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Asymptotic model of electroporation

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Electroporation is described mathematically by a partial differential equation (PDE) that governs the distribution of pores as a function of their radius and time. This PDE does not have an analytical solution and, because of the presence of disparate spatial and temporal scales, numerical solutions are hard to obtain. These difficulties limit the application of the PDE only to experimental setups with a uniformly polarized membrane. This study performs a rigorous, asymptotic reduction of the PDE to an ordinary differential equation (ODE) that describes the dynamics of the pore density $N(t)$. Given $N(t)$, the precise distribution of the pores in the space of their radii can be determined by an asymptotic approximation. Thus, the asymptotic ODE represents most of the phenomenology contained in the PDE. It is easy to solve numerically, which makes it a powerful tool to study electroporation in experimental setups with significant spatial dependence, such vesicles or cells in an external field. [S1063-651X(99)10603-2]

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I. INTRODUCTION

When a membrane is exposed to a high transmembrane potential, it exhibits a rapid increase in its conductivity by 5–6 orders of magnitude [1]. Such an electrical breakdown of the barrier function of the membrane is generally attributed to the creation of pores, which are the aqueous pathways in the lipid bilayer of the membrane. This process, called electroporation, can be irreversible, leading to a mechanical rupture of the membrane, or reversible, in which case pores reseal and the same membrane can experience multiple episodes of the high conductivity state. The transient state of high conductivity has important practical applications, allowing the fusion of cells and the introduction of the biologically active substances (drugs or genetic material) into cells [2–4]. On the other hand, electroporation occurs as an undesirable side affect following the delivery of defibrillation shocks to the heart [5–7] and may be responsible for the late necrosis after the accidental exposure to high voltage [8].

The theoretical understanding of the electroporation process is based on the Smoluchowski equation, a partial differential equation (PDE) that governs the distribution of pores as a function of their radius and time. Let $n(r,t)$ denote the pore density distribution function such that at a given time t, the number of pores (per unit area) with radii between r and $r+dr$ is $n(r,t)dr$. According to the literature, $n(r,t)$ satisfies the equation

$$n_t+D\partial_r\left(-\frac{\varphi_r}{kT}n-n_r\right)=S(r), \tag{1}$$

where D is the diffusion constant of pores, $\varphi(r)$ is the pore energy, k is the Boltzmann constant, T is the absolute temperature, and $S(r)$ is the source term that represents the creation and the destruction of pores. Subscript t denotes differentiation with respect to time; differentiation with respect to pore radius is denoted by ∂_r or by a subscript r. This equation was first used to describe electroporation in 1979 by Chizmadzhev and colleagues [9]. Further development of the theory of electroporation was undertaken by Weaver and colleagues [10–13]. A joint review by Weaver and Chizmadzhev gives a thorough summary of current understanding of electroporation and the relation between the theory and experiments [14].

Investigating the electroporation process using PDE (1) has several drawbacks. First, this equation requires the knowledge of several constants whose values cannot be measured directly. Most of these constants were estimated by theoretical arguments and are known only by order of magnitude [12]. Because of the uncertainties in the values of the parameters, the solution to Eq. (1) gives only a qualitative picture of the electroporation process. Second, the connection between the variables of the PDE and the quantities that are available from the experiments is far from obvious. Experimental studies use simplified, partial descriptions of the electroporation process to interpret the collected data [15,16]. Finally, the PDE (1), with its variable coefficients, does not have an analytical solution and must be solved numerically. However, the exponential dependence of the creation and destruction rates on the pore energy and the existence of disparate spatial and temporal scales makes the numerical solution hard to obtain. Hence, numerical solutions of the PDE (1) have been obtained only for a spatially clamped, uniformly polarized membrane patch [12,13,17]. Application of Eq. (1) to an experimental situation with significant spatial dependence, such a vesicle or a cell in an external field [18–20], would be impractical.

This paper presents a rigorous, asymptotic reduction of PDE (1) to an ordinary differential equation (ODE). This ODE describes the dynamics of the pore density $N(t)$, which is related to the pore distribution function $n(r,t)$ by

$$N(t)\equiv\int_0^\infty n(r,t)dr. \tag{2}$$

Given $N(t)$, the distribution of the pores in the space of their radii can be determined by an asymptotic approximation.

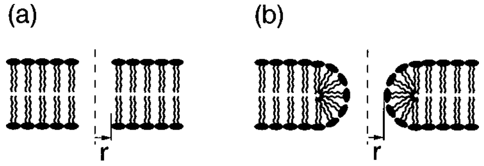

FIG. 1. The structure of (a) hydrophobic and (b) hydrophilic pores (based on Refs. [16,21,22]).

However, the essential features of the electroporation process are well reflected by the pore density $N(t)$ and the asymptotic ODE represents most of the phenomenology contained in the PDE.

The organization of this paper is as follows. Section II gives a physical background of the electroporation process and introduces the initial-boundary value problem governing the pore density $n(r,t)$. Section III converts the initial-boundary value problem to dimensionless form. Small parameters in the dimensionless equations lead to a reduced problem which is presented in Sec. IV. Section V uses order of magnitude estimates to develop a heuristic overview of the asymptotic dynamics of the pore density $N(t)$. The actual derivation is carried out in Sec. VI. Section VII uses the asymptotic ODE to demonstrate an explicit dependence of pore creation rate on the transmembrane potential. Finally, Sec. VIII discusses the limitations and practical implications of this study.

II. PHYSICAL BACKGROUND

This paper assumes the existence of two types of pores [16,21,22]. The hydrophobic pores [Fig. 1(a)] are simply gaps in the lipid bilayer of the membrane, formed as a result of its thermal fluctuations. The hydrophilic or inverted pores [Fig. 1(b)] have their walls lined with the water-attracting heads of lipid molecules. Hence, the hydrophilic pores allow the passage of water-soluble substances, such as ions, while the hydrophobic pores do not. In the remainder of this paper, the hydrophilic pores will be referred to as conducting and the hydrophobic pores, as nonconducting.

The crucial assumption in studying the behavior of pores is the relationship between the pore radius and its energy. This paper assumes the pore energetics proposed by Abidor *et al.* [16,21] depicted in Fig. 2. This energy function consists of two curves, $u(r)$ for the nonconducting pores and $w(r)$ for the conducting pores. The energy $E(r)$ of a pore of radius r is the lesser of $u(r)$ and $w(r)$ as shown in the inset of Fig. 2. $E(r)$ has two maxima, at r_* and r_d and a local minimum at r_m. The pore energies at r_*, r_m, and r_d are denoted by

$$E_* = E(r_*), \quad E_m = E(r_m), \quad \text{and } E_d = E(r_d). \tag{3}$$

Typical values of radii and energies are given in Table I.

The energy $u(r)$ of the nonconducting pores is given in the literature in terms of Bessel's functions [16]. However, for the values of parameters in Table I, the Bessel's functions expression is well approximated by the quadratic function

$$u(r) \approx E_* \left(\frac{r}{r_*} \right)^2. \tag{4}$$

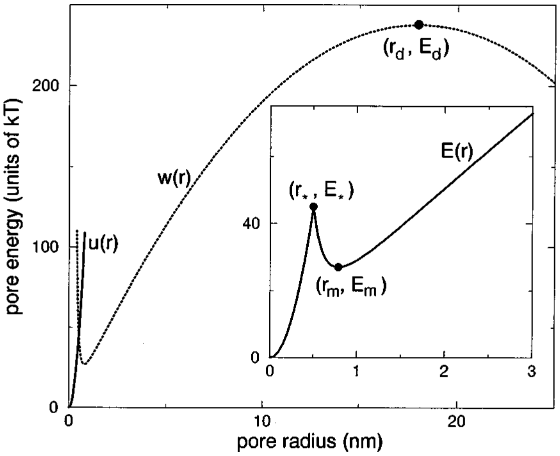

FIG. 2. The energy function of a pore at the transmembrane potential $V_m = 0$ (based on Refs. [16,21]). This plot uses the values of parameters in Table I and the form of $w(r)$ in Eq. (5).

The energy $w(r)$ of conducting pores is given by the formula

$$w(r) = 2\pi\gamma r - \pi\sigma r^2 + \left(\frac{C}{r} \right)^4, \tag{5}$$

where γ is the energy per unit length of the pore perimeter and σ is the energy per unit area of the intact membrane. The first two terms in Eq. (5) are identical to the energy of the conducting pores given in the literature [10,16,21]. The third term, added by us, represents the steric repulsion [14,23] between lipid heads lining the pore and is responsible for the increase in pore energy with shrinking radius $r \to r_*^+$. Constant C and the power are chosen so that r_*, E_*, and r_m are close to values measured by Glaser *et al.* [16].

The pore energy $E(r)$ of Fig. 2 corresponds to the situation when there is no externally applied transmembrane potential. In the presence of a transmembrane potential V, the pore energy, denoted by $\varphi(r,t)$, is given by

$$\varphi(r,t) = E(r) - \pi a_p V^2 r^2, \tag{6}$$

where the time dependence in φ arises through the temporal variations of V. The component $-\pi a_p V^2 r^2$ is the capacitive contribution [10,21]. The coefficient a_p is a property of the membrane and its aqueous environment. The simplest estimate, based on a continuum model [11,16], gives a_p in terms of membrane thickness h and dielectric constants κ_w and κ_m of water and membrane:

$$a_p = \frac{1}{2h} (\kappa_w - \kappa_m) \epsilon_0, \tag{7}$$

where ϵ_0 is the permittivity of vacuum. A nominal value of a_p is given in Table I.

For a sufficiently small V, $\varphi(r,t)$ has the same qualitative structure as $E(r)$: a cusplike maximum at r_*, a local minimum at r_m, and a maximum at r_d. However, r_m and r_d are now functions of V. The energies at these radii are denoted by

$$\varphi_* \equiv \varphi(r_*, t), \quad \varphi_m \equiv \varphi(r_m, t), \quad \varphi_d \equiv \varphi(r_d, t). \tag{8}$$

TABLE I. Values of parameters.

Parameter	Symbol	Value	Source
Diffusion coefficient	D	5×10^{-14} m^2 s^{-1}	[13]
Edge energy of a pore	γ	1.8×10^{-11} Jm^{-1}	[13,16]
Energy of an intact membrane	σ	10^{-3} Jm^{-2}	[13]
Constant in Eq. (5)	C	9.67×10^{-15} J$^{1/4}$ m	est. from Eq. (5)
Radii:			
at local maximum	r_*	0.5 nm	[16]
at local minimum	r_m	0.8 nm	[16]
at global maximum	r_d	18 nm	est. from Eq. (5)
Energies:			
at local maximum	E_*	45 kT	[16]
at local minimum	E_m	25.6 kT	est. from Eq. (5)
at global maximum	E_d	238 kT	est. from Eq. (5)
Dielectric constant of water	κ_w	80	[13,16]
Dielectric constant of lipid	κ_m	2	[13,16]
Permittivity of vacuum	ϵ_0	8.85×10^{-12} Fm^{-1}	
Thickness of the membrane	h	5 nm	[16]
Constant in Eq. (6)	a_p	6.9×10^{-2} Fm^{-2}	est. from Eq. (7)
Fluctuation rate per unit volume	ν_c	2×10^{38} m^{-3} s	[10]
Fluctuation rate per lipid molecule	ν_d	10^{11} s^{-1}	[16]

Figure 3 shows graphs of $\varphi(r,t)$ parametrized by V. As V increases to a critical value of $V_c\approx0.5$ V, the local minimum r_m and local maximum r_d coalesce and disappear.

The last term in the Smoluchowski equation (1) is the source term $S(r)$ that represents the creation and the destruction of pores. The formulation for $S(r)$ assumes that the formation of pores is a two-step process [16,21,22]. All pores are initially created as nonconducting [Fig. 1(a)]. According to Ref. [10], nonconducting pores with energy between U and $U+dU$ are created at a rate

$$\nu_c h e^{-U/kT} d\left(\frac{U}{kT}\right) \tag{9}$$

per unit area of the membrane. Here, ν_c is the "attempt rate density" [10] with units s^{-1}m^{-3} and

$$U(r,t)\equiv u(r)-\pi a_p V^2 r^2 \tag{10}$$

denotes the energy of nonconducting pores at nonzero transmembrane potential. The differential dU of energy in Eq. (9) is related to a corresponding differential dr of the pore radius by $dU=U_r dr$. Hence, the pores with radii between r and $r+dr$ are created at a rate

$$\nu_c h \frac{U_r}{kT} e^{-U/kT} dr \tag{11}$$

per unit area of the membrane.

If a nonconducting pore is created with the radius $r>r_*$, it spontaneously changes its configuration and forms an inverted, conducting pore [Fig. 1(b)]. This conducting pore survives as long as its radius remains above r_*. Hence, with $r>r_*$, Eq. (11) is effectively a creation rate density of conducting pores. If a nonconducting pore is created with the radius $r<r_*$, the pore remains nonconducting and it has a lifetime "on the order of the lipid fluctuations" [16]. That implies that in time dt, the fraction of nonconducting pores with radii less than r_* which are destroyed is $\nu_d dt$, where ν_d is the frequency of lipid fluctuations. Hence, the nonconducting pores with radii between r and $r+dr$, $r<r_*$, are destroyed at a rate

$$\nu_d n dr \tag{12}$$

per unit area of the membrane.

Based on the creation rate (11) and destruction rate (12), the source density $S(r)$ can be written as

$$S(r)=\nu_c h \frac{U_r}{kT} e^{U/kT} - \nu_d n H(r_* - r), \tag{13}$$

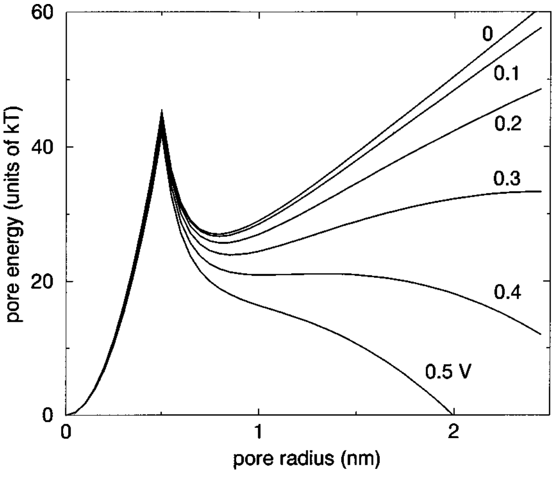

FIG. 3. The change in the energy function of a pore at the transmembrane potential $V_m\neq0$.

where $H(r)$, the Heavyside's step function, represents the fact that only nonconducting pores $(0<r<r_*)$ are being destroyed. With Eq. (13), PDE (1) is complete.

The standard initial and boundary value problem associated with PDE (1) requires that an initial condition $n(r,0)$ be given in $r>0$, and that a boundary condition on n at $r=0$ be specified for all t. This paper assumes that $r=0$ is an absorbing boundary, i.e.,

$$n(0,t)=0. \tag{14}$$

Intuitively, condition (14) means that when pores shrink to zero, they disappear.

III. DIMENSIONLESS FORM OF THE BOUNDARY VALUE PROBLEM

The initial-boundary value problem (1),(14) of pore dynamics is amenable to asymptotic solution. A preliminary nondimensionalization leads to the recognition of relevant gauge parameters. The variables are scaled according to the system of units in the scaling table:

$$\begin{array}{lllll} \text{Variable} & r & t & \varphi, U & n \\ \text{Unit} & r_* & r_*^2/D & E_* & 1/r_*^3 \end{array} \tag{15}$$

The units of pore radius and energy clearly come from the structure of pore energy φ as a function of r (Fig. 2). The unit of time is the duration required for pore radius to diffuse a distance on the order of r_*. It is assumed that the characteristic time associated with the temporal variation of φ is equal or larger than this unit of time. The unit of pore distribution function n amounts to comparing the density of pores to the density of lipid heads: Let $\overline{n}(\overline{r})$ be the nondimensional pore distribution as a function of nondimensional radius $\overline{r} \equiv r/r_*$. Given the unit $1/r_*^3$ of n in Eq. (15), n and $\overline{n}$ are related by

$$n(r)=\frac{1}{r_*^3}\overline{n}\left(\frac{r}{r_*}\right). \tag{16}$$

Hence, the pore number N per unit area (2) can be expressed as

$$N=\int_0^\infty \frac{1}{r_*^2}\overline{n}\left(\frac{r}{r_*}\right)d\left(\frac{r}{r_*}\right)=\frac{1}{r_*^2}\int_0^\infty \overline{n}(\overline{r})d\overline{r}. \tag{17}$$

If the dimensionless integral $\int_0^\infty \overline{n}(\overline{r})d\overline{r}$ is of the order of unity, then N has the order of magnitude $1/r_*^2$. Assuming that r_*^2 is comparable to the area of a lipid head group [12,14], $1/r_*^2$ corresponds to ''one pore per lipid molecule.'' So indeed, measuring n in units of $1/r_*^3$ leads to a dimensionless distribution function which is the density of pores per density of lipid molecules. Physically reasonable solutions are expected to have n much smaller then unity.

The dimensionless version of the Smoluchowski equation (1) is

$$n_t+\partial_r\left(-\frac{1}{\varepsilon}\varphi_r n-n_r\right)=\frac{1}{\mu}\left(\frac{U_r}{\varepsilon}e^{-U/\varepsilon}-\gamma n H(1-r)\right). \tag{18}$$

Here, ε, μ, and γ are dimensionless parameters

$$\frac{1}{\varepsilon}\equiv\frac{E_*}{kT}\approx 45,$$

$$\frac{1}{\mu}\equiv\frac{r_*^2}{D}\nu_d\approx 5\times 10^5,$$

$$\gamma\equiv\frac{\nu_d}{\nu_c h r_*^2}\approx 0.4. \tag{19}$$

The nominal values of ε and μ are small while γ is of order unity. This suggests an asymptotic analysis in which ε, μ are treated as gauge parameters tending to zero and γ is treated as a constant independent of ε,μ.

The quantity

$$f\equiv-\frac{1}{\varepsilon}\varphi_r n-n_r \tag{20}$$

in the left hand side of Eq. (18) is the dimensionless flux. The right hand side of Eq. (18) contains the dimensionless source, with the creation rate

$$\frac{1}{\mu\varepsilon}U_r e^{-U/\varepsilon} \tag{21}$$

and the destruction rate

$$\frac{\gamma}{\mu}nH(1-r). \tag{22}$$

This destruction rate indicates that a lifetime of a nonconducting pore is on the order of μ.

IV. REDUCTION OF THE BOUNDARY VALUE PROBLEM TO $r\geqslant 1$

A. Pore creation and destruction near $r=1$

Simple order of magnitude estimates based on the dimensionless PDE (18) provide physical insight into the creation of conducting pores. There are two routes for the creation of conducting pores.

(i) The ''indirect'' route, in which a nonconducting pore is created with radius $r<1$ and then diffuses into $r>1$ before it is destroyed. Once in $r>1$, it turns into a conducting pore.

(ii) The ''direct'' route, in which a nonconducting pore is created with radius $r>1$ and immediately converts into a conducting pore.

Since the dimensionless creation rate (21) decays exponentially with length constant ε as r increases, it is clear that the creation of nonconducting pores in $r<1$ is much more prolific than the creation of conducting pores with $r>1$. Can it be that even though there is a voracious destruction of the nonconducting pores at dimensionless rate (22) in $r<1$, enough of them cross over into $r>1$ to make the indirect route dominant? The answer depends on the relative magnitudes of ε and μ.

Consider nonconducting pores created with radius $r=1-x$, where $0<x<1$. Can they be transported into $r>1$ during their dimensionless lifetime μ? The drift velocity in $r<1$ is seen from Eq. (18) to be negative, $-\varphi_r/\varepsilon=-U_r/\varepsilon<0$, so the only transport process that increases r is diffusion. The dimensionless time required to diffuse distance x (in order to cross into $r>1$) is $O(x^2)$, and the fraction of pores which make it have order of magnitude $e^{-x^2/\mu}$. Hence, the rate ν' at which conducting pores are created by the indirect route is estimated in order of magnitude by the integral

$$\int_0^1 \frac{U_r(1-x)}{\varepsilon\mu} e^{-U(1-x)/\varepsilon} e^{-x^2/\mu} dx. \tag{23}$$

Here, the Gaussian factor $e^{-x^2/\mu}$ represents the fraction of pores making it to $r>1$ and the reminder of the integrand is the creation rate at $r=1-x$. Since for the nominal values of ε and μ in Eq. (19), $\sqrt{\mu}\ll\varepsilon$, the Gaussian factor dominates in Eq. (23) and the integral is estimated by Laplace's method to give the order of magnitude of ν',

$$\nu'=O\left(\frac{1}{\varepsilon\sqrt{\mu}} e^{-\varphi_*/\varepsilon}\right). \tag{24}$$

Direct creation of conducting pores with $r>1$ proceeds at rate

$$\nu=\int_1^\infty \frac{U_r}{\varepsilon\mu} e^{-U/\varepsilon} dr=\frac{1}{\mu} e^{-\varphi_*/\varepsilon}. \tag{25}$$

Note that the main contribution to ν comes from a "1^+ boundary layer," $0<r-1=O(\varepsilon)$. Comparison of Eqs. (24) and (25) reveals that

$$\frac{\nu'}{\nu}=O\left(\frac{\sqrt{\mu}}{\varepsilon}\right)=0.064. \tag{26}$$

Since $\nu'\ll\nu$ it is the *direct* route that dominates the creation of conducting pores.

B. Absorbing boundary condition at $r=1$

In the PDE (18), the pore distribution function $n(r,t)$ is defined in $0<r<\infty$. In practical problems, only conducting pores with radius $r>1$ are of interest. Pore density in $0<r<1$ matters only through its effect on pore density in $r>1$. The proceeding analysis determines whether the $r<1$ pores really matter by comparing the magnitude of the pore distribution function n in the 1^+ and 1^- boundary layers.

In the 1^+ boundary layer, $0<r-1=O(\varepsilon)$, the convective component $-n\varphi_r/\varepsilon$ and the diffusive component $-n_r$ of the flux f (20) balance each other and the creation rate ν (25) in the order of magnitude. This leads to the order of magnitude estimate n^+ of n in the 1^+ boundary layer

$$n^+=O\left(\frac{\varepsilon}{\mu} e^{-\varphi_*/\varepsilon}\right) \quad \text{in} \quad 0<r-1=O(\varepsilon). \tag{27}$$

Now consider pore distribution in the 1^- boundary layer, $0<1-r=O(\sqrt{\mu})$. The $O(\sqrt{\mu})$ thickness of this boundary layer is equal to the distance a nonconducting pore diffuses in its lifetime μ. Pores are introduced into the 1^- boundary layer by three processes.

(i) Conducting pores cross over from $r>1$ into $r<1$ at a rate whose order of magnitude is $O[(1/\mu)e^{-\varphi_*/\varepsilon}]$, the same as the magnitude of ν (25).

(ii) Spontaneous creation at a rate

$$\int_{1-\sqrt{\mu}}^1 \frac{U_r}{\varepsilon\mu} e^{-U/\varepsilon} dr=O\left(\frac{1}{\varepsilon\sqrt{\mu}} e^{-\varphi_*/\varepsilon}\right). \tag{28}$$

The estimate of the integral in the left hand side is based on $\sqrt{\mu}\ll\varepsilon$ as in Eq. (19).

(iii) Pores also enter the 1^- boundary layer across the left endpoint $r=1-\sqrt{\mu}$. This rate is estimated the same way ν' was. In fact, its order of magnitude $O[(1/\varepsilon\sqrt{\mu})e^{-\varphi_*/\varepsilon}]$ is the same as that of ν' (24).

In the limit $\sqrt{\mu}\ll\varepsilon$, process (i), representing the crossover from $r>1$, dominates. Hence, pores are introduced into the left boundary layer at rate ν (25). This rate must be balanced by the rate of pore destruction in this interval, which has order of magnitude

$$O\left(\left(\frac{n^-}{\mu}\right)\sqrt{\mu}\right)=O\left(\frac{n^-}{\sqrt{\mu}}\right). \tag{29}$$

Here, n^- represents the order of magnitude of n in the 1^- boundary layer, to be determined by balance of Eqs. (25) and (29). This balance gives

$$n^-=O\left(\frac{1}{\sqrt{\mu}} e^{-\varphi_*/\varepsilon}\right) \tag{30}$$

and, consequently,

$$\frac{n^-}{n^+}=O\left(\frac{\sqrt{\mu}}{\varepsilon}\right)=0.064. \tag{31}$$

The magnitude n^- in the 1^- boundary layer is much less than magnitude n^+ in the 1^+ boundary layer. Hence, in the limit ε, $\sqrt{\mu}/\varepsilon\to 0$, the pore distribution function n in $r>1$ is subject to the effective, absorbing boundary condition and the full boundary value problem can be replaced by a reduced boundary value problem in $r>1$. It consists of the Smoluchowski equation

$$n_t+\partial_r\left\{\frac{-\varphi_r}{\varepsilon}n-n_r\right\}=\frac{U_r}{\varepsilon\mu}e^{-U/\varepsilon} \quad \text{in} \quad r>1, \tag{32}$$

together with absorbing boundary condition

$$n(1,t)=0. \tag{33}$$

V. HEURISTIC OVERVIEW OF THE ASYMPTOTIC DYNAMICS OF PORE DENSITY

A heuristic overview of phenomena contained in the boundary value problem (32),(33) can be given in advance of detailed derivation. Consider the situation in which the local energy minimum at $r=r_m$ exists. A significant fraction of the pores created in the 1^+ boundary layer congregate near this energy minimum. Pores cannot accumulate around r_m indefinitely. Eventually, saturation must set in. One mode of saturation consists of pores crossing the diffusion barrier at r_d and expanding without bounds. For artificial bilayers such as in Chernomordik's experiments [1], this is catastrophic: Mechanical rupture of the membrane is imminent. In actual biological cell membranes, unbound pore growth is generally arrested by mechanical structures in the membrane such as the cytoskeletal network [24,25]. In any case, the fate of pores after crossing r_d is beyond the scope of the analysis presented here. There is only one relevant question about breakdown: How long does it take? This topic has been extensively studied [1,9,26]. The expected time to breakdown scales with the energy barrier $\varphi_d-\varphi_m$ as $e^{-(\varphi_d-\varphi_m)/\varepsilon}$.

A second mode of saturation consists of pores near r_m climbing back the energy barrier at $r=1$ and crossing over into $r<1$ where they are destroyed. This process is called resealing. The time for resealing scales with energy barrier $\varphi_*-\varphi_m$ as $e^{-(\varphi_*-\varphi_m)/\varepsilon}$.

If $\varphi_d>\varphi_*$, resealing is seen long before breakdown. In this case, a first guess at the ODE for $N(t)$ follows from simple order of magnitude estimates. Suppose that the accumulation of pores about r_m is initially negligible. The net flux of pores from the 1^+ boundary layer to r_m is approximated by the creation rate ν (25). For times much shorter than the breakdown time, pores continue to accumulate around r_m with none crossing into $r>r_d$. The pore distribution $n(r,t)$ in $1<r<r_d$ is approximated in order of magnitude by the Boltzmann distribution

$$n(r,t)\sim n_m e^{-(\varphi(r,t)-\varphi_m)/\varepsilon}, \tag{34}$$

where $n_m\equiv n(r_m,t)$. There is a sharp peak about $r=r_m$ which accounts for most of the total pore density $N(t)$. In fact, n_m in Eq. (34) is related to N according to

$$N=n_m\int_1^{r_d} e^{-(\varphi-\varphi_m)/\varepsilon}dr. \tag{35}$$

The integral may be evaluated by the Laplace's method

$$\int_1^{r_d} e^{-(\varphi-\varphi_m)/\varepsilon}dr\sim\sqrt{\frac{2\pi\varepsilon}{\varphi_m''}}=O(\sqrt{\varepsilon}), \tag{36}$$

where $\varphi_m''\equiv\varphi_{rr}(r_m,t)$. Combining Eqs. (35) and (36) gives

$$n_m=O\left(\frac{N}{\sqrt{\varepsilon}}\right). \tag{37}$$

The Boltzmann distribution (34) implies that the sharp peak at r_m is surrounded by exponentially small "tails" in the rest of $1<r<r_d$. The tail amplitude at $r=1$ has order of magnitude

$$n_m e^{-(\varphi_*-\varphi_m)/\varepsilon} \quad \text{or} \quad \frac{N}{\sqrt{\varepsilon}}e^{-(\varphi_*-\varphi_m)/\varepsilon} \tag{38}$$

on account of Eq. (37). If there were no pore creation in the 1^+ boundary layer, crossover into $r<1$ would proceed at a rate whose order of magnitude is

$$\frac{N}{\varepsilon^{3/2}}e^{-(\varphi_*-\varphi_m)/\varepsilon}. \tag{39}$$

This is simply the tail amplitude (38) divided by thickness ϵ of the 1^+ boundary layer. In the presence of pore creation (25), the net flux of pores from the 1^+ boundary layer to the accumulation at r_m should be the difference between Eqs. (25) and (39). This gives the conjectured ODE for $N(t)$,

$$\dot{N}\approx\frac{1}{\mu}e^{-\varphi_*/\varepsilon}-\frac{N}{\varepsilon^{3/2}}e^{-(\varphi_*-\varphi_m)/\varepsilon}. \tag{40}$$

It turns out that Eq. (40) is in fact correct up to factors of $O(1)$, so it properly captures the dependence on gauge parameters ε, μ. From Eq. (40), it is evident that net pore production is turned off, $\dot{N}=0$, when N achieves the equilibrium value N_{eq} with order of magnitude

$$N_{\mathrm{eq}}=O\left(\frac{\varepsilon^{3/2}}{\mu}e^{-\varphi_m/\varepsilon}\right). \tag{41}$$

VI. SINGULAR PERTURBATION ANALYSIS

A. Regularizing transformation

This section presents the rigorous derivation of the ODE (40) governing the pore density $N(t)$. The analysis assumes that the local energy minimum at r_m exists, that $\varphi_*<\varphi_d$, and that the dimensionless time constant of φ's temporal variations is of order unity. The first step is a preliminary regularization of the boundary value problem (32),(33). In intervals of r where the total flux f is much smaller than its components $-n_r$ and $-n\varphi_r/\varepsilon$, one can determine the approximate form of n by setting $f=0$ in Eq. (20) and integrating the resulting ODE. The result is

$$n\sim e^{-[\varphi(r,t)-C(t)]/\varepsilon}, \tag{42}$$

where $C(t)$ is a function of time, as yet undetermined. The $1/\varepsilon$ in the exponent indicates that solutions for $n(r,t)$ generally have length and time constants of size $O(\varepsilon)$. The exponential is factored out by a transformation from n to a new variable g, defined by

$$n(r,t)=g(r,t)e^{-[\varphi(r,t)-C(t)]/\varepsilon}. \tag{43}$$

The idea is that solutions for g should not contain length and time constants of size $O(\varepsilon)$, at least not in whole regions of the (r,t) plane of size unity.

The curve $r=r_m(t)$ in (r,t) plane represents the position of the energy minimum as a function of time. Suppression of short length and time constants from g in a neighborhood of this curve leads immediately to the determination of the function $C(t)$ in Eq. (43). Assuming that length and time constants of g in a neighborhood of $r_m(t)$ are $O(1)$, the local behavior of n in an $O(\sqrt{\varepsilon})$ neighborhood of $r_m(t)$ is well approximated by the following asymptotic form:

$$n\sim g_m e^{-(\varphi_m-C)/\varepsilon}e^{-\varphi_m''(r-r_m)^2/\varepsilon}, \tag{44}$$

which was obtained by replacing $g(r,t)$ in Eq. (43) by $g_m\equiv g[r_m(t),t]$ and substituting $\varphi(r,t)$ in the exponential by its Taylor series around r_m. Equation (44) has a Gaussian factor $e^{-\varphi_m''(r-r_m)^2/\varepsilon}$, which shows that $n(r,t)$ is sharply peaked about r_m and that almost all the total pore density N is due to pores with $|r-r_m|=O(\sqrt{\varepsilon})$. Integrating Eq. (44) in r and using Eq. (36), one finds an asymptotic form of N,

$$N\sim g_m e^{-(\varphi_m-C)/\varepsilon}\sqrt{\frac{2\pi\varepsilon}{\varphi_m''}}. \tag{45}$$

N generally does not contain short time constants of duration ε. In fact, the usual situation is quite the opposite. Recall from Eq. (40) that the resealing time constant is exponentially large in ε. Given absence of time constant ϵ in $N(t)$, it is clear from Eq. (45) that in order for g_m not to have an ε time constant, $C(t)$ must be

$$C(t)=\varphi_m\equiv\varphi[r_m(t),t]. \tag{46}$$

With Eq. (46), transformation (43) is completely defined

$$n(r,t)=g(r,t)e^{-(\varphi-\varphi_m)/\varepsilon}. \tag{47}$$

In addition, Eq. (45) relates g_m to N by

$$g_m=\sqrt{\frac{\varphi_m''}{2\pi\varepsilon}}N. \tag{48}$$

Substituting the representation (47) of n into Eq. (20) gives the flux f in terms of g

$$f=-g_r e^{-(\varphi-\varphi_m)/\varepsilon}. \tag{49}$$

Substitution of Eq. (47) into Smoluchowski equation (32) gives a PDE for g,

$$\begin{aligned} g_t-\frac{\varphi_t-\dot{\varphi}_m}{\varepsilon}g-g_{rr}+\frac{\varphi_r}{\varepsilon}g_r \\ =\frac{U_r}{\varepsilon\mu}e^{-\varphi_m/\varepsilon}e^{-(U-\varphi)/\varepsilon}\quad \text{in}\ \ r>1, \end{aligned} \tag{50}$$

and the absorbing boundary condition (33) translates into

$$g(1,t)=0. \tag{51}$$

In addition, the initial distribution function $n(r,0)$ induces the corresponding initial distribution function $g(r,0)$.

B. Outer solution

Asymptotic analysis of problem (50),(51) for g begins with the *outer* limit process in which length and time constants of g are $O(1)$, independent of ε. The leading order approximation to the PDE (50) comes from balance of $O(1/\varepsilon)$ terms

$$\varphi_r g_r\sim(\varphi_t-\dot{\varphi}_m)g$$

or

$$\frac{g_r}{g}\sim\frac{\varphi_t-\dot{\varphi}_m}{\varphi_r}. \tag{52}$$

The right hand side of Eq. (52) is possibly singular at r_m and r_d, where $\varphi_r=0$. The singularity at r_m is removable. Note that

$$\dot{\varphi}_m=\frac{d}{dt}\varphi_t[r_m(t),t]=(\varphi_t+\dot{r}\varphi_r)|_{r=r_m}=\varphi_t(r_m,t)$$

so

$$\frac{\varphi_t-\dot{\varphi}_m}{\varphi_r}=\frac{\varphi_t(r,t)-\varphi_t(r_m,t)}{\varphi_r(r,t)}\to\frac{\varphi_{tr}(r_m,t)}{\varphi_{rr}(r_m,t)}\quad \text{as}\ \ r\to r_m. \tag{53}$$

The limit of the indeterminate form is computed by L'Hospital's rule. Since the right-hand side of Eq. (52) is regular at r_m, Eq. (52) may be integrated to give

$$g\sim g_m\lambda,$$

with

$$\lambda=\lambda(r,t)\equiv\exp\left(\int_{r_m}^{r}\frac{\varphi_t-\dot{\varphi}_m}{\varphi_r}\,dr\right)\text{in}\ \ 1<r<r_d. \tag{54}$$

There are immediate questions about this outer solution: At any time t, its values in the entire interval of r, $1<r<r_d$ are set by its value g_m at $r=r_m$. It is clear that this outer solution cannot match arbitrary initial values of $g(r,0)$, nor does it satisfy the absorbing boundary condition $g(1,t)=0$ unless $g\equiv 0$. The leading order outer solution exhibits nonuniform validity in the following.

(i) An *initial layer* where $0<t=O(\varepsilon)$. This layer resolves the rapid relaxation of the initial data $g(r,0)$ to the $t=0^+$ limit of outer solution.

(ii) The 1^+ *boundary layer* where $0<r-1=O(\varepsilon)$. This boundary layer solution resolves the noncompliance of the outer solution with the absorbing boundary condition at $r=1$ and determines the effective flux of pores from the 1^+ boundary layer to r_m.

(iii) An *internal layer* about the diffusion barrier, where $|r-r_d|=O(\sqrt{\varepsilon})$, due to the generally nonremovable singularity of $(\varphi_t-\dot{\varphi}_m)/\varphi_r$ at r_d. This singular behavior is resolved by matching with an internal layer solution which describes the flux across the diffusion barrier.

This paper addresses only (ii), the 1^+ boundary layer solution. As argued in the Discussion, (i) and (iii) are either negligible or irrelevant to the scenarios of pore dynamics addressed here. Relaxation of the initial distribution to the outer solution and escape over a *time-dependent* diffusion barrier are addressed in a separate paper, currently in preparation.

C. Boundary layer solution in $r=1^+$ and asymptotic ODE for pore density

In the 1^+ boundary layer, $0<r-1=O(\varepsilon)$, g generally exhibits $O(\varepsilon)$ length and time constants. In this case, the "time terms"

$$g_t-\frac{\varphi_t-\dot{\varphi}_m}{\varepsilon}g \tag{55}$$

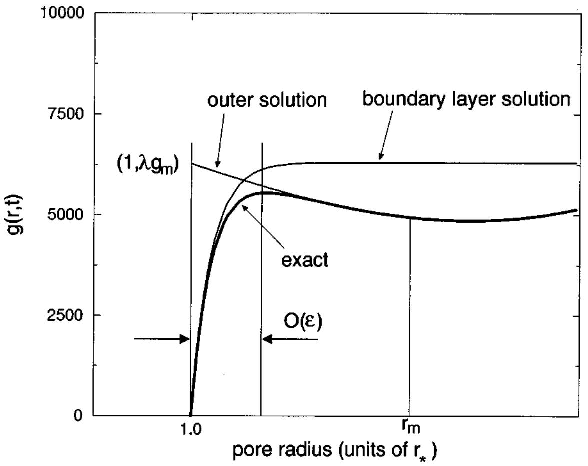

FIG. 4. Approximation of function $g(r,t)$ by the outer and boundary layer solutions.

are one power of ε smaller than the "space terms"

$$-g_{rr}+\frac{\varphi_r}{\varepsilon}g_r \tag{56}$$

in Eq. (50). Hence, in the 1^+ boundary layer, time terms (55) may be dropped from the leading order approximation to Eq. (50). What survives is the ODE

$$-g_{rr}+\frac{\varphi_r}{\varepsilon}g_r\sim\frac{U_r}{\varepsilon\mu}e^{-\varphi_m/\varepsilon}e^{-(U-\varphi)/\varepsilon}. \tag{57}$$

Introducing to Eq. (57) the boundary layer coordinate

$$R\equiv\frac{r-1}{\varepsilon} \tag{58}$$

and taking the limit $\varepsilon\to 0$, $R>0$ fixed, one obtains

$$-g_{RR}-|\varphi'_*|g_R\sim\frac{\varepsilon}{\mu}U'_*e^{-\varphi_m/\varepsilon}e^{-(U'_*+|\varphi'_*|)R}\quad\text{in } R>0. \tag{59}$$

Here, $\varphi'_*\equiv\varphi_r(1^+,t)$ and similarly, $U'_*\equiv U_r(1^+,t)$. Since φ'_* is generally negative, it is represented by $-|\varphi'_*|$ so no minus sign hides in Eq. (59). Effective boundary conditions are

$$g=0\quad\text{at } R=0, \tag{60}$$

$$g\to g_m\lambda(1,t)\quad\text{as } R\to\infty. \tag{61}$$

Equation (60) is the absorbing boundary condition and Eq. (61) follows from matching with the outer solution (54). Figure 4 gives an illustration of the matching condition (61). The boundary layer and outer solutions are depicted in relation to each other and to the exact solution for g.

The complete solution for g can be easily obtained from the boundary value problem (59)–(61). However, g itself is not relevant. What is really needed is the net flux of pores out of the 1^+ boundary layer. This flux can be extracted from Eqs. (59)–(61) as follows. The first integral of Eq. (59) is

$$e^{|\varphi'_*|R}g_R=\frac{\varepsilon}{\mu}e^{-\varphi_m/\varepsilon}e^{-U'_*R}+F, \tag{62}$$

where F is a constant of integration. It is related to the net flux out of the 1^+ boundary layer. In the limit $\varepsilon\to 0$, $R>0$ fixed, the flux (49) reduces to

$$f\sim-\frac{1}{\varepsilon}e^{-(\varphi_*-\varphi_m)/\varepsilon}(g_Re^{-|\varphi'_*|R}). \tag{63}$$

The expression in parentheses is equal to the left-hand side of Eq. (62). Hence, after substituting in its place the right-hand side of Eq. (62), it is seen that this boundary layer approximation to the flux converges to

$$f_\infty\equiv-\frac{1}{\varepsilon}e^{-(\varphi_*-\varphi_m)/\varepsilon}F\quad\text{as } R\to\infty. \tag{64}$$

Expressing F in terms of f_∞ in Eq. (62), and dividing by $e^{|\varphi'_*|R}$ gives

$$g_R=\frac{\varepsilon}{\mu}e^{-\varphi_m/\varepsilon}e^{-(U'_*+|\varphi'_*|)R}-\varepsilon e^{(\varphi_*-\varphi_m)/\varepsilon}e^{-|\varphi'_*|R}f_\infty\quad\text{in } R>0. \tag{65}$$

Integrating Eq. (65) in R from 0 to ∞ and using the boundary conditions (60),(61) gives

$$g_m\lambda(1,t)=\frac{\varepsilon}{\mu}\frac{e^{-\varphi_m/\varepsilon}}{U'_*+|\varphi'_*|}-\frac{\varepsilon}{|\varphi'_*|}e^{(\varphi_*-\varphi_m)/\varepsilon}f_\infty. \tag{66}$$

This obscure looking relation is actually the asymptotic ODE for $N(t)$: The net flux f_∞ is of course $\dot{N}$, the rate of change of the pore density $N(t)$, and g_m is related to N by Eq. (48). With these substitution and further rearrangement, Eq. (66) becomes

$$\dot{N}=K\left(1-\frac{N}{N_{\text{eq}}}\right), \tag{67}$$

where K and N_{eq} are functions of time given by

$$K=\frac{a}{\mu}e^{-\varphi_*/\varepsilon}, \tag{68}$$

$$a\equiv\frac{|\varphi'_*|}{U'_*+|\varphi'_*|}, \tag{69}$$

$$N_{\text{eq}}=\frac{\varepsilon^{3/2}}{\mu}be^{-\varphi_m/\varepsilon}, \tag{70}$$

$$b\equiv\frac{1}{U'_*+|\varphi'_*|}\sqrt{\frac{2\pi}{\varphi''_m}}e^{\int_1^{r_m}(\varphi_t-\dot{\varphi}_m)/\varphi_r dr}. \tag{71}$$

In the expressions for K and N_{eq}, the dependence on gauge parameters ε, μ and exponentially small factors $e^{-\varphi_*/\varepsilon}$, $e^{-\varphi_m/\varepsilon}$ are written out explicitly. The remaining factors a and b are $O(1)$ functions of time. Note further that the terms

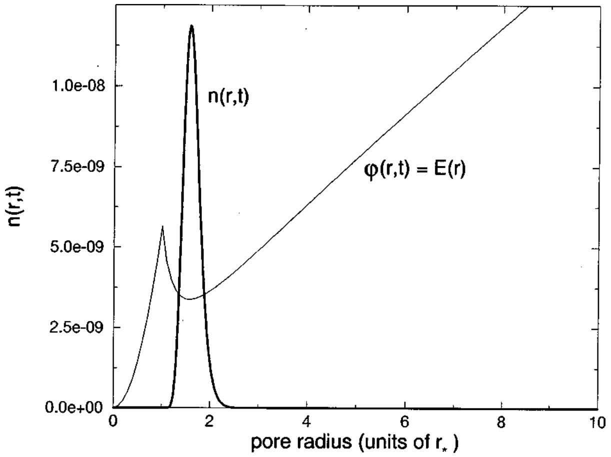

FIG. 5. Asymptotic approximation of the pore density distribution function $n(r,t)$ at transmembrane potential $V_m=0$ for which pore energy $\varphi=E(r)$.

of the ODE (67) and N_{eq} (70) indeed have the orders of magnitude predicted in Eqs. (40),(41).

If the dimensionless characteristic time of temporal variations of pore energy φ is much longer that unity, then $|\varphi_t|$, $|\dot{\varphi}_m| \ll 1$ and the exponential factor

$$e^{\int_1^{r_m}(\varphi_t-\dot{\varphi}_m)/\varphi_r dr} \tag{72}$$

in Eq. (71) is nearly unity. In this ‘‘quasistatic limit,’’ the asymptotic ODE (67) for $N(t)$ is equivalent to the ODE that would results if φ were assumed time independent. In this case, N_{eq} would represent the saturation value of N at which the net pore creation rate is zero.

Given pore density $N(t)$, the pore distribution function $n(r,t)$ is recovered asymptotically from Eq. (47) with g given by Eq. (54). The resulting formula is

$$n(r,t) \sim N\sqrt{\frac{\varphi_m''}{2\pi\varepsilon}}\, e^{\int_{r_m}^{r}(\varphi_t-\dot{\varphi}_m)/\varphi_r dr} e^{-(\varphi-\varphi_m)/\varepsilon}. \tag{73}$$

Figure 5 depicts the asymptotic distribution n at transmembrane potential $V_m=0$.

VII. PORE CREATION RATE COUPLED TO TRANSMEMBRANE POTENTIAL

The coefficients K and N_{eq} in the ODE (67) depend on the energy functions φ and U. These in turn depend on transmembrane potential V. The dimensionless version of Eq. (6) is

$$\varphi(r,t) = E(r) - \pi r^2 V^2, \tag{74}$$

where V is dimensionless potential, measured in units of $(\sqrt{E_*/a_p})/r_*$. An analogous dimensionless relation holds for $U(r,t)$. Substituting $\varphi=\varphi(r,t)$, $U=U(r,t)$ into formulas (68)–(71) gives the dependence of K and N_{eq} upon V. For instance, consider K: The main dependence on V comes from the exponential factor

$$e^{-\varphi_*/\varepsilon} = e^{-E_*/\varepsilon} e^{\pi V^2/\varepsilon} = e^{-E_*/\varepsilon} e^{(V/V_{ep})^2}, \tag{75}$$

where

$$V_{ep} = \sqrt{\varepsilon/\pi}. \tag{76}$$

The $O(1)$ coefficient a in Eq. (68) also depends on V but this dependence is much weaker than that of the exponential $e^{(V/V_{ep})^2}$ and is unlikely to be detectable experimentally. In practice, a is treated as a constant independent of V, resulting in an approximation for K,

$$K \approx \alpha e^{(V/V_{ep})^2}. \tag{77}$$

The approximation to N_{eq} based on the same reasoning is

$$N_{\mathrm{eq}} \approx N_0 e^{r_m^2 (V/V_{ep})^2}. \tag{78}$$

In Eqs. (77),(78), α and N_0 are nominal ‘‘constants’’ in which weak V dependence has been suppressed. The minimum energy radius r_m also depends on V but, as Fig. 3 makes clear, r_m changes very little as V is increased from 0 to V_c. Hence, r_m is also treated as a nominal ‘‘constant.’’ The ODE (67) with K and N_{eq} given by (77),(78) has the same form as the model proposed by DeBruin and Krassowska [27], in which constants α, V_{ep}, N_0, and r_m were chosen to fit experimental data.

The constant V_{ep}, defined in Eq. (76), is a characteristic voltage of electroporation. For ε defined in Eq. (19), $V_{ep}=0.084$ or, in dimensional units, 0.267 V. At a glance, this estimate appears to be significantly smaller than experimental evidence, which puts threshold for observable electroporation between 0.5 and 1.2 V, depending on the type of the membrane [1,28]. However, if V increases from 0 to V_{ep}, the rate of pore creation increases only by a factor of e. Such rate is too small to produce during a typical few milliseconds shock pore density sufficient for the experimentally detectable increase in membrane conductance. To cause an experimentally observable effect, V must exceed V_{ep} by a factor of 2 or 3. Hence, threshold voltage for electroporation measured in experiments is actually $2-3V_{ep}=0.53-0.8\,\mathrm{V}$, well within the range of experimental estimates.

VIII. DISCUSSION

The analysis presented in this paper reveals several interesting features of the electroporation process. First, regarding the source of the conducting pores: Essentially all conducting pores are initially created as hydrophobic pores with radius $r>r_*$ (more precisely, in a thin boundary layer just right of r_*) and immediately convert to hydrophilic pores. Because of a very fast destruction rate, pores created with $r<r_*$ have practically no chance of increasing their radius and converting to conducting pores. Naively, one might expect the interval $0<r<r_*$ to act as a source of pores because pore creation rate is exponentially larger for the smaller pores. In fact, the opposite is true. A certain number of pores created with $r>r_*$ cross over to $r<r_*$, where they are destroyed. Hence, instead being a source of pores, the interval $0<r<r_*$ acts as a sink, causing a drop in the pore distribution function $n(r,t)$ in a thin boundary layer to the right of r_*. This feature of the electroporation process allows the analysis to focus only on conducting pores living in the $r>r_*$ interval and to treat r_* as an absorbing boundary.

Second, regarding the pore distribution function: A vast majority of pores accumulate around the energy minimum r_m. The pore distribution function $n(r,t)$ is well approximated by a Gaussian distribution with a sharp peak at r_m and exponentially small ''tails'' in the rest of the interval (Fig. 5). The interaction of these tails with the energy barriers at r_* and r_d is responsible for pore creation, saturation, resealing, and rupture. Which events will take place, depends on the relative magnitude of the two energy barriers. If $\varphi_* < \varphi_d$, then the interaction of the left tail with energy barrier at r_* slows and shuts down the creation. If the number of pores is larger than the equilibrium, excess pores reseal by climbing back the energy barrier at r_* and crossing over into $r < r_*$ where they are destroyed. If $\varphi_d < \varphi_*$, then the pores of the right tail cross the energy barrier at r_d and expand without bounds, possibly leading to irreversible breakdown and mechanical rupture of the membrane. A qualitatively different situation occurs if the energy minimum at r_m and the energy barrier at r_d do not exist. In this case, the pores created near r_* all expand without bounds, similar to a ''mudslide,'' again setting up a stage for a mechanical rupture.

For $\varphi_* < \varphi_d$, the asymptotic ODE (67) derived in this study describes pore creation when pore density $N(t) \ll N_{\rm eq}$, saturation and turning the creation rate off when $N(t)$ approaches $N_{\rm eq}$, and resealing when $N(t) > N_{\rm eq}$. In this case, the ODE (67) determines $N(t)$ and the pore distribution function $n(r,t)$ is computed from Eq. (73). Having $n(r,t)$, one can determine the current flowing through pores and, using an appropriate circuit equation, follow the temporal evolution of the transmembrane potential V.

The asymptotic ODE (67) can also be used in cases in which the diffusion barrier φ_d is lower than φ_* or altogether absent. If $\varphi_d < \varphi_*$, saturation is achieved for N much less than $N_{\rm eq}$ in Eq. (70). This is because escape over the energy barrier at r_d is energetically much easier than climbing up the energy barrier at $r = r_*$. With $N \ll N_{\rm eq}$, the resealing term in Eq. (67) may be dropped. If a local energy minimum r_m does not exist, there is no mechanism at all to generate a tail of $n(r,t)$ near $r = r_*$. Without the significant resealing induced by this tail, the resealing term in Eq. (67) can again be dropped. Hence, for both cases discussed above, the rate of pore production is approximated by omitting the resealing term $-N/N_{\rm eq}$ in Eq. (67). However, $n(r,t)$ no longer has a simple approximation such as a Gaussian about r_m. Consequently, one has no means of computing the current through pores and its effect on the transmembrane potential V. Hence, for $\varphi_d < \varphi_*$, the pore density $N(t)$ computed from the ODE is valid only if V is set by an external circuit, such as during voltage-clamp experiments [1,16].

For the values of parameters in Table I, the diffusion barrier φ_d disappears at $V_c \approx 0.5\,\mathrm{V}$. Does it mean that the ODE loses validity at V close to 0.5 V? Not necessarily. According to PDE (1), all pores created near r_* should expand without bounds, leading to the mechanical rupture of the membrane. However, such catastrophic scenarios are not observed in practice. In typical electroporation experiments, majority of the cells reseal and survive, even if they are exposed to V of 1 V and larger. The explanation is that, in most experimental situations, the diffusion barrier disappears only transiently, during the time when V reaches its peak. Rapid creation of pores leads to an increase of the transmembrane current, which in turn leads to the decrease of V below V_c. The diffusion barrier at r_d is restored, and the fact that most of the cells survive implies that the restoration of φ_d happens sufficiently fast and that φ_d is sufficiently high to prevent the escape of pores. Therefore, the ODE derived in this study may be applied even under transmembrane potentials larger than V_c. There will be some loss in accuracy: during the short transient when the diffusion barrier disappears, the pore distribution $n(r,t)$ is not Gaussian and this model probably underestimates the magnitude of current flowing through the membrane.

The asymptotic ODE (67) was derived under the assumption that the temporal variations of energy $\varphi(r,t)$, $U(r,t)$ have characteristic time on the order of $r_*^2/D \approx 5\,\mu\mathrm{s}$. When the characteristic time of φ, U is much longer than 5 μs, $n(r,t)$ responds ''quasistatically'' to the temporal variations: Except for the boundary layer near r_*, the distribution is nearly proportional to the Boltzmann factor $e^{-\varphi(r,t)/kT}$ in Eq. (47). In this case, the quasistatic limit of the ODE (67) as discussed in Sec. VI is valid. When the characteristic time of φ, U approaches and decreases below the 5 μs limit, the tails of $n(r,t)$ away from r_m manifest significant deviations from the Boltzmann factor. Still, the response of $n(r,t)$ to temporal variation of φ, U is nearly instantaneous and the pore creation rate is described by the time-dependent version of the ODE (67). Note that even in this case, the main dependence of coefficients K and $N_{\rm eq}$ in Eqs. (68)–(71) upon $V(t)$ is quasistatic, via exponential factors $e^{(V/V_{\rm eq})^2}$, $e^{r_m^2 (V/V_{\rm eq})^2}$. The non-Boltzmann tail effect manifests itself as an exponential factor (72). It is buried in the $O(1)$ coefficient b in Eq. (70) and, as such, it is barely discernible in any practical sense.

The lower bound on the admissible temporal variations of φ, U is set by the relaxation time of $n(r,t)$ from arbitrary initial data to Gaussian form (73). As argued in Sec. VI B, this relaxation occurs within an initial layer of dimensionless duration ε. The dimensional time corresponding to ε is $\varepsilon r_*^2/D \approx 0.1\,\mu\mathrm{s}$. Hence, the time-dependent version of the ODE (67) remains valid when the time constants of φ, U are 0.1 μs or longer.

Setting limits on temporal variations of pore energy φ, U imposes the same limits on the transmembrane potential V, which is the quantity available for experimental manipulation. During a typical electroporation experiment, changes to V occur due to two mechanisms:

(i) Direct charging of the membrane adjacent to the electrode. This process has a time constant $R_m C_m \approx 1-10\,\mathrm{ms}$, where R_m and C_m are the surface resistance and surface capacitance of the membrane [29].

(ii) Polarization of the individual cells by an electric field. Here, the time constant depends on the shape of the cell. An order of magnitude estimate is $C_m d/\sigma_i = 0.25 - 2.5\,\mu\mathrm{s}$, where d is the dimension of the cell and σ_i is the conductivity of the cytoplasm [30].

Comparing these estimates with the 5 and 0.1 μs limits shows that the asymptotic ODE (67) and its quasistatic approximation are both valid for case (i): Even if the stimulating current has frequency in the MHz range, the response of the membrane is slow enough to shield pore energy from fast changes. The frequency of the stimulus may be an issue in case (ii). Small cells polarize and depolarize very rapidly in the external field, these rapid changes are reflected in φ and U, and the use of the quasistatic version of ODE (67) may be ''pushing the envelope.'' Still, even in this case, one can expect only a modest difference between solutions using the quasistatic and time-dependent ODE. As argued above, the time dependence affects only an $O(1)$ coefficient b (70). Therefore, for most experimental setups, the ODE (67) should give an adequate description of the electroporation process. The only exception may be voltage clamp experiments, in which the rate of rise of V does not depend on the intrinsic properties of the cells and membranes but is enforced by an external circuit.

The advantages of the asymptotic ODE are fourfold. First, the simple form of the ODE (67) makes it amenable to analytical examination that can elucidate the most important qualitative features of the electroporation process. The same qualitative features exist in the PDE (1) but are buried and, because of the difficulties associated with solving the PDE and its analysis, are much harder to uncover. Second, the ODE contains a smaller number of parameters and most of them are related in a straightforward way to experimental measurements [16]. Thus, the ODE provides a convenient conceptual framework for the design and interpretation of the electroporation experiments. Third, formulas (68)–(71) presented in this paper provide the connection between the parameters of the ODE and the molecular-level constants appearing in the PDE. This connection provides a way of using experimental results to evaluate the value of these constants which at present are known only to the order of magnitude. Fourth, the ODE (67) is easy to solve numerically, at a minimal computational cost and may serve as a tool to study electroporation in the spatially distributed systems.

The ODE (67) is an asymptotic reduction of the Smoluchowski equation (1) assuming a specific form of the pore energy function φ (6). In this formulation, the factors contributing to the energy are the surface tension of the membrane, the line tension of the pore edge, and the membrane capacitance, which introduces dependence on the transmembrane potential. Pore energy (6) is at present the most widely used in the literature. However, there exist formulations that account for different factors, such as osmotic pressure [31], electrocompression of the lipid bilayer [32], interaction with the membrane cytoskeleton [25], or deformation of cells by electric field [33]. To electroporation theories based on these alternative formulations, the exact form of the ODE (67) obviously does not apply. However, if these theories are based on the Smoluchowski equation and if they contain small parameters (which is quite likely), then an asymptotic reduction similar to the one presented here should be feasible. In such cases, the present study can provide a ''blueprint'' for deriving simplified approximations for the theories of electroporation based on different pore energetics.

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