated with eq 6.

\[
\rho_{\text{solution}} = x_\text{glycerol} \cdot \rho_\text{glycerol} + (1-x) \rho_\text{water}
\]

where \(\rho_{\text{solution}}, \rho_{\text{glycerol}}\) and \(\rho_{\text{water}}\) are the densities for a mixed solution of glycerol and water, a solution of pure glycerol, and a solution of pure water, respectively; \(x\) is the weight ratio of glycerol in the mixed solution.

\[
\varepsilon_{\text{solution}} = x_\text{glycerol} \cdot \varepsilon_\text{glycerol} + (1-x) \varepsilon_\text{water}
\]

where \(\varepsilon_{\text{solution}}, \varepsilon_{\text{glycerol}}\) and \(\varepsilon_{\text{water}}\) are the dielectric constants for a mixed solution of glycerol and water, a solution of pure glycerol, and a solution of pure water, respectively.

\[
\mu_{\text{solution}} = \mu_{\text{water}} \left[ \frac{1 - x/C_m}{1 - (k_0C_m - 1)x/C_m} \right]^{2.5 \varepsilon_{\text{w}}/(12 - k_0 \varepsilon_{\text{w}})}
\]

where \(\mu_{\text{solution}}\) and \(\mu_{\text{water}}\) are the viscosities for a mixed solution of glycerol and water and a solution of pure water, respectively. \(C_m\) is as the volume fraction of dispersed particles, such as glycerol molecules, at which the solution viscosity reaches an infinite value; \(k_0\) accounts for the repulsive colloidal forces among dispersed particles, which is related to the hydrodynamic forces.

\[
k_0 = -0.012T + 4.74
\]

where \(T\) is the absolute temperature, and \(C_m = 0.74\) and \(C_m = 1.2\) were used for solutions with glycerol weight ratios less than 60% and equal to 60%, respectively. The obtained viscosity values with different glycerol percentages are shown in Figure S1 with a comparison to experimental data.

The diffusion coefficient of glycerol molecules in mixed solutions was described by eq 5, which was a linear fitting of the experimental results.

\[
D = (9.986 - 9.802\alpha) \times 10^{-10} \text{ (m}^2/\text{s})
\]

On the basis of the Stokes–Einstein equation,

\[
D = \frac{RT}{N_A \pi a^2 \mu_{so}}
\]

in which \(R\) is the gas constant, \(N_A\) is Avogadro’s number, and \(a\) is the radius of a moving particle, the diffusion coefficient of ions in a mixed solution can be calculated as follows:

\[
D_{\text{solution}} = \frac{\mu_{\text{water}}}{\mu_{\text{solution}}} D_{\text{water}}
\]

where \(D_{\text{solution}}\) and \(D_{\text{water}}\) are the diffusion coefficients of ions in a mixed solution and in water, respectively.

### RESULTS AND DISCUSSIONS

Behaviors of large-aspect-ratio pores in contact with viscosity gradients were recently probed experimentally and numerically with single mesopores in polymer films. Electroosmotic flow (EOF) caused by the negatively charged pore walls could drag the solution of the reservoir on the entrance side into the mesopores to induce voltage-dependent electrical resistance. These pores exhibited ionic-current rectification equal to the ratio of the two solutions’ conductivities. This electroosmotically driven ionic-current rectification could be fully predicted and easily tuned by changing the conductivity of one solution and holding that for the other solution steady. The experiments showed that for long pores, the liquid–liquid miscible region between water and a 40% glycerol solution could be ignored because of its minute length. Herein, we considered simplified cases with pores under different viscosity gradients without the consideration of the liquid–liquid miscible regions. Hence, theoretical prediction for ionic-current rectification could be given via equations of access resistance on one side \((R_{ac})\) and pore resistance \((R_p)\):

\[
R_{ac} = \frac{1}{2kD}
\]

\[
R_p = \frac{4L}{\pi k D^2}
\]

where \(k\) is the conductivity of the solution, \(D\) is the diameter of the pore, and \(L\) is the length of the pore.

Pores were negatively charged so that the direction of electroosmotic flow was expected to be determined by the direction of cation migration. In the electrode configuration chosen, the working electrode was placed in the medium of low conductivity, as shown in Figure 1. Consequently, positive voltages were expected to fill the pore with the low-conductivity medium, and \(|I_+|\) was expected to be larger than 1. The ionic-current-rectification (ICR) ratio could be calculated as

\[
\text{ICR} = \left| \frac{I_+}{I_-} \right| = \left| \frac{V/R_+}{V/R_-} \right| = \frac{R_+}{R_-}
\]

\[
= \frac{R_{ac-water} + R_{p-water} + R_{ac-solution}}{R_{ac-water} + R_{p-water} + R_{ac-solution}}
\]

\[
\frac{k_{\text{water}}}{k_{\text{solution}}} = \frac{D_{\text{water}}}{D_{\text{solution}}} = \frac{\mu_{\text{solution}}}{\mu_{\text{water}}}
\]

in which \(I_+, I_-, R_+, \text{ and } R_-\) are the currents (I) and resistances (R) under positive and negative voltages. \(V\) is the applied voltage. \(R_{ac-water}\) and \(R_{ac-solution}\) stand for the access resistances on the sides in contact with aqueous solution and with mixed solution, respectively. \(R_{p-water}\) and \(R_{p-solution}\) are the pore resistances assuming the pore is filled with water and with mixed solution, respectively. \(k_{\text{water}}\) and \(k_{\text{solution}}\) are the conductivities of the aqueous and mixed solutions, respectively. The viscosity parameters were taken from the literature.
Figure 2 shows the predicted ICR ratios under viscosity gradients between an aqueous solution on one side of the pore and a mixed glycerol solution with a 20, 40, or 60% weight ratio of glycerol on the other side. When the pore length is short ($L/D < 0.1$), access resistance on both sides of the pore dominates the total resistance. In this case, eq 9 predicts an ICR $\approx 1$ (i.e., no current rectification appears). This case has been experimentally determined in ultrathin MoS$_2$ pores subjected to a viscosity gradient.\textsuperscript{22} As the pore length increases ($0.1 < L/D < 10$), the pore resistance becomes more significant, leading to an ICR $> 1$, which means more obvious current rectification. Finally, when the pore length is $\sim$10 times its diameter, the ICR ratio reaches its maximum value equal to the viscosity ratio across the pore, as shown before.\textsuperscript{20,21}

However, when the length of the pore belongs to an intermediate range, such as less than a hundred nanometers with a diameter as 20 nm, the liquid–liquid miscible region may extend across the pore because of diffusion of solvent molecules. In this case, the solution property is not uniform, that means the conductivity of the solution in the pore can be higher than that of the glycerol solution but lower than that of the aqueous solution. From the literature, there is little consideration of the cases of pores with medium lengths under viscosity gradients.\textsuperscript{20} We will show that because of its sensitivity to applied voltages, new transport phenomena can be observed.

In this work, ionic-current behaviors through nanopores under viscosity gradients were investigated through experiments and COMSOL simulations. For the case with a viscosity gradient between aqueous and 40% glycerol solutions, Figure S2 shows the distribution of glycerol molecules in the pore and the corresponding viscosity distributions from simulations. When a positive voltage was applied as 0.4 V, the EOF dragged the 40% glycerol solution to the pore, which made the concentration of glycerol molecules higher than in cases without a voltage or with a negative voltage. Therefore, the solution was more viscous. Because the pore was very short, there were no plateau regions in the concentration distribution of glycerol, which changed monotonously and was different from that in long pores.\textsuperscript{16}

Experimental investigation of ionic-current behaviors under viscosity gradients had been done with SiN pores $\sim$20 nm in diameter and 50 nm in length. As shown in Figure 3a, in the cases without viscosity gradients, the current depended linearly on the applied voltages. When the viscosity gradient appeared between aqueous and 40% glycerol solutions, current...
rectification happened correspondingly.\textsuperscript{20} The ICR ratio is plotted in Figure S3. The peak ratio was found to be $\sim$1.9 in 1 M KCl solutions, which was lower than the conductivity ratio (Table S1) and viscosity gradient, suggesting the pore was filled with a mixture of water and glycerol.\textsuperscript{26} For negative voltages, the EOF filled the pore with aqueous solution, which resulted in a current similar to that of the aqueous case. Under positive biases, EOF dragged the glycerol solution into the pore, which induced a smaller current. Under lower voltages (<1.25 V), the current-rectification ratio increased with voltage.\textsuperscript{11} However, an interesting phenomenon appeared when the voltage reached $\sim$1.25 V: a second, steeper current slope appeared, which suppressed the current-rectification ratio to 1. Both the increased current and the decreased ICR ratio were unexpected, because usually, a larger EOF\textsuperscript{2} under a stronger electric field can fill the pore with more viscous solutions.\textsuperscript{20,21} Here, we call it abnormal current rectification. In order to roughly evaluate the location of the appearance of the second slope (i.e., the turning point), the ICR ratio was plotted with voltage. The phenomenon of abnormal current rectification could be easily repeated with different SiN pores, as shown in Figure S4. Please note that because of differences in pore geometry and opening diameters, the locations of the turning points for different pores may be slightly different.

Different electrolyte concentrations and viscosity gradients were considered in the experiment. The abnormal current rectification happened with KCl concentration from 0.5 to 1.0 M (Figures S5 and S6). The results in Figures 3b and S3a indicate that a lower salt concentration could provide a larger ICR ratio. This was due to the stronger electroosmotic flow in the pore,\textsuperscript{25} which was related to a thicker Debye layer and a higher surface potential.\textsuperscript{25} The turning point in a more dilute solution was located at a voltage that was a little higher, according to the comparison of ICR ratios shown in Figure 3b. For the cases with different viscosity gradients across the pore, higher viscosity gradients produced more obvious current rectification because of the larger differences in conductivities between the viscous and aqueous solutions. From the ICR-ratio plot, the turning point depended closely on the weight ratio of the glycerol. (Figures S3b and S6)

In order to understand the mechanism of the abnormal current rectification, COMSOL simulations were conducted with a consideration of viscosity gradients across the nanopore. 20 nm in diameter and 50 nm thick with $-0.01$ C/m$^2$ in surface charge density. The same electrolyte concentrations and viscosity gradients as those studied experimentally in Figure 3 were considered. Current—voltage curves obtained from simulations are shown in Figure 4. The ICR ratios under different KCl concentrations and viscosity gradients are plotted in Figure S7. The simulation results were in excellent agreement with the experiment. Please note that the absolute values of the current-rectification ratios in the simulations were a little lower than those obtained in the experiments. This may be due to a small difference in the pore shape or surface charge density used in the simulations from those of the real cases.\textsuperscript{35,35,48}

In the next step, the current—voltage curves with detailed contributions of the cations and anions under a viscosity gradient with aqueous and 40% glycerol solutions were analyzed, as shown in Figure 5a,b. The contributions of K$^+$ ions under no viscosity gradient shared almost the same value at different voltages (i.e., $\sim$51%, a little larger than 50% because of the enhanced concentration caused by the negatively charged surfaces).\textsuperscript{25} In the presence of a viscosity gradient, from $-1$ to 3 V the contribution of K$^+$ ions monotonously decreased to a level significantly below 50% and remained at a relative constant value of 52.5% at voltages below $-1$ V. Similar results had also been obtained with different KCl concentrations and viscosity gradients. For the cases with different KCl concentrations, the percentage distributions of K$^+$ ions had the same trend, whereas for the cases with different viscosity gradients, a higher viscosity gradient resulted in a larger decrease in the K$^+$-ion contribution to the total current. The decreased contribution of cations to the total ion current under viscosity gradients was surprising, because SiN nanopores were expected to be negatively charged. Counter-ions were attracted to the pore surface to form electric double layers,\textsuperscript{25} which made K$^+$ ions the dominant current carriers (i.e., 51% current contribution in the aqueous case and in the 40% glycerol cases without gradients). Different electrolytes had been used to consider the influence of ionic mobility. As
shown in Figure S8, ionic mobility did not have obvious effect on the abnormal current-rectification phenomenon. In order to explain why the contribution of K⁺ ions to the total current decreased at high positive biases, we investigated the ionic-concentration distributions and electroosmotic flow in the pore, which could influence the ionic current directly.11,49,50 As shown in Figure 6a,b, the concentrations of both ions were strongly voltage-dependent, and at high voltages, concentration polarization became obvious. With an increase in the applied positive voltage, the concentration of Cl⁻ ions became higher.

Figure 5. Simulation data of ionic current through a nanopore. (a) IV curves with detailed contributions from cations and anions in a 1 M KCl solution under a viscosity gradient between aqueous and 40% glycerol solutions. (b) Contributions of K⁺ ions to the total current in a 1 M KCl solution in aqueous and glycerol solutions, as well as under a viscosity gradient. (c) Contributions of K⁺ ions to the total current in KCl solutions of different concentrations under a viscosity gradient between aqueous and 40% glycerol solutions. (d) Contributions of K⁺ ions to total current in a 1 M KCl solution under different viscosity gradients.

Figure 6. Simulation results from a nanopore 50 nm in length and 20 nm in diameter. (a,b) Concentration distributions of K⁺ (solid lines) and Cl⁻ (dashed lines) ions (a) along the pore axis and (b) in the radial direction at the center cross-section of the pore. (c,d) Electroosmotic flow (c) along the axis of the pore and (d) in the radial direction at the center cross-section of the pore. The viscosity gradient was set between 40% glycerol and aqueous solutions. The surface charge density of the pore walls was set as −0.01 C/m². The pore region is shown in yellow.
than that of K⁺ ions. The increase in the co-ion concentration was indeed induced by the viscosity gradient, because in symmetric aqueous conditions, counterions shared almost the same concentrations as the co-ions at all voltages (Figure S9c,d). We thought this was caused by the different ionic-mobility changes along the pore axis for K⁺ and Cl⁻ ions. Under positive voltages, K⁺ ions moved from the solution with a higher viscosity to the aqueous solution. Their ionic mobility increased along the axis. Cl⁻ ions passed through the pore in the opposite direction, with their mobility decreasing monotonously during the translocation process. Consequently, Cl⁻ ions could have a larger concentration than K⁺ ions because of the slower depletion at the end of the pore connected with a high-viscosity solution, as shown in Figures 6a and S9a. We also found that compared with the case of symmetric solutions, higher depletion of K⁺ ions happened at the pore entrance under viscosity gradients because of an increase of their mobility along the pore axis (Figure S10).

However, K⁺ and Cl⁻ ions shared almost the same mobility at the same locations in the pore. A small difference in their concentrations cannot produce such an obvious current increase. The voltage-dependent relative concentrations of cations and anions prompted us to look into details of magnitude and direction of electroosmotic flow in the pore, which can control the viscosity of the solution and then the ionic mobility. As another important physical property of the solution in the pore, the EOF was investigated in axial and radial directions. The radial-speed distribution in the center cross-section of the pore is plotted in Figure 6d. At low positive voltages, such as 0.2 and 0.4 V, the EOF followed the forward direction, which went from the positive electrode to the negative electrode, and was weak because of the high viscosity of the solution in the pore. As the voltage increased, the magnitude of the EOF first decreased to nearly 0 mm/s at 0.6 V and then switched direction and increased with the voltage. Because the solution viscosity was relatively high, the fluid flow caused by the directional movement of ions in electric double layers could not affect the flow velocity in the center of the pore in a significant manner. The reversed electroosmotic flow induced in the center of the pore moved from the negative electrode to the positive electrode, which brought the pore a solution of lower viscosity from the exit side (Figure S9b). As a result, the ionic mobility increased under high positive voltages which produced a much larger current as we found in the experiments. In Figure 6d, the velocity distribution of the EOF in the radial direction was similar to that found before under concentration gradients. On the basis of the ionic-concentration distributions, the reversed EOF was caused by more Cl⁻ ions being in the center of the pore than K⁺ ions. From the Navier–Stokes equations, the net force of the fluid in the center region followed the moving direction of Cl⁻ ions. The 2D EOF flow in the pore was plotted in Figure 7, which revealed that the direction of electroosmotic flow was dependent on the radial position at voltages higher than 0.6 V. Consequently, formation of vortices was expected, which would lead to more efficient mixing of the two solutions.

In order to confirm the voltage-dependent direction of electroosmotic flow in short nanopores subjected to viscosity gradients, resistive-pulse detection using Au nanoparticles with 10 nm cores and PEG5000 modification was conducted to show the direction of fluidic movement. The mean particle diameter was determined as 33.8 nm through the dynamic-light-scattering method (Figure S11). Because of the presence of PEG molecules on the particle surfaces, we assumed the particles were neutral. Experiments were conducted using small pores ~20 nm in diameter because the limitation of the current range was ±200 nA in the Axopatch 200B. Consequently, under symmetric aqueous conditions, we did not observe particle passages through the pore, as is typically done, but rather their approach to the pore opening, which also caused a small current decrease, as shown in Figure S12. This current drop was due to the change of resistance caused by the occupation of the particle in the access-resistance regions. Because the particles were only put in the cis chamber, the current drops were observed only for one voltage polarity, consistent with the direction of electroosmotic flow. Please note that there may be a few spikes under positive voltages because of the passed ultrasmall particles. When the solution in the trans chamber was replaced with a 40% glycerol solution, at low positive voltages, similar current blockades were observed as those in aqueous solutions for one voltage polarity only (Figure S13a,b). With the voltage increasing to 1.5 V, resistive pulses appeared under both negative and positive voltages. In order to avoid the influence of the ultrasmall particles passing through the pore, detection was also conducted with a newly changed 40% glycerol solution on the trans side under reversed voltages. As shown in Figure S13d, resistive pulses also appeared. The passage of particles for both voltage polarities could be explained by the radially inhomogeneous direction of electroosmotic flow shown in Figure 7. If a particle approached the pore close to the walls, it would be moved toward the pore opening in the direction of cations; if a particle approached the pore closer to the pore center, it would experience the direction of reversed electroosmotic flow.

Finally, we wanted to identify parameters that would allow us to tune the occurrence of the reversed electroosmotic flow and the rectification degree. Note that for the 50 nm long and 20 nm in diameter nanopore considered in Figure 3, the maximum rectification degrees obtained in the experiment and simulation (~1.9 and ~1.5) were lower than the viscosity ratio of 3.98 and conductivity ratio of 2.87. Because of the dependence of the solution distribution in the pore on the EOF, which could be tuned with pore geometry and surface charge densities, ionic transport was investigated with different pore geometries (i.e., length and diameter), as well as different surface charge densities under a viscosity gradient between 40% glycerol and aqueous solutions. As shown in Figure S14, under the same voltage, as we increased the...
narrow nanopore length and kept the diameter at 20 nm, ICR ratios increased as well, in accordance with our prediction of a smaller influence of the liquid–liquid miscible region on the pore conductance. For pores with the same length (50 nm) but different diameters, the ICR ratios became lower as the diameter increased, which was caused by a lowered percentage of the pore resistance in larger pores because of the increased access resistance, as well as the weaker viscosity gradient caused by the higher flux of EOF (Figure S15). Through changing the pore geometry, the ICR ratio still cannot reach the viscosity gradient within 3 V. Higher voltages and surface charge densities were also considered because the EOF depended on the electric-field strength and surface potential linearly. Under the same electric-field strength (applied voltage divided by pore length), we found that the current-rectification ratio reached the viscosity ratio under strong electric fields for pores longer than 500 nm only, although super-large voltages may not be feasible in experiments. By using higher surface charge densities on pore surfaces, much stronger current rectification appeared across the pore within 3 V. For the case of a pore with ~0.04 C/m², the ICR ratio could reach 3.5, approaching the viscosity gradient. As shown in Figure S16, the strong EOF filled most regions inside the pore with viscous solution, which also increased the viscosity outside the pore on the exit side. From the ICR-ratio plots, the voltage corresponding to the peak value can roughly represent the turning point. With increased pore lengths, enhanced surface charge densities, and decreased pore diameters, the voltage corresponding to the peak value can roughly represent the turning point. With increased pore lengths, enhanced surface charge densities, and decreased pore diameters, the turning point became located at larger voltages (Figure S14d), which was caused by the balance between electroosmotic flow and the reversed electroosmotic flow.

**CONCLUSIONS**

Nanopores with low aspect ratios present a good model system to investigate liquid–liquid miscible regions in confined spaces. Herein, with the method of current detection, ionic behaviors were explored under viscosity gradients between aqueous and glycerol solutions. With a viscosity gradient across thin nanopores, abnormal current rectification was found when the pore was filled with solutions of high viscosities under relatively high electric-field strengths. From the current–voltage curves, it was shown that the ionic current had a turning point, after which the current value increased much faster and the current-rectification ratio decreased, in a different trend from normal current rectifications. With help from simulations, this abnormal current rectification was found to be caused by the reversed electroosmotic flow, which dragged the aqueous solution into the pore to lower the viscosity. Excessive co-ions can be responsible for the unexpected reversed electroosmotic flow in a negatively charged, confined channel because of their slower depletion compared with that of counterions along the pore. Through enhancing the surface charge density of the pore, the ionic-current-rectification ratio could reach the viscosity gradient across thin nanopores.

The experiments and modeling presented in this manuscript also revealed that enhanced mixing of solutions could occur in nanoconfined spaces if reversed EOF appeared. The induced mixing could find applications in designing nanoreactors and probing chemical reactions at the nanoscale. Meanwhile, the enhanced sensitivity of the ICRs of short nanopores to viscosity makes them a very attractive tool for dynamically probing local changes in solution viscosity induced, for example, by products of chemical reactions or the presence of molecules or particles.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.8b04225.

Conductivity of the solutions in the experiments, boundary conditions and parameters in the simulations, viscosity obtained from the simulations, repeatability of abnormal ionic-current rectification, ionic and fluidic behaviors from the simulation, resistive-pulse detection of nanoparticles, simulation results from pores with different sizes and surface charges, and simulation report (PDF)

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: yinghua.qiu@utah.edu or yinghua.qiu@hotmail.com (Y.Q.).
*E-mail: wanunu@neu.edu (M.W.).

**ORCID**

Yinghua Qiu: 0000-0003-2489-0784
Zuzanna S. Siwy: 0000-0003-2626-7873
Meni Wanunu: 0000-0002-9837-0004

**Present Address**

Y.Q.: Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We acknowledge funding from the National Institutes of Health (HG009186). We thank M. Alibakhshi at Northeastern University for help in fabrication of SiN-membrane chips and Professor S. Sridhar at Northeastern University for use of the dynamic-light-scattering instrument.

**REFERENCES**

(1) Siwy, Z. S.; Howorka, S. Chem. Soc. Rev. 2010, 39 (3), 1115–1132.
(2) Schoch, R. B.; Han, J. Y.; Renaud, P. Rev. Mod. Phys. 2008, 80 (3), 839–883.
(3) Luo, L.; German, S. R.; Lan, W.-J.; Holden, D. A.; Mega, T. L.; White, H. S. Annu. Rev. Anal. Chem. 2014, 7 (1), 513–535.
(4) Bayley, H.; Martin, C. R. Chem. Rev. 2000, 100 (7), 2575–2594.
(5) Lepoitevin, M.; Ma, T.; Bechelany, M.; Janot, J.-M.; Balme, S. Adv. Colloid Interface Sci. 2017, 250, 195–213.
(6) Wu, X.; Ramiah Rajasekaran, P.; Martin, C. R. ACS Nano 2016, 10 (4), 4637–4643.
(7) Oyarzua, E.; Walther, J. H.; Megaridis, C. M.; Koutouetskos, P.; Zambrano, H. A. ACS Nano 2017, 11 (10), 9997–10002.
(8) van der Heyden, F. H. J.; Bonthuis, D. J.; Stein, D.; Meyer, C.; Dekker, C. Nano Lett. 2006, 6 (10), 2232–2237.
(9) Ji, J.; Kang, Q.; Zhou, Y.; Feng, Y.; Chen, X.; Yuan, J.; Guo, W.; Wei, Y.; Jiang, L. Adv. Funct. Mater. 2017, 27 (2), 1603623.
(10) Feng, J.; Graf, M.; Liu, K.; Ovchinnikov, D.; Dumencio, D.; Heirianian, M.; Sandigand, V.; Aluru, N. R.; Kis, A.; Radenovic, A. Nature 2016, 536, 197.
(11) Siwy, Z. S. Adv. Funct. Mater. 2006, 16 (6), 735–746.
(12) Guo, W.; Tian, Y.; Jiang, L. Acc. Chem. Res. 2013, 46 (12), 2834–2846.

**AUTHOR INFORMATION**

**Corresponding Authors**

*E-mail: yinghua.qiu@utah.edu or yinghua.qiu@hotmail.com (Y.Q.).
*E-mail: wanunu@neu.edu (M.W.).

**ORCID**

Yinghua Qiu: 0000-0003-2489-0784
Zuzanna S. Siwy: 0000-0003-2626-7873
Meni Wanunu: 0000-0002-9837-0004

**Present Address**

Y.Q.: Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States

**Notes**

The authors declare no competing financial interest.

**ACKNOWLEDGMENTS**

We acknowledge funding from the National Institutes of Health (HG009186). We thank M. Alibakhshi at Northeastern University for help in fabrication of SiN-membrane chips and Professor S. Sridhar at Northeastern University for use of the dynamic-light-scattering instrument.

**REFERENCES**

(1) Siwy, Z. S.; Howorka, S. Chem. Soc. Rev. 2010, 39 (3), 1115–1132.
(2) Schoch, R. B.; Han, J. Y.; Renaud, P. Rev. Mod. Phys. 2008, 80 (3), 839–883.
(3) Luo, L.; German, S. R.; Lan, W.-J.; Holden, D. A.; Mega, T. L.; White, H. S. Annu. Rev. Anal. Chem. 2014, 7 (1), 513–535.
(4) Bayley, H.; Martin, C. R. Chem. Rev. 2000, 100 (7), 2575–2594.
(5) Lepoitevin, M.; Ma, T.; Bechelany, M.; Janot, J.-M.; Balme, S. Adv. Colloid Interface Sci. 2017, 250, 195–213.
(6) Wu, X.; Ramiah Rajasekaran, P.; Martin, C. R. ACS Nano 2016, 10 (4), 4637–4643.
(7) Oyarzua, E.; Walther, J. H.; Megaridis, C. M.; Koutouetskos, P.; Zambrano, H. A. ACS Nano 2017, 11 (10), 9997–10002.
(8) van der Heyden, F. H. J.; Bonthuis, D. J.; Stein, D.; Meyer, C.; Dekker, C. Nano Lett. 2006, 6 (10), 2232–2237.
(9) Ji, J.; Kang, Q.; Zhou, Y.; Feng, Y.; Chen, X.; Yuan, J.; Guo, W.; Wei, Y.; Jiang, L. Adv. Funct. Mater. 2017, 27 (2), 1603623.
(10) Feng, J.; Graf, M.; Liu, K.; Ovchinnikov, D.; Dumencio, D.; Heirianian, M.; Sandigand, V.; Aluru, N. R.; Kis, A.; Radenovic, A. Nature 2016, 536, 197.
(11) Siwy, Z. S. Adv. Funct. Mater. 2006, 16 (6), 735–746.
(12) Guo, W.; Tian, Y.; Jiang, L. Acc. Chem. Res. 2013, 46 (12), 2834–2846.
(13) Cao, L.; Guo, W.; Wang, Y.; Jiang, L. Langmuir 2012, 28 (4), 2194–2199.
(14) Hsu, W.-L.; Harvie, D. J. E.; Davidson, M. R.; Jeong, H.; Goldys, E. M.; Inglis, D. W. Lab Chip 2014, 14 (18), 3539–3549.
(15) Lin, C.-Y.; Yeh, L.-H.; Hsu, J.-P.; Tseng, S. Small 2015, 11 (35), 4594–4602.
(16) Qiu, Y.; Dawid, A.; Siwy, Z. S. J. Phys. Chem. C 2017, 121 (11), 6255–6263.
(17) Möller, F. M.; Kriegel, F.; Kieß, M.; Sojo, V.; Braun, D. Angew. Chem., Int. Ed. 2017, 56 (9), 2340–2344.
(18) Wang, L.; Guo, W.; Xie, Y. B.; Wang, X. W.; Xue, J. M.; Wang, Y. G. Radiat. Meas. 2009, 44 (9–10), 1119–1122.
(19) Herr, A. E.; Molho, J. I.; Santiago, J. G.; Mungal, M. G.; Kenny, T. W.; Garguilo, M. G. Anal. Chem. 2000, 72 (5), 1053–1057.
(20) Yuskov, E. C.; An, R.; Mayer, M. ACS Nano 2010, 4 (1), 477–487.
(21) Qiu, Y.; Lucas, R. A.; Siwy, Z. S. J. Phys. Chem. Lett. 2017, 8 (16), 3846–3852.
(22) Feng, J.; Liu, K.; Bulushev, R. D.; Khlybov, S.; Dumcenco, D.; Kis, A.; Radenovic, A. Nat. Nanotechnol. 2015, 10 (12), 1070–1076.
(23) de Haan, H. W.; Slater, G. W. Phys. Rev. E 2013, 87 (4), 042604.
(24) Hille, B. Ion channels of excitable membranes, 2nd ed.; Sinauer Associates Inc.: Sunderland, MA, 2001.
(25) Israelachvili, J. N. Intermolecular and Surface Forces, 3rd ed.; Academic Press: Burlington, MA, 2011.
(26) Tuvia, S.; Almagor, A.; Bitler, A.; Levin, S.; Korenstein, R.; Yedgar, S. Proc. Natl. Acad. Sci. U. S. A. 1997, 94 (10), 5045–5049.
(27) Kuimova, M. K.; Yahioolu, G.; Levitt, J. A.; Suhling, K. J. Am. Chem. Soc. 2008, 130 (21), 6672–6673.
(28) Suhling, K.; Siegel, J.; Lanigan, P. M.; Lévéque-Fort, S.; Webb, S. E.; Phillips, D.; Davis, D. M.; French, P. M. Opt. Lett. 2004, 29 (6), 584–586.
(29) Pramanik, S.; Mishra, M. Phys. Fluids 2016, 28 (8), 084104.
(30) Fologea, D.; Uplinger, J.; Thomas, B.; McNabb, D. S.; Li, J. Lab Chip 2005, 5 (9), 1734–1737.
(31) Liu, B.; Stolovitzky, G.; Martyna, G. Nanoscale 2012, 4 (4), 1068–1077.
(32) Luo, L.; Holden, D. A.; Lan, W.-J.; White, H. S. ACS Nano 2012, 6 (7), 6507–6514.
(33) Carson, S.; Wilson, J.; Aksimentiev, A.; Wanunu, M. Biophys. J. 2014, 107 (10), 2381–2393.
(34) Qiu, Y.; Vlassiakou, I.; Chen, Y.; Siwy, Z. S. Anal. Chem. 2016, 88 (9), 4917–4925.
(35) Sa, N.; Lan, W.-J.; Shui, W.; Baker, L. A. ACS Nano 2013, 7 (12), 11272–11282.
(36) Wang, J.; Ma, J.; Ni, Z.; Zhang, L.; Hu, G. RSC Adv. 2014, 4 (15), 7601–7610.
(37) Ho, C.; Qiao, R.; Heng, J. B.; Chatterjee, A.; Timp, R. J.; Aluru, N. R.; Timp, G. Proc. Natl. Acad. Sci. U. S. A. 2005, 102 (30), 10445–10450.
(38) Qiu, Y. Analyst 2018, 143, 4638.
(39) Cussler, E. L. Diffusion: mass transfer in fluid systems; Cambridge University Press: Cambridge, 2009.
(40) Robinson, R. A.; Stokes, R. H. Electrolyte solutions; Courier Corporation, 2002.
(41) Takamura, K.; Fischer, H.; Morrow, N. R. J. Pet. Sci. Eng. 2012, 98–99, 50–60.
(42) Akerlof, G. J. Am. Chem. Soc. 1932, 54 (11), 4125–4139.
(43) Ternström, G.; Sjöström, A.; Aly, G.; Jernqvist, A. J. Chem. Eng. Data 1996, 41 (4), 876–879.
(44) Einstein, A. Ann. Phys. 1905, 322 (8), 549–560.
(45) Lide, D. R. CRC Handbook of Chemistry and Physics, 90th ed.; CRC Press: Boca Raton, FL, 2009.
(46) Hall, J. E. J. Gen. Physiol. 1975, 66 (4), 531–532.
(47) Ghosal, S. Electrophoresis 2004, 25 (2), 214–228.
(48) Davenport, M.; Healy, K.; Pevarnik, M.; Teslich, N.; Cabrini, S.; Morrison, A. P.; Siwy, Z. S.; Létant, S. E. ACS Nano 2012, 6 (9), 8366–8380.
(49) Lin, D.-H.; Lin, C.-Y.; Tseng, S.; Hsu, J.-P. Nanoscale 2015, 7 (33), 14023–14031.
(50) Ai, Y.; Zhang, M.; Joo, S. W.; Cheney, M. A.; Qian, S. J. Phys. Chem. C 2010, 114 (9), 3883–3890.
(51) Coulter, W. H. Means for Counting Particles Suspended in a Fluid. U.S. Patent 2,656,508, 1953.
(52) Rahme, K.; Chen, L.; Hobbs, R. G.; Morris, M. A.; O’Driscoll, C.; Holmes, J. D. RSC Adv. 2013, 3 (17), 6085–6094.
(53) Qiu, Y.; Siwy, Z. Nanoscale 2017, 9 (36), 13527–13537.
(54) Hyun, C.; Rollings, R.; Li, J. Small 2012, 8 (3), 385–392.