The current-voltage relation for electropores with conductivity gradients

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In electroporation, an electric field transiently permeabilizes the cell membrane to gain access to the cytoplasm, and to deliver active agents such as DNA, proteins, and drug molecules. Past work suggests that the permeabilization is caused by the formation of aqueous, conducting pores on the lipid membrane, which are also known as electropores. The current-voltage relation across the membrane-bound pores is critical for understanding and predicting electroporation. In this work, we solve the Nernst–Planck equations in a geometry encompassing an isolated electropore to investigate this relation. In particular, we study cases where the intra- and extracellular electrical conductivities differ. We first derive an analytical solution, which is subsequently validated with a direct numerical simulation using a finite volume method. The main result of the current work is a formula for the effective pore resistance as a function of the pore radius, the membrane thickness, and the intra- and extracellular conductivities. This formula can be incorporated into whole-cell or planar-membrane electroporation models for system-level prediction and understanding. © 2010 American Institute of Physics.
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I. INTRODUCTION

In electroporation, one or multiple electric pulses are applied to permeabilize the cell membrane. The permeabilization can be reversible, such that the cytoplasm becomes transiently accessible to biologically active molecules while the cell maintains viability. This mechanism finds wide applications in cell transfection, protein insertion (proteomics), gene and drug delivery, and stem cell research, among others.^{1–3} When the permeabilization is irreversible, permanent cell membrane damage results, which leads to cell apoptosis. In this case, electroporation can be harnessed to develop biofouling control, debacterialization, and novel cancer treatment techniques.^{4–7}

In addition to technological developments, research in the past two decades has also made significant progress in model understanding of the complex processes involved in electroporation. The in-depth insight and prediction capability provided is critical for the improvement and optimization of this technique. In the current literature, the permeabilization process is understood as the nucleation and evolution of aqueous, conducting pores (also known as the electropores) on the membrane, and is described by the Smoluchowski equation.^{8–10} The application of an electric field leads to the buildup of a transmembrane potential (TMP). When the TMP reaches a critical threshold, stable hydrophilic pores begin to form on the membrane. Ionic conduction through these pores subsequently limits the growth of the TMP due to the reduced effective membrane resistance.¹¹ The evolution of the TMP in turn affects further evolution of the pores. A key element coupling these two processes is the current-voltage relation across the membrane-bound elec-

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FIG. 1. Schematic of the problem. (a) The geometry of an isolated pore. (b) The rotational elliptic coordinates for the extracellular space: ξ (solid) and η (dashed) contours. Here, σ_m^e and Φ_m^e are the conductivity and potential at the extracellular-pore interface, respectively.

tropores. This relation, however, is difficult to measure directly due to the typical nanometer pore sizes, although measurements of the integrated currents over the entire cell/membrane can be achieved.^{12–14}

Various modeling approaches on the current-voltage relation can be found in the literature. In some of their earlier works, Joshi and co-workers used a simple, linear correlation between membrane resistance and the fractional area occupied by the pores.^{15,16} Krassowska and co-workers calculated the pore resistance based on the electrical conductivity of the pore-filling electrolyte solution; they have also included the effects of the “input” (or “spreading”) resistance which is due to the contributions from the half-spaces surrounding the pore.^{11,17} A more sophisticated formula was first proposed by Chernomordik and co-workers which was based on a one-dimensional Nernst–Planck model for the electrolyte solutions within the pore.^{12,18} This model was further more rigorously developed by Barnett,¹⁹ and has been widely used by various groups pursuing electroporation modeling research.^{20–28} A salient feature of this model is the inclusion of the effects due to membrane polarization (the Born energy) and steric hindrance. The most complete formula following this approach is that by Vasilkoski *et al.*,²⁶ in which the authors also take into account the spreading resistance in a manner similar to Krassowska and co-workers.^{11,17} It is important to note that nearly all these models require the specification of the electrical conductivity for the electrolyte solution in and/or around the pore as an input parameter. For some applications where the intra- and extracellular conductivities are equal and known, this task is straightforward, as the conductivity field around the pore can be set to be uniform. For most cases, however, the conductivities do differ. An example is sea urchin electroporation where the conductivity of the buffer is an order of magnitude higher than that of the cytoplasm.^{11,13} In this situation, strong conductivity and electrolyte concentration gradients exist across the membrane-bound pore, and the effective pore conductivity (or resistance) needs to be calculated as a function of the specific environmental parameters. The current work attempts to achieve this goal.

We solve the Nernst–Planck equations for a binary electrolyte solution in a three-dimensional geometry describing a cylindrical, membrane-bound electropore and its surrounding spaces [see Fig. 1(a) below]. Our approach follows the direct solution method of Neu *et al.*²⁹ However, instead of assuming a uniform conductivity field, we allow different electrolyte concentrations, and hence electrical conductivities, in the intra- and extracellular spaces. As a consequence, the conductivity gradient may induce charge separation, and both electrically neutral and non-neutral regimes exist. In Sec. II, the governing equations are first solved analytically in the electrically neutral limit. The resulting formula, an effective pore resistance as a function of the ambient conductivities, the pore radius, and the membrane thickness, is a main contribution of the present work. In Sec. III, the analytical solution is compared with the results from direct numerical simulations (DNSs) of the original Nernst–Planck equations. We find that the agreement between the two solutions is excellent when charge separation is negligible, and reasonable even when electroneutrality is violated, in particular in terms of the effective pore resistance. The results suggest the use of the analytical

solution as a convenient and practical formula to calculate the current-voltage relation across an electropore for system-level electroporation modeling. In Sec. IV, we briefly discuss the present results in relation to the class of methods following Glaser *et al.*¹⁸ and Barnett.¹⁹ A main difference is that the electric potential in the present work is solved consistently from the ionic current conservation equations, and does not require an empirical specification. We also discuss the limitations of the present model and possible approaches for extension. Finally in the Appendix, we document the detailed arguments leading to the boundary conditions. These boundary conditions implicitly include the effect of membrane polarization within the continuum framework.

II. THEORY

In Fig. 1(a), an isolated pore is idealized as a cylindrical, electrolyte-filled channel embedded in an impermeable membrane. The radius of the pore is r_0 , the membrane thickness is h , and the geometry is axisymmetric with respect to the z -axis. σ_∞ and Φ_∞ denote the ambient electrical conductivity and electric potential, and the superscripts i and e denote intra- and extracellular, respectively. Specific experimental conditions often give rise to differences in σ_∞^i and σ_∞^e , resulting in nonuniform conductivity distributions. The difference in the ambient potentials defines the TMP,

$$V_m \equiv \Phi_\infty^e - \Phi_\infty^i. \quad (1)$$

An effective pore resistance is defined as

$$R_{\text{tot}} = R_p + R_i = V_m / i_p, \quad (2)$$

where i_p is the *total* ionic current through the pore, R_p denotes the resistance due to the electrolyte solution within the pore, and R_i is the *spreading* or *input* resistance due to contributions from the half-spaces surrounding the pore.^{17,26,30} The effective resistance characterizes the current-voltage relation across the pore-embedded membrane, which is the subject of the current study.

A. Formulation

We use a binary electrolyte model to describe the fluid around the electropore. The steady-state Nernst–Planck equation for ionic transport reads

$$\nabla \cdot \mathbf{J}_\pm = 0, \quad \mathbf{J}_\pm \equiv \omega_\pm F z_\pm c_\pm \nabla \Phi + D_\pm \nabla c_\pm. \quad (3)$$

Here the subscripts $+$ and $-$ denote the cation and anion, respectively. $\mathbf{J}$ denotes the molecular flux which includes contributions from both electrophoresis and diffusion. ω is the ion electrical mobility, F is the Faraday constant, z is the ion valence number, c is the molar concentration, D is the molecular diffusivity, and Φ is the electric potential. Equation (3) can be rewritten in terms of the electrical conductivity and charge density, which are defined as

$$\sigma \equiv F^2(\omega_+ z_+^2 c_+ + \omega_- z_-^2 c_-), \quad \rho_E \equiv F(z_+ c_+ + z_- c_-), \quad (4)$$

where ω denotes the electrophoretic mobility. For simplicity, we shall assume that the ions are monovalent with symmetric properties, i.e., $z_+ = -z_- = 1$, $\omega_+ = \omega_- = \omega$. Using Einstein's relation $D = \omega RT$, where R is the molar gas constant and T is the temperature, we also have $D_+ = D_- = D$. Straightforward manipulation reveals

$$\nabla \cdot [\omega^2 F^2 \rho_E \nabla \Phi + D \nabla \sigma] = 0, \quad (5)$$

$$\nabla \cdot [\sigma \nabla \Phi + D \nabla \rho_E] = 0. \quad (6)$$

These equations are completed with Gauss's law relating the charge density to the electric potential

$$\nabla \cdot \epsilon \nabla \Phi = -\rho_E, \quad (7)$$

with ϵ denoting the electrical permittivity of the aqueous solution. For boundary conditions we assume

$$\begin{cases} \sigma \rightarrow \sigma_\infty^{e,i}, & \rho_E \rightarrow 0, & \Phi \rightarrow \Phi_\infty^{e,i}, & \text{far from the pore,} \\ \partial\sigma/\partial n = \partial\rho_E/\partial n = \partial\Phi/\partial n = 0, & & & \text{on the membrane.} \end{cases} \quad (8)$$

That is, the ambient solution is assumed to be electrically neutral, and the membrane is impenetrable for both the electric field and ionic transport. Note that the ambient conditions are specified in a manner similar to that of a boundary layer (BL) problem in fluid mechanics.³¹ The Neumann conditions on the membrane are derived from a leaky-dielectric model,³² and include implicitly the effects of charge accumulation and membrane polarization. Further details are presented in the Appendix. Equations (5)–(8) define our governing equation system.

Further simplifications can be made if we consider a scaling argument. We nondimensionalize the equations with the following scales:

$$[x, y, z] = L, \quad [\sigma] = \sigma_o, \quad [\Phi] = E_o L, \quad [\rho_E] = \epsilon E_o / L. \quad (9)$$

Here L is a length scale, σ_o is a characteristic electrical conductivity, and E_o is a characteristic electric field strength. The dimensionless equations read

$$\nabla^* \cdot \left[\left(\frac{\tau_r \tau_D}{\tau_{ep}^2} \right) \rho_E^* \nabla^* \Phi^* + \nabla^* \sigma^* \right] = 0, \quad (10)$$

$$\nabla^* \cdot \left[\sigma^* \nabla^* \Phi + \left(\frac{\tau_r}{\tau_D} \right) \nabla^* \rho_E^* \right] = 0, \quad (11)$$

where

$$\tau_r \equiv \epsilon / \sigma_o, \quad \tau_{ep} \equiv L / \omega F E_o, \quad \tau_D \equiv L^2 / D \quad (12)$$

are, respectively, the time scales for charge relaxation, ion electrophoresis, and molecular diffusion. Note that $u_{ep} = \omega F E_o$ is the characteristic electrophoretic velocity. The dimensionless variables are denoted by the superscript *. For a physiological solution with an electrical conductivity in the S/m range, τ_r is on the order of a few nanoseconds or less. On the other hand, using $L = 20$ nm and $D = 2 \times 10^{-9}$ m²/s (the value for chloride in aqueous solutions), τ_D is estimated to be 200 ns, much greater than τ_r . Equation (6) can be therefore simplified to the Ohmic equation (we hereby will always work with the dimensional equations, although the simplifications are argued in the dimensionless form)

$$\nabla \cdot \sigma \nabla \Phi = 0. \quad (13)$$

Furthermore, using $\omega = 8 \times 10^{-13}$ mol s/kg (the mobility of a chloride ion), and an electric field strength of $E_o = 10^5$ V/m, the electrophoretic time scale τ_{ep} is estimated to be a few microseconds. Under this circumstance, the condition $\tau_r \tau_D \ll \tau_{ep}^2$ is satisfied, and Eq. (5) can be reduced to a pure diffusive equation for the conductivity

$$\nabla \cdot D \nabla \sigma = 0. \quad (14)$$

Equations (13) and (14) constitute the *reduced governing equations* for the potential and the conductivity and are decoupled from Gauss's law (7). Note that the reduction from Eqs. (5) and (6) to Eqs. (13) and (14) is often (equivalently) achieved by assuming the electroneutrality condition, $c_+ \approx c_-$. This assumption is valid when the criterion

$$\beta \equiv (c_+ - c_-)/(c_+ + c_-) = (\rho_E/F)/(\sigma/\omega F^2) \sim \tau_r/\tau_{ep} \ll 1 \quad (15)$$

is satisfied. Here β is a dimensionless variable characterizing the degree of charge separation. In fact, Eqs. (13) and (14) are in general valid for an arbitrary binary electrolyte (i.e., not restricted to the *symmetric* binary electrolyte assumed here), with D replaced by an effective diffusivity, $D_{\text{eff}} \equiv D_+D_-(z_+ - z_-)/(D_+z_+ - D_-z_-)$. We refer the interested readers to further details in Refs. 33–35.

We remark that in the case of $\sigma = \text{const}$ (corresponding to $c_+ = c_- = \text{const}$), Eq. (3) becomes trivial, and the charge density ρ_E is automatically zero. For this case Eq. (6) is simplified to be the Laplace equation for Φ . This equation, together with boundary conditions similar to Eq. (8), has been solved to obtain the electric potential by Neu *et al.*²⁹ in a study of the electrical energy required for pore formation.

In what follows, we will first derive an approximate analytical solution using the reduced equation system (13) and (14). The solution is rigorously valid for electrically neutral regime, where the reduced governing equations are valid. For parameters relevant to electroporation applications, the effective electric field is often much greater than the estimate we used above, and non-negligible charge separation is often present. For these cases, we solve the original Eqs. (5)–(8) directly using numerical methods. The details of the numerical solution, as well as comparisons to the analytical solution, are presented in Sec. III.

B. Analytical solution

For the geometry described in Fig. 1, the reduced Eqs. (13) and (14) can be solved with a rotational elliptic coordinate transformation³⁰ to obtain analytical expressions for σ , Φ , and R_{tot} . For the extracellular space, the new coordinates are defined in relation to the cylindrical coordinates as

$$r = r_0 \sqrt{(1 + \xi^2)(1 - \eta^2)}, \quad z = r_0 \xi \eta + \frac{h}{2}. \quad (16)$$

The contour lines for ξ and η are shown in Fig. 1(b). Note that under such transformation, the $\xi=0$ contour demarcates the pore and the extracellular space. The governing Eqs. (13) and (14) become

$$\frac{\partial}{\partial \xi} \left[(1 + \xi^2) \frac{\partial \sigma}{\partial \xi} \right] + \frac{\partial}{\partial \eta} \left[(1 - \eta^2) \frac{\partial \sigma}{\partial \eta} \right] = 0, \quad (17)$$

$$\frac{\partial}{\partial \xi} \left[(1 + \xi^2) \sigma \frac{\partial \Phi}{\partial \xi} \right] + \frac{\partial}{\partial \eta} \left[(1 - \eta^2) \sigma \frac{\partial \Phi}{\partial \eta} \right] = 0. \quad (18)$$

We further assume that the variables at the extracellular-pore interface σ_m^e and Φ_m^e are constant. This assumption is an approximation which we discuss below. This simplification eliminates the variable dependence on η , and the equations can be directly integrated with respect to ξ to yield

$$\sigma(\xi) = \sigma_m^e + 2(\sigma_\infty^e - \sigma_m^e) \frac{\tan^{-1}(\xi)}{\pi}, \quad (19)$$

$$\Phi(\xi) = \frac{\Phi_\infty^e \ln(\sigma(\xi)/\sigma_m^e) - \Phi_m^e \ln(\sigma(\xi)/\sigma_\infty^e)}{\ln(\sigma_\infty^e/\sigma_m^e)}. \quad (20)$$

The intracellular space can be solved similarly. The solution is obtained by simply replacing the superscripts e with i in the above expressions, and not presented here.

For the electrolytes solution in the pore, the assumption that σ and Φ are constant at the intra- and extracellular interfaces makes the governing equations one dimensional,

$$\begin{cases} \frac{\partial}{\partial z} \left(D \frac{\partial \sigma}{\partial z} \right) = 0, & \frac{\partial}{\partial z} \left(\sigma \frac{\partial \Phi}{\partial z} \right) = 0, \\ \sigma(z = \pm h/2) = \sigma_m^{e,i}, & \Phi(z = \pm h/2) = \Phi_m^{e,i}. \end{cases} \quad (21)$$

The solutions are

$$\sigma = \sigma_m^e + (\sigma_m^e - \sigma_m^i) \left(\frac{z}{h} - \frac{1}{2} \right), \quad \Phi = \Phi_m^i + (\Phi_m^e - \Phi_m^i) \frac{\ln(\sigma/\sigma_m^i)}{\ln(\sigma_m^e/\sigma_m^i)}. \quad (22)$$

The interfacial variables $\sigma_m^{e,i}$ and $\Phi_m^{e,i}$ remain unknown. These values are determined by matching the total conductivity flux and the Ohmic current

$$F \equiv \int_0^{r_0} \left(-D \frac{\partial \sigma}{\partial z} \Big|_{z=\pm h/2} \right) 2\pi r dr, \quad i_p \equiv \int_0^{r_0} \left(-\sigma \frac{\partial \Phi}{\partial z} \Big|_{z=\pm h/2} \right) 2\pi r dr \quad (23)$$

from the respective (intracellular, pore, and extracellular) spaces at the upper and lower pore interfaces. The calculation yields

$$\sigma_m^i = \sigma_\infty^i + \frac{\sigma_\infty^e - \sigma_\infty^i}{2(1 + 2h/\pi r_0)}, \quad \sigma_m^e = \sigma_\infty^e - \frac{\sigma_\infty^e - \sigma_\infty^i}{2(1 + 2h/\pi r_0)}, \quad (24)$$

$$\Phi_m^i = \Phi_\infty^i + V_m \frac{\ln(\sigma_m^i/\sigma_\infty^i)}{\ln(\sigma_\infty^e/\sigma_\infty^i)}, \quad \Phi_m^e = \Phi_\infty^e - V_m \frac{\ln(\sigma_\infty^e/\sigma_m^e)}{\ln(\sigma_\infty^e/\sigma_\infty^i)}. \quad (25)$$

Equations (19), (20), (22), (24), and (25) provide the complete solution for σ and Φ . Finally, with these expressions available, the effective resistance can be readily computed from the definition (2). The result is

$$R_{\text{tot}} = R_p + R_i = \frac{h}{\pi r_0^2} \frac{\ln(\sigma_m^e/\sigma_m^i)}{(\sigma_m^e - \sigma_m^i)} + \frac{1}{4r_0} \frac{\ln(\sigma_\infty^e \sigma_m^i/\sigma_\infty^i \sigma_m^e)}{\sigma_\infty^e - \sigma_m^e} = \frac{(\pi r_0 + 2h)}{2\pi r_0^2 \sigma_{\text{eff}}}, \quad (26)$$

where

$$\sigma_{\text{eff}} \equiv \frac{\sigma_\infty^e - \sigma_\infty^i}{\ln(\sigma_\infty^e/\sigma_\infty^i)}. \quad (27)$$

As stated earlier, R_p is the resistance from the electrolyte solution within the pore, and the spreading resistance R_i combines contributions from both the intra- and extracellular spaces. This final expression is a function of the membrane thickness h , the pore radius r_0 , and the ambient conductivities σ_∞^i and σ_∞^e . Note that Eq. (26) recovers the formula used in Refs. 11 and 17 where we have replaced the pore conductivity with the newly derived quantity, σ_{eff} .

We remark that although Eqs. (19), (20), and (22) are rigorous solutions of Eqs. (13) and (14) in the respective subspaces, the combined solutions need to be understood as an approximate solution for the entire domain. The approximation arises from the fact that we assume $\sigma_m^{e,i}$ and $\Phi_m^{e,i}$ are constant along the pore interface. In addition, we match the total fluxes, Eq. (23), not the fluxes at each point. However, as we demonstrate below with DNSs, these assumptions are well validated. The analytical and numerical solutions agree well with each other, especially in the electrically neutral regime from which the former is derived.

III. NUMERICAL SIMULATION

In this section, we solve the governing Eqs. (5)–(8) numerically. We will denote the results as DNS in comparison to the analytical solution developed above. Note that a rigorous prescription of the boundary condition (8) requires an infinite computational domain which is impossible. Instead, we choose a large computational domain (relative to both the pore radius r_0 and the

FIG. 3. Contour plots of σ_* and Φ_* from both [(a) and (b)] analytical and [(c)–(f)] numerical solutions. In each graph the blank space encompassed by the dashed lines is the membrane. For this case $\delta=0.16$ and $\gamma=11$.

cal constraints. We present studies with two typical values of TMP: $V_m=0.05$ and 1 V, respectively. The former represents a low-TMP regime where the electroneutrality is mostly observed. The latter represents a high-TMP regime, and is a typical critical value required to induce significant membrane electroporation.^{36–38}

Figure 3 shows typical results in contours of σ_* and Φ_* . For this case, we choose $r_0=0.8$ nm ($\delta=0.16$) and $\sigma_\infty^e=5$ S/m ($\gamma=11$). The value for the pore size corresponds to that of an equilibrium hydrophilic pore used in standard electroporation theories,^{39,40} the value for the extracellular conductivity is taken from sea urchin egg electroporation experiments,¹³ which has been studied by various groups.^{11,20,24} Figures 3(a) and 3(b) show the analytical solution developed in Sec. II B. Note that the result does not depend on the magnitude of the TMP. Figures 3(c) and 3(d) show the DNS using Eqs. (5)–(8) and $V_m=0.05$ V. The result provides an excellent agreement with the analytical solution. The maximum errors for the entire computational domain are 0.018 and 0.095 for σ_* and Φ_* , respectively. Figures 3(e) and 3(f) show the DNS with $V_m=1$ V. More obvious deviations from the analytical solution are observed. The maximum errors are 0.38 and 0.19 for σ_* and Φ_* , respectively.

The good agreement between the analytical solution and the DNS for $V_m=0.05$ V is primarily attributed to a small degree of charge separation, shown in Fig. 4(a). β is negligibly small for most of the computational domain and reaches a maximum value of 0.14 around the lower pore inter-

FIG. 4. Contour plots of β from the DNS for the case of $\delta=0.16$ and $\gamma=11$.

face. For this case, electroneutrality is a consistent approximation, and the reduced governing Eqs. (13) and (14) hold. In addition, the assumption that σ_m and Φ_m (the conductivity and potential at the upper and lower pore interfaces) are constants is also approximately observed, and the flow in the pore is indeed close to one dimensional. This effect is suggested in Figs. 3(c) and 3(d) and is more directly shown in Fig. 6 below. On the other hand, a more significant charge separation is present for the case of $V_m=1$ V, shown in Fig. 4(b). This effect is induced by a strong electric field present (correlated with the high value of V_m), as we analyze below. By temporarily ignoring the diffusive current $\nabla \cdot D \nabla \rho_E$, Eq. (6) becomes the Ohmic equation (13), which can be rewritten as

$$\rho_E = \epsilon \frac{\nabla \sigma}{\sigma} \cdot \nabla \Phi. \quad (29)$$

Here we have used the Gauss law (7). Because β is proportional to the charge density ρ_E , it is subsequently correlated with both the strength of the electric field $-\nabla \Phi$ and the conductivity gradient $\nabla \sigma$. For large values of β , the governing Eqs. (5)–(8) are more deviant from the reduced Eqs. (13) and (14), and so are the solutions.

Figure 5 shows more results along the pore centerline (z -axis) for the case of $V_m=0.05$ V, and for two pore radii, $r_0=0.8$ nm ($\delta=0.16$) and $r_0=50$ nm ($\delta=10$), representing the small and large pore size regimes. We study five conductivity ratios, $\gamma=0.1, 0.5, 2, 5$, and 11 , respectively. For each graph, the pore extends from $z_*=-0.5$ to 0.5 . In the σ_* and Φ_* plots, the dashed lines are from the analytical solution for comparison. It is straightforward to verify that the analytical solution for σ_* depends only on δ , not γ .

For $\delta=0.16$ [Figs. 5(a) and 5(b)], the analytical solution agrees well with the DNS. The degree of deviation is clearly correlated with the degree of charge separation β plotted in Fig. 5(c). The worst case occurs for $\gamma=0.1$ due to two combined effects. First, a strong conductivity gradient (correlated with a large conductivity difference such as in this case) introduces an increased amount of net charge, as indicated by Eq. (29). Equation (29) also correctly predicts that the charge density is positive for $\gamma>1$ and negative for $\gamma<1$. Second, from Eq. (15), the magnitude of β is also inversely proportional to $c_+ + c_-$, or, equivalently, σ [see Eq. (15)]. For $\gamma=0.1$, the extracellular conductivity assumes the smallest value for all cases studied ($\sigma_\infty^e=0.0455$ S/m), resulting in a relatively large β . This argument also explains why β always reaches the maximum on the side of lower conductivity, i.e., the extracellular side for $\gamma<1$ and the intracellular side for $\gamma>1$.

In contrast, the case of $\delta=10$ shows a uniformly excellent agreement with the analytical solution. This effect is again explained by Eq. (29) in terms of the correlation between charge density and electric field strength. The relatively large pore size ($r_0=50$ nm) results in a weaker electric field for the same value of V_m . Estimating from Figs. 5(b) and 5(e) (and noting $z_*=z/h$, where $h=5$ nm), the electric field strength for $\delta=0.16$ is on the order of 10^7 V/m, whereas for $\delta=10$, 10^5 V/m. The *effective* electric field is therefore nearly two orders of magnitude lower in

FIG. 5. Centerline plots (at $r_*=0$) for σ_* , Φ_* , and β for $V_m=0.05$ V. Two δ values are presented: [(a)–(c)] 0.16 and [(d)–(f)] 10. For both cases, $\gamma=0.1, 0.5, 2, 5$, and 11. Solid lines denote the DNS; dashed, analytical; dotted, the position of the pore boundaries.

this latter case. For all γ -values considered, the maximum absolute value for β does not exceed 8.7×10^{-4} . The maximum deviation does not exceed 0.013 for both σ_* and Φ_* .

Figure 6 plots σ_* and Φ_* from the DNS at the extracellular-pore interface ($z_*=1/2$) for $\delta=0.16$ and various values of γ . The analytical solution gives constant values (dashed) by assump-

FIG. 6. Comparison of the numerical (solid) and analytical (dashed) solutions for (a) σ_* and (b) Φ_* at the extracellular-pore interface ($z_*=0.5$) for $\delta=0.16$ and $\gamma=0.1, 0.5, 2, 5$, and 11. For σ_* , the analytical solution does not depend on γ , and the DNSs for all γ values are close to each other.

FIG. 7. Centerline plots (at $r_*=0$) for σ_* , Φ_* , and β for $V_m=1$ V and for [(a)–(c)] $\delta=0.16$ and [(d)–(f)] 10. For all cases $\gamma=0.1, 0.5, 2, 5$, and 11. Solid lines denote the DNS; dashed, analytical; dotted, the position of the pore boundaries.

tion, which are calculated according to Eqs. (24) and (25). In general, the numerical solutions are near-uniform close to the pore centerline ($r_*=0$) and vary more toward the pore edge ($r_*=1$). The analytical solution, although missing the detailed variations due to the simplification, provides a reasonable approximation to the DNS (considered more accurate). The only exception is for Φ_* and for the case of $\gamma=11$. As we explained earlier, this deviation is caused by the deviation from electroneutrality. For this case, and despite the difference in the absolute value when compared with the analytical solution, the solution from DNS for Φ_* does not show a significant variation along the radial direction. Similar agreements and trends are observed for all cases studied and are not presented for brevity.

For the same parametric set studied in Fig. 5, Fig. 7 presents the results for $V_m=1$ V, i.e., for the high-TMP regime. For $\delta=0.16$ [Figs. 7(a) and 7(b)] and when compared with Fig. 5 ($V_m=0.05$ V), the DNS shows an obvious deviation from the analytical solution for all γ values due to the high degree of charge separation present [Fig. 7(c)]. Note that the distribution of β tends to be uniform within the pore. Interestingly, the DNS for $\delta=10$ still well agrees with the analytical solution. The reason for the agreement is due to a relatively low effective electric field and degree of charge separation, same as we have previously explained.

Last but not least, we compare the total resistance calculated from the DNS and the analytical solution for both of the TMP values, $V_m=0.05$ and 1 V. We present the results for a wide range of δ and γ values, namely, $\delta=0.16, 1, 2, 5$, and 10 and $\gamma=0.1, 0.2, 0.5, 1, 2, 5$, and 11. The results

FIG. 8. Comparison of the total resistance R_{tot*} from the analytical (solid) and numerical (symbols) solutions [(a) and (b)]. The conductivity ratios are set to be $\gamma=0.1, 0.2, 0.5, 1, 2, 5$, and 11 . (c) and (d) show the percentage error between the two calculations.

are presented in Fig. 8. In Figs. 8(a) and 8(b), we show the dimensionless resistance as calculated with the two methods and for the two TMP values. In Figs. 8(c) and 8(d), we plot the relative percentage errors between the two. The analytical result is calculated from Eq. (26) and then nondimensionalized using Eq. (28) to yield

$$R_{*,ana} = \frac{(\pi\delta + 2)\ln \gamma}{2(\gamma - 1)}. \quad (30)$$

This expression shows that the dimensionless resistance has a linear dependence on δ . For the DNS, the resistance is first calculated with Eq. (2), then nondimensionalized in the same manner. Note that for this case the total current is calculated as

$$i_p = \int_0^{r_0} \left(-\sigma \frac{\partial \Phi}{\partial z} - D \frac{\partial \rho_E}{\partial z} \right)_{z=\pm h/2} 2\pi r dr.$$

That is, the total current now includes both the Ohmic and the diffusive components [cf. Eq. (23)]. The result demonstrates similar general trends we previously observe in detailed comparisons. The two solutions agree better for smaller TMP, larger δ , and larger γ values. For most cases, the difference is within 5%. It is interesting to note that a good agreement is observed for some cases where the analytical and numerical calculations differ in the details, e.g., for $V_m=1$ V, $\delta=0.16$, and $\gamma=0.5-11$. This observation is not surprising, as the integration process (to obtain the total current) tends to ameliorate and suppress differences. Based on these comparisons, we conclude that the analytical formula (26) and (27) can be used to characterize the current-voltage relation across an electropore. However, caution should be exercised for a few exceptional cases where the difference exceeds 10%. Notably, for $\delta=0.16$, $\gamma=0.1$, and both TMP values, the discrepancy approaches 25%. These cases represent a regime where charge separation is the most prominent (see Fig. 7), and the details of the analytical and numerical calculations differ the most. In such a situation, the former best serves as an estimate for the real solution which is more accurately captured by the latter.

IV. DISCUSSION

In this work we have presented a Nernst–Planck model for the steady-state ion and current transport processes around an electropore. The main purpose is to study the current-voltage relation, or, equivalently, the effective resistance across the pore-embedded membrane. We have developed an analytical solution for the electrically neutral regime. We have also performed the DNS of the full Nernst–Planck equations to provide comparison and validation. We found that the analytical solution and the DNS agree well when charge separation is negligible, and deviate more obviously when otherwise.

For the effective resistance, the analytical solution provides an accurate prediction for most of the cases studied. The availability of the analytical expression (26) is of practical importance for electroporation modeling and applications, and is one of the main contributions of the current work. In a typical case, the electropores form a large and dynamic population with variable sizes. Performing DNS for each individual pore is prohibitively expensive. Using a compact formula such as Eq. (26) to compute the total membrane resistance and electric current is the best viable way for whole-cell or planar-membrane electroporation model calculations.

The current work differs from the class of approaches following Glaser *et al.*¹⁸ and Barnett.¹⁹ In these works, the pore conductance is derived from a one-dimensional Nernst–Planck model and is expressed in terms of an electric potential. This electric potential in general includes a linear, applied component and a contribution from the membrane polarization effect. The latter is specified empirically and is different for the respective cations and anions. Such difference in the electric potential “felt” by the different ions is possibly accounting for the noncontinuum effects, e.g., when the ions are passing through the pore one by one (see also a discussion below). The model by Kakorin and Neumann²¹ also considers the ion concentration difference across the pore, but follows a similar approach otherwise. The present work solves the Nernst–Planck system directly in a three-dimensional geometry surrounding the pore. The electric potential is obtained from the governing Eqs. (5)–(8). Consistent with the continuum assumption, the electric field is the same for both the cations and anions. The membrane polarization effect is implicitly taken into account by setting appropriate boundary conditions on the membrane (see the Appendix for further details).

The present work extends from that of Neu *et al.*²⁹ by considering a gradient in the electrical conductivity instead of a uniformly distributed conductivity field. A salient difference between the physical behaviors of these two cases is that charge separation is possible in the former case [Eq. (29)] and theoretically zero in the latter. The presence of a net charge in the fluid bulk may have an interesting implication on molecular translocation which we speculate below.

Inevitably, the present model is a significant idealization of a problem involving great complexities. We have made simplifications such that the model is tractable, but meanwhile have ignored effects that may have important impacts on the results. These effects are the subject of future explorations, and a few of them are discussed below.

A. Noncontinuum effects

The present work employs a continuum model to capture the physical processes at the nanometer scale. As pointed out by previous authors, the flow variables in this case are understood as ensemble averages.¹⁹ Within this understanding, the continuum model is assumed to be valid. Even the smallest pore size considered ($r_0=0.8$ nm) is a few times greater than the typical ion radii, hence allowing the passing of several ions at the same time. Noncontinuum effects are expected to be more prominent for very dilute solutions, where the ions may pass through the pore one by one. For this case, the electric field that each ion experiences depends on the charge it carries and its instantaneous position with respect to the membrane. Such effects can be approximated by including a potential of trapezium shape in a modified continuum model,^{18,21} or can be better captured via molecular simulations, to which the continuum models offer a reference of comparison.

B. Multi-ion effects

We have assumed that the electrolyte solution is composed of two symmetric, monovalent ions. This approximation is appropriate for salt-based solutions, such as in vesicle electroporation experiments.³⁶ For biological cells, and in particular in the cytoplasm, the electrolyte solution has a more complex composition involving both small ions⁴¹ and charged macromolecules (proteins, DNA, etc.). The latter often possess relatively large charge numbers and small diffusivities. An extension to a multispecies Nernst–Planck model can take into account possible nonbinary behavior. However, the main challenge would be to properly specify the properties of the macromolecules, such as the number of species, the intracellular concentration, the diffusivity and mobility, etc.

C. Electrohydrodynamic flow

Fluid motion induced by the electrostatic (Maxwell) stress has been ignored in the present model. The consideration of this effect requires the coupling of the Nernst–Planck system with the Navier–Stokes equations describing fluid motion, such as in an earlier work by one of us (H.L.³⁵). Without solving these detailed equations here, it is interesting to speculate the impact of such flow should the effect becomes important. The driving force of the flow, the electrical body force, is derived from the divergence of the Maxwell stress and equals $\rho_E \mathbf{E}$ (a vector). This forcing term determines the direction of the induced flow. That is, if $\rho_E > 0$, the flow points to the direction of the electric field; if $\rho_E < 0$, the opposite. In the specific geometric configuration considered here, the flow would jet toward the intracellular space if $\gamma > 1$, and the opposite if $\gamma < 1$. Note that the directions do not change if the direction of the applied electric field reverts. The induced flow always points into the lower conductivity region. This trend has interesting implications for electroporation-mediated molecular delivery: The electrohydrodynamic flow has a favorable effect if transport into the lower conductivity side is desired, and an adverse effect if the opposite.

Finally, as a concluding remark, the direct validation of the model with experimental measurement is possible. Single and stabilized pores have been generated in previous experiments on planar membrane electroporation.^{42,43} However, no conductivity gradient was present in either of the cases. In order to pursue a direct comparison to the present theory, similar experiments need to be performed with controlled and different conductivities across the membrane. On the other hand, indirect evidence does support the present prediction. In the sea-urchin-egg electroporation model study by Krassowska and co-workers, the authors used $\sigma_\infty^i = 0.455$ S/m and $\sigma_\infty^e = 5$ S/m.^{11,20,41} The conductivity for the pore was adopted to be $\sigma = 2$ S/m following the previous experiments.⁴⁴ Using Eq. (27) and these intra- and extracellular conductivity values, we can calculate the effective conductivity for this case, $\sigma_{\text{eff}} = 1.9$ S/m. This value is very close to the above one, with which the model provided favorable prediction on the whole-cell level.

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APPENDIX: THE BOUNDARY CONDITIONS FOR THE GOVERNING EQUATIONS

1. The ambient boundary conditions

In Eq. (8), we have prescribed that the conductivity and electric potential approach the ambient values at an infinite distance away from the pore. This treatment follows a BL-type philosophy commonly found in fluid mechanics problems.³¹ In a standard BL theory, the fluid velocity is solved assuming that it converges to an ambient value (obtained from the so-called “external solution”) at infinity. However, the solution often yields that the velocity reaches (asymptotically) the ambient condition within a small, finite distance which defines the BL thickness. A similar situation is found in the present problem. Theoretically, $\Phi_\infty^{e,i}$ and $\sigma_\infty^{e,i}$ have to be obtained from a

global solution (see, e.g., Ref. 11) which assumes discontinuities across an infinitesimally thin membrane. They are then used as ambient boundary conditions to solve the “inner” problem, as described by Fig. 1. Indeed, most of the transition between the intra- and extracellular values occurs within a finite distance (Figs. 5 and 7). In fact, the analytical solution (19) and (20) can be used to estimate the BL thickness: The conductivity and potential converge to their respective ambient values following an arctangent function in both of the half-spaces. Equation (19) can be written as

$$\frac{\sigma(\xi) - \sigma_m^e}{\sigma_\infty^e - \sigma_m^e} = \frac{\tan^{-1}(\xi)}{\pi/2}. \quad (\text{A1})$$

For $\xi \rightarrow \infty$, we observe $\sigma(\xi) \rightarrow \sigma_\infty^e$. For $\xi = 10$, which corresponds to a distance of approximately $10r_0$ from the pore, Eq. (A1) yields

$$\sigma(\xi = 10) - \sigma_m^e = 0.94(\sigma_\infty^e - \sigma_m^e). \quad (\text{A2})$$

This calculation suggests that 94% of the extracellular variation in conductivity occurs within a $10r_0$ radius range from the pore. The same conclusion applies to the intracellular space. We may therefore use $10r_0$ as a reasonable estimate for the thickness of the intra- and extracellular BLs. For $r_0 = 10$ nm, this length scale is on the order of 100 nm, which is much smaller than a typical cell size (~ 10 μm). This disparity in scales justifies the BL approach and boundary condition prescription. However, for macropores whose BL thickness approaches the cell size, the current theory no longer applies.

2. The boundary conditions on the membrane

In this section, we argue that the nonpenetration boundary condition on the membrane (8) is an approximation of the so-called Taylor–Melcher leaky dielectric model,³² and that the membrane polarization effect has been implicitly accounted for in this model.

The leaky dielectric model states that at every point on the liquid-membrane interface,

$$\mathbf{n} \cdot (\sigma_l \mathbf{E}_l - \sigma_m \mathbf{E}_m) = -\frac{\partial q_s}{\partial t}, \quad (\text{A3})$$

$$\mathbf{n} \cdot (\epsilon_l \mathbf{E}_l - \epsilon_m \mathbf{E}_m) = q_s. \quad (\text{A4})$$

Here $\mathbf{n}$ is the unit normal vector on the membrane pointing into the liquid; σ , ϵ , $\mathbf{E}$, and q_s are, respectively, the conductivity, permittivity, electric field, and surface charge (presumably in the Stern layer due to ion adsorption onto the membrane). The subscripts l and m denote the liquid and the membrane, respectively. At steady state, $\partial q_s / \partial t = 0$, and Eq. (A3) becomes

$$\mathbf{n} \cdot (\sigma_l \mathbf{E}_l - \sigma_m \mathbf{E}_m) = 0. \quad (\text{A5})$$

Typically σ_l is in the range of S/m, whereas σ_m is on the order of 10^{-7} S/m.^{45,46} We may therefore safely assume $\sigma_m \approx 0$, and Eq. (A5) becomes the Neumann condition

$$\mathbf{n} \cdot \sigma_l \mathbf{E}_l = 0. \quad (\text{A6})$$

This equation is the nonpenetration condition for the electric field which we use in Eq. (8). Furthermore, we consider the no-flux condition for the ions on the membrane

$$\mathbf{n} \cdot (\omega_\pm F z_\pm c_\pm \nabla \Phi + D_\pm \nabla c_\pm) = 0. \quad (\text{A7})$$

Because now $\mathbf{n} \cdot \nabla \Phi = 0$, the above condition reduces to

$$\mathbf{n} \cdot \nabla c_{\pm} = 0.$$

The definitions for σ and ρ_E give rise to their respective Neumann boundary conditions on the membrane.

It is interesting to note that Eq. (A6) does not imply a zero surface charge ρ_s . Although the normal electric field on the liquid side at the liquid-membrane interface is zero, the counterpart on the membrane side is not. The electric field within the membrane can be solved with the following steps. First the complete solution in the liquid is obtained as outlined in this paper. This solution provides the potential on the membrane surface, which serves as the boundary condition to solve the Laplace equation governing the electric potential within the membrane. In fact, this approach has been implemented by Neu *et al.*²⁹ to obtain the complete solution within both the liquid and the membrane. Finally, the surface charge is calculated according to Eq. (A4),

$$-\mathbf{n} \cdot \epsilon_m \mathbf{E}_m = q_s.$$

In this regard, the membrane charge is implicitly taken into account and the membrane polarization effect is implicitly included. However, in the present work the electric field within the membrane is not required to calculate that within the liquid due to the approximated Neumann boundary condition in Eq. (8).

Note that in the calculation of the Born energy by Parsegian^{47,48} (from which the later model by Glaser *et al.*¹⁸ was in part derived), the membrane is considered a perfect dielectric where $\mathbf{n} \cdot (\epsilon_l \mathbf{E}_l - \epsilon_m \mathbf{E}_m) = 0$. In this model the surface charge is zero, and there is no Stern layer. Such assumption is appropriate when the calculation concerns a single ion in an otherwise de-ionized environment. When the membrane is in contact with electrolytic liquids with high ion concentrations, the authors of the present work believe that the leaky dielectric model (A3) and (A4) is more appropriate in describing the ion-membrane interactions.

Last but not least, we have validated the use of Eq. (A6) instead of Eq. (A5) as the boundary condition. We solved the Nernst–Planck equation system with Eq. (A5) and in conjunction with the Laplace equation for the electric field within the membrane. The result (not shown) yields a negligible difference when compared with that from using the simplified boundary condition (A6).

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