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Estimation of the parameters of the Smoluchowski equation describing the occurrence of pores in a bilayer lipid membrane under soft poration

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Abstract. The conductive lipid pores occurring in planar bilayer membranes are known to manifest themselves experimentally as current fluctuations. Reliable recording of such fluctuations during phase transitions, as well as in membranes with various additives (for example, SDS), allows one to determine the characteristics of hypothetical hydrophilic pores, namely, their number, sizes, lifetimes, and duration of time intervals between pores. Because, in contrast with electroporation, the emergence of pores in a membrane does not require high voltages, this process is called soft poration. Studying the characteristics of pores under soft poration allows us to estimate the parameters of the Smoluchowski equation and compare them with the corresponding parameters used to describe electroporation. In this work, the experimental characteristics of current fluctuations in the membrane with the addition of SDS to the bulk solution were used to estimate the parameters of the Smoluchowski equation: the pore edge tension, the energy of the hydrophobic pore/hydrophilic pore barrier, the coefficient of pore diffusion in the radius space, the initial distribution density of the number of pores, and the attempt rate density of the lipids in a membrane. The obtained estimates are close to the parameter values used in studies of electroporation.

1 Introduction

The increase in the lipid bilayer permeability in response to the application of electric field [1], under phase transitions [2] or with the addition of detergents [3,4] is usually associated with the formation of conductive lipid pores. Lipid pores can be investigated experimentally by registering the current fluctuations in planar bilayer lipid membranes [4–6] or by observing the leakage of fluorescent markers from the liposomes [7]. The molecular mechanisms of the formation of conductive pores under electroporation are studied by the methods of molecular dynamics [8].

To analyze the evolution of such pores, the Smoluchowski equation is used which describes the diffusion of pores in the radius space [9]. This model assumes that both hydrophobic and hydrophilic pores can exist in a membrane. The electric field reduces the potential barrier between them thus facilitating the formation of hydrophilic pores. We proposed [10–12] using the Smoluchowski equation with a source term to analyze the hydrophilic pores occurring under phase transition in a dipalmitoylphosphatidylcholine membrane. The introduction of a hydrophobic pore source to the equation was motivated by the possibility of the emergence, under phase transition, of additional structural defects in a membrane due to the increased fluctuation of the area per one lipid molecule. In [12], a good agreement was shown between the experimental characteristics of the current fluctuations in membranes and the distributions of pore lifetimes and the inter-impulse intervals as calculated using the Smoluchowski equation.

To describe the behavior of pores, the equation uses parameters whose values cannot be measured directly in experiment. These parameters are estimated using indirect approaches which leads to a considerable spread in their values. For example, the pore edge tension is conventionally estimated from 9 [13], 9.2 [14], 10 [15–17], up to 18 [18], 20 [19,20] and even 40 [21] pN. The energy of the hydrophobic pore/hydrophilic pore barrier is estimated at 50 [9], 51 [20], or 45 [16] kT, where k is the Boltzmann constant, and T is the temperature.

There is a significant difference in the estimates of the coefficient of pore diffusion in the radius space: cited are

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the values of 50 [20], 200 [22], and 1100 [23] $\mathrm{nm^2\,ms^{-1}}$. The Appendix in [9] contains a discussion of the physical meaning of some of the parameters involved in the Smoluchowski equation. In particular, the authors extrapolate the experimental and computational results obtained for electroporation under high voltages to the case of a small, ideally zero membrane potential.

It is known that, under low voltages, conductive hydrophilic pores which manifest themselves experimentally in the form of current fluctuations in the membrane can occur under phase transitions [2,4,5] as well as in the presence of detergents in subsolubilizing concentrations [4, 24]. These effects which, in contrast with electroporation, do not require high voltages on the membrane have been named soft poration [4]. The study of pore characteristics under soft poration permits obtaining experimental data to enable the estimation the Smoluchowski equation parameters and comparison of those with the parameters computed for electroporation.

In this work, experimental data are used as basis for the estimation of the following parameters: pore edge tension, energy of the hydrophobic pore/hydrophilic pore barrier, coefficient of the diffusion of pores in the radius space, initial distribution density of the number of pores, and the attempt rate density for the lipids in a membrane.

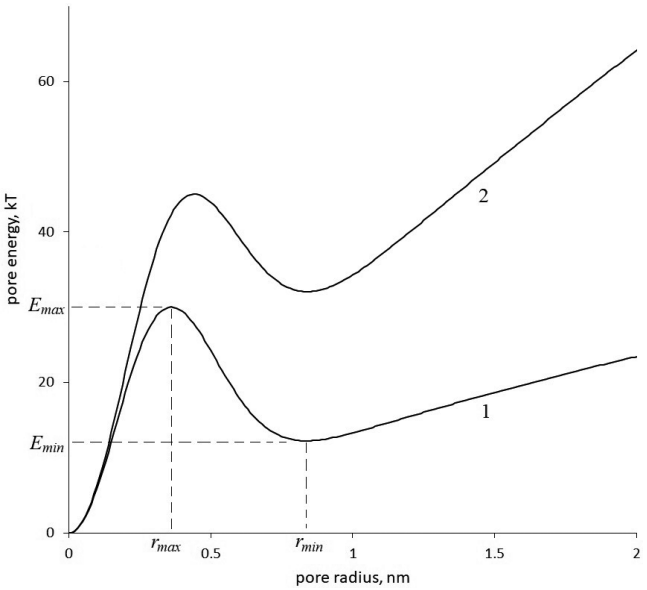

Fig. 1. Dependence of the pore energy on the radius under different pore edge tensions and energies of the hydrophobic pore/hydrophilic pore barrier 9.2 pN, 30 kT (1) and 22.7 pN, 45 kT (2). Other computational parameters are presented in table 1.

2 Materials and methods

2.1 Computation using the Smoluchowski equation

In the literature (for example, [9]), an accepted approach is to consider the diffusion of pores in the radius space using the Smoluchowski equation for the distribution density of the number of pores with respect to the radius $n(r,t)$:

$$\frac{\partial n}{\partial t} = D\left\{\frac{\partial^2 n}{\partial r^2} + \frac{\partial}{\partial r}\left[n\frac{\partial}{\partial r}\left(\Delta E/kT\right)\right]\right\}, \qquad (1)$$

where r is the pore radius, t is the time, $\Delta E(r)/kT$ is the pore energy profile, D is the diffusion coefficient for pores in the radius space, $n(r,t)\mathrm{d}r$ is the number of pores with radii falling between r and $r+\mathrm{d}r$.

Pores are conventionally divided into hydrophobic and hydrophilic ones [16,25]. In [18,26], energy profiles are presented separately for hydrophobic and hydrophilic pores. We will consider, following the logic of [27], another model of lipid pores, in which it is proposed to abandon the rigid separation of pores into hydrophobic and hydrophilic ones. The part of the inner surface of any through pore in the membrane located at the edges of the pore closer to the surface of the membrane is hydrophilic, while a hydrophobic belt is retained in its middle. The height of the hydrophobic belt L, which determines the hydrophobicity of the pore, depends on the radius of the pore. This dependence, presented in [27] on the basis of studying the methods of molecular dynamics, can be represented as $L = h\exp(-r^2/{r_{phob}}^2)$, where h is the membrane thickness, r_{phob} is the characteristic pore radius determining the degree of its hydrophobicity.

Within this approach, the pore energy can be given by

$$\Delta E = 2\pi r L\sigma_{phob}(r) + 2\pi r(h-L)\sigma_{phil} - \pi r^2\sigma_{phil}, \quad (2)$$

where σ_{phil} is the interphase tension of the hydrophilic pore surface between the phospholipid heads and water, $\sigma_{phob} = r/2\rho\sigma_\infty$ is the interphase tension between the hydrophobic pore surface and water depending on the pore size, σ_∞ is the interphase tension between hydrophobic lipid tails and water, ρ is the characteristic radius. A linear relation between the interphase tension and radius was proposed [25] for pores of small radius. A more general expression contains the Bessel functions. In this case, one can also restrict oneself to the linear approximation, since the first term of eq. (2) contains the factor $\exp(-r^2/{r_{phob}}^2)$, which at large radii reduces its contribution to the pore energy. Equation (2) can be transformed into

$$\Delta E = \frac{\pi r^2}{\rho}\sigma_\infty h\exp\left(-\frac{r^2}{{r_{phob}}^2}\right) + 2\pi r\gamma\left(1-\exp\left(-\frac{r^2}{{r_{phob}}^2}\right)\right) - \pi r^2\sigma_{phil}, \qquad (3)$$

where $\gamma \sim h\sigma_{phil}$ is the pore edge tension. Equation (3) describes the pore energy profiles presented in fig. 1. Note that eq. (3) is analogous to the energy profile that we found empirically in our previous studies [10–12,28] to describe the temporal characteristics of pores under phase transition. Although, within the model used, pores cannot be uniquely divided by radius into hydrophobic and hydrophilic ones, we assume for simplicity that the location of the local maximum r_{max} (see fig. 1) separates hydrophobic ($r < r_{max}$) and hydrophilic pores ($r > r_{max}$), while

E_{max} is the energy of the hydrophobic pore/hydrophilic pore barrier, E_{min} is the energy of a hydrophilic pore, r_{min} is the radius of a hydrophilic pore (as determined in experiment). Thus, all pores with $r > r_{max}$, that can exist in a metastable state with minimal energy E_{min}, and which we can register by current traces, are considered hydrophilic pores. $R = \gamma/\sigma$ is the maximum possible pore radius not leading to the destruction of the pore. With $r \gg r_{phob}$, the energy of a hydrophilic pore is given by the known equation, $\Delta E = 2\pi\gamma r - \pi\sigma_{phil} r^2$ [25]. Note that the integration of the distribution of the number of pores

$$N_{phob}(t) = \int_0^{r_{max}} n(r,t)\mathrm{d}r, \tag{4a}$$

$$N_{phil}(t) = \int_{r_{max}}^{R} n(r,t)\mathrm{d}r \tag{4b}$$

yields the total of hydrophobic pores and the total of hydrophilic pores in the membrane, a quantity determined in experiment.

As boundary conditions, the derivatives with respect to radius were set to zero:

$$\frac{\partial n(0)}{\partial r} = \frac{\partial n(R)}{\partial r} = 0, \tag{5}$$

which has the following physical meaning: at the left boundary ($r = 0$), the pores cannot transfer into the region of negative radii, while at the right boundary ($r = R$), the pore radius cannot exceed R, or the membrane will cease to exist.

Equation (1) has the stationary solution,

$$n_{st}(r) = n_0 \exp\left(-\Delta E(r)/kT\right), \tag{6}$$

where n_0 determines the distribution density of the number of pores at $r = 0$.

Consider the stationary process of hydrophilic pore formation (fig. 2): pores occur at random points in time but the probability of their occurrence does not vary over time. Assume that the process of the occurrence of pores is a Poisson process characterized by the parameter Θ which is the mean time between the occurrence of two successive pores. Assume that the closure of the existing pores is also a Poisson process characterized by the mean pore lifetime τ. The mean number of pores in a membrane is then determined by the Little formula [29],

$$N_{phil} = \tau/\Theta. \tag{7}$$

This permits using experimental data and eqs. (4b) and (6), with a specified energy profile (3), to determine the value of n_0,

$$n_0 = \frac{\tau}{\Theta \int_{r_{max}}^{R} \exp(-\Delta E(r)/kT)\mathrm{d}r}. \tag{8}$$

We will solve eq. (1) for two situations: a) at the time of the start of calculation, there are no hydrophilic pores in the membrane, b) at the time of the start of calculation, only one hydrophilic pore is present in the membrane. For hydrophobic pores, the initial conditions are the same in both cases, while for hydrophilic pores, the initial conditions differ:

$$n(r,0)\Big|_{N_{phil}(0)=0} = \begin{cases} n_{st}(r), & 0 \le r \le r_{max}, \\ 0, & r_{max} < r \le R, \end{cases} \tag{9a}$$

$$n(r,0)\Big|_{N_{phil}(0)=1} = \begin{cases} n_{st}(r), & 0 \le r \le r_{max}, \\ n_{st}(r) \Big/ \int_{r_{max}}^{R} n_{st}(r)\mathrm{d}r, & r_{max} < r \le R. \end{cases} \tag{9b}$$

Integration of the initial conditions (9) from r_{max} to R gives $N_{phil}(0) = 0$ or $N_{phil}(0) = 1$, corresponding to our two cases: the membrane does not have hydrophilic pores or it has a single pore. Note that the initial conditions (9) are not solutions to eq. (1), but their sum with the weights $\frac{\Theta-\tau}{\Theta}$ and $\frac{\tau}{\Theta}$ is equal to the stationary solution (6). Note also that both solutions of eq. (1) with the boundary conditions (5) and initial conditions (9) tend to the stationary solution (6). The computation results for the integral (4b) with the initial conditions (9a) increase from 0 to the mean value of the number of hydrophilic pores τ/Θ, while with initial conditions (9b), they decrease from 1 to the value τ/Θ.

Equation (1) was solved numerically on the interval $[0, R]$ and at every time step, eq. (4b) was used to calculate the number $N(t)$ of hydrophilic pores in the membrane.

2.2 Experiment: comparison of calculations with experimental data

The experimental data processed in this work were presented in [4]. The current fluctuations of planar BLMs of azolectin (Avanti Polar Lipids, Alabaster) formed on an aperture of area 1 mm^2 in a 0.1 M NaCl solution with the addition of 0.07 mM SDS (Sigma) were measured. The measurements were carried out in symmetrical conditions. Transmembrane currents were detected in voltage clamp mode, at a discretization frequency of 1000 Hz.

With the addition of SDS to the solution, current impulses were observed in the current traces (see fig. 2), which were assumed to be connected to the occurrence of hydrophilic pores [4]. To compare the computation results with the experiment, we assumed that we dealt with an ergodic process and considered the temporal dependence as an ensemble of "membranes". In this text, to distinguish the hypothetical "membranes" in the ensemble from real membranes, the former will be placed in quotation marks. We divided the whole of the current trace into portions of size Δt. Each successive portion is shifted in time with respect to the preceding one by the discretization step δt. Each portion we considered as the current trace of a separate "membrane" from the ensemble.

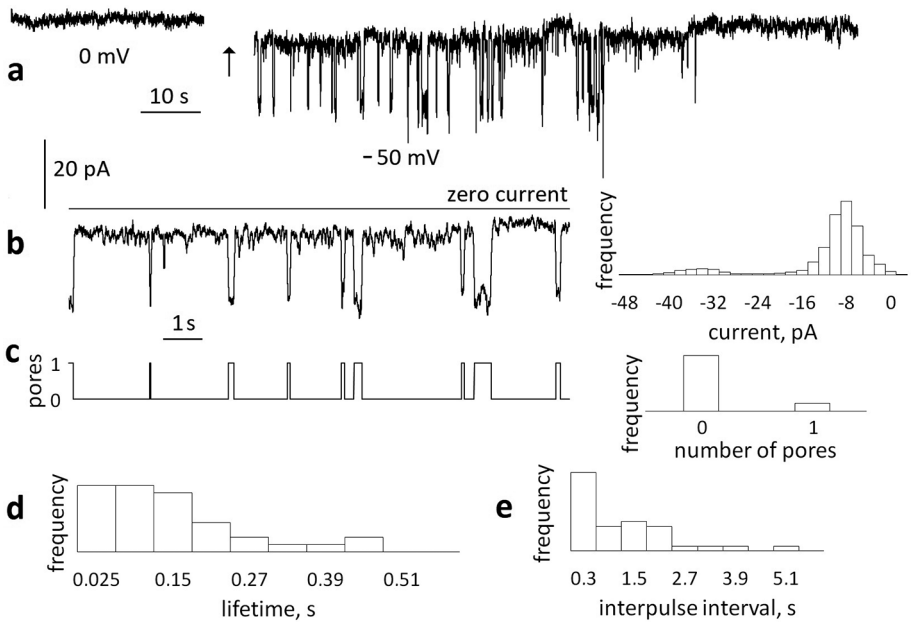

Fig. 2. Analysis of the current trace of an azolectin membrane under the addition of 0.07 mM SDS to 0.1 M NaCl bathing solution. (a) Current trace. The arrow indicates the moment of switching the voltage on the membrane from 0 to −50 mV. (b) Fragment of the current trace and current histogram. The horizontal line refers to zero current. (c) The result of processing the current trace – the number of hydrophilic pores and histogram of those pores. (d) and (e): histograms of the pore lifetime and inter-impulse interval.

In the ensemble, two groups of "membranes" can be identified: those that initially have no pores and those that have a single pore. Sooner or later any open pores will close, and new pores will occur in the "membranes" that initially do not have any. With time, the "membranes" in both groups will intermingle, and the specificity of the groups will vanish. For each group of "membranes", we averaged current traces of length Δt. As a result, the averaged trace for the group that initially had had no pores increased from 0 to the mean number of hydrophilic pores, τ/Θ, and the averaged trace for the group that initially had had a pore decreased from 1 to the same mean value, τ/Θ.

Note that the total volume of the "membrane" ensemble was determined by $\frac{T_{all}-\Delta t}{\delta t}$, where T_{all} is the duration of a membrane current trace. Meanwhile, the number of independent traces was equal to the integer part of the fraction $\frac{T_{all}}{\Delta t}$. With averaging, this effect did not lead to a shift in the averaged value; rather, the averaging error was determined not by the square root of the number of "membranes" but by the square root of the number of independent traces.

It was those two averaged traces that we compared with the results of computing the integral (4b) of the function $n(r,t)$ obtained through solving eq. (1).

The process can be represented by a scheme describing the transition of the "membrane" from state 0 (no pores) to state 1 (one pore) at a rate of $1/\Theta$, and back, at a rate of $1/\tau$. We have $M = M_0(t) + M_1(t)$, where $M_0(t)$ ($M_1(t)$) is the time-variable number of "membranes" which initially had no pores (had one pore), and M is the total number of "membranes":

$$M_0 \underset{1/\tau}{\overset{1/\Theta}{\longleftrightarrow}} M_1$$

This scheme is described by the following system of equation:

$$\begin{cases} \dfrac{\mathrm{d}M_0(t)}{\mathrm{d}t} = -\dfrac{M_0(t)}{\Theta} + \dfrac{M_1(t)}{\tau}, \\ \dfrac{\mathrm{d}M_1(t)}{\mathrm{d}t} = \dfrac{M_0(t)}{\Theta} - \dfrac{M_1(t)}{\tau}, \\ M = M_0(t) + M_1(t), \end{cases}$$

which is solved for two cases: under the initial condition where all the membranes have a pore, $M_1(0) = M$, and under the initial condition where all the membranes have no pores $M_0(0) = M$. The resulting mean number of pores in the membrane is given by

$$N_{phil} = \frac{\tau}{\tau+\Theta} + \frac{\Theta}{\tau+\Theta}\exp\left[-\left(\frac{1}{\tau}+\frac{1}{\Theta}\right)t\right], \quad (10a)$$

for the case where initially all the "membranes" have a pore, or

$$N_{phil} = \frac{\tau}{\tau+\Theta} - \frac{\tau}{\tau+\Theta}\exp\left[-\left(\frac{1}{\tau}+\frac{1}{\Theta}\right)t\right], \quad (10b)$$

for the case where initially the "membranes" have no pore.

3 Results

3.1 Experimental results

In total, 7 membranes were investigated, in which 202 pores were registered. A characteristic record of the current fluctuations under fixation regime is shown in fig. 2(a). The length of the record is about 2 minutes. During the initial part, no voltage was applied to the membrane, then the voltage was $-50\,\mathrm{mV}$. On the application of the voltage, the record showed current impulses which ceased after $\sim 1\,\mathrm{min}$. Our interpretation was that the discrete current impulses were due to the occurrence of hydrophilic pores. A characteristic trace fragment of length $\sim 15\,\mathrm{s}$ is shown in fig. 2(b). The time dependence of the number of hydrophilic pores in the membrane obtained in the analysis of the current trace is shown in fig. 2(c): the membrane either has no pores or has one pore. The transition from the trace to pores was made by to the rule: if the current was less than $-25\,\mathrm{pA}$, then we implied that a pore existed; otherwise, we implied that there was no pore.

In the experiment, 36 pores were registered. The mean lifetime of a hydrophilic pore was $\tau = 0.17 \pm 0.16\,\mathrm{s}$, and the mean time between the occurrences of successive pores was $\Theta = 1.25 \pm 1.24\,\mathrm{s}$. The approximate equality of the means and standard deviations of the pore lifetime and the time between the occurrences of pores suggests exponential distribution laws (see the histograms in figs. 2(d) and 2(e) for a selection of 36 pores). The validity of exponential laws testifies to stationary Poisson processes.

The radius of a toroidal pore can be estimated as the radius of the cylinder with the same volume [30]:

$$r_{min} = \sqrt{Ih/\pi gU} = 0.84 \pm 0.07\,\mathrm{nm}, \tag{11}$$

where $h = 5\,\mathrm{nm}$, I is the mean amplitude of current impulses, U is the voltage on the membrane, and $g = 1.07\,\mathrm{S\,m^{-1}}$ is the specific conductivity of 0.1 M NaCl solution at room temperature.

Figure 3 shows experimental data obtained through the analysis of the time dependence of the number of hydrophilic pores presented in fig. 2(c). The length of the record was $T_{all} = 43.543\,\mathrm{s}$. With a discretization step of $\delta t = 1\,\mathrm{ms}$ and the trace duration of one "membrane" from the ensemble equal to $\Delta t = 0.5\,\mathrm{s}$, 43043 "membrane" traces were obtained. The time during which there were no pores amounted to 37.937 s, while the time during which there was a pore amounted to 5.606 s.

We assumed that we dealt with an ensemble of 43043 "membranes", which we divided into two groups: "membranes" that initially have no pores (the group counting 37437 "membranes"), and "membranes" that have one pore (5606 "membranes"). We observed the varying number of pores in each "membrane" for 0.5 s. After that, we averaged the data separately for the "membranes" with a pore (fig. 3, markers 1), and for the "membranes" without a pore (fig. 3, markers 0). The averaged dependences thus obtained start at 1 or at 0, but with time, they all tend to the stationary value 0.136.

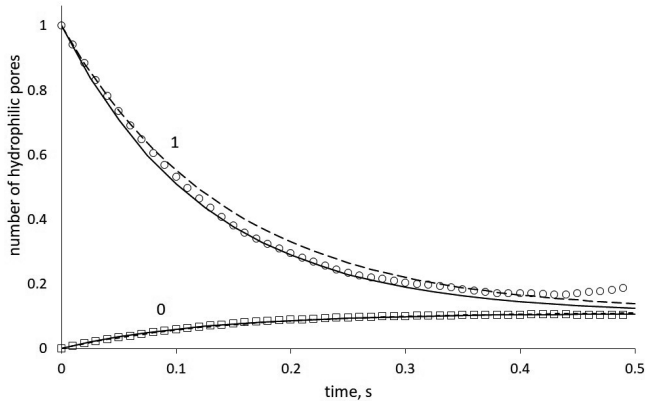

Fig. 3. Time variation of the mean number of hydrophilic pores in the groups of "membranes" which initially had a pore (1), or did not have a pore (0), obtained from the experimental data (markers) and from the computation results (curves) for the Smoluchowski equation (solid curves) and for eqs. (10) (dashed curves).

3.2 Computation results

The parameters involved in solving the Smoluchowski equation are presented in table 1.

Table 1 presents the parameters obtained with the use of the experimental data, T, r_{min}, and data from the literature, σ_{phil}, σ_∞, h, D.

To estimate the edge tension γ of a hydrophilic pore, we used the following relations [9] connecting the pore energy to its lifetime τ and the time Θ between the occurrences of pores:

$$\frac{1}{\tau} = \nu V_{10} \exp\left(-\frac{E_{max} - 2\pi\gamma r_{min}}{kT}\right), \tag{12a}$$

$$\frac{1}{\Theta} = \nu V_{01} \exp\left(-\frac{E_{max}}{kT}\right), \tag{12b}$$

where ν is the attempt rate density of membrane lipids, V_{10} and V_{01} are membrane volumes whose molecule fluctuations can lead to the transition from hydrophilic pore to hydrophobic pore, $1 \to 0$, or from hydrophobic pore to hydrophilic pore, $0 \to 1$. According to [9], $V_{01} = Sh$ is the total volume of the membrane (S being the membrane area), and $V_{10} \approx 2\pi r_{min}^2 h$ is the volume of the membrane portion immediately connected with a pore. Dividing expressions (12) into each other

$$\frac{\Theta}{\tau} = \frac{2\pi r_{min}^2}{S} \exp\left(\frac{2\pi\gamma r_{min}}{kT}\right), \tag{13}$$

allows one to estimate the edge energy of a hydrophilic pore: $\gamma = 22.7\,\mathrm{pN}$. For this estimate, we also used data from the literature: $\gamma = 9.2\,\mathrm{pN}$ [14].

The fitted parameters r_{phob} and ρ determine the radius r_{min} and allow us to change the magnitude of the energy barrier.

Equation (8), with the experimental parameters τ and Θ, were used to compute the initial distribution density of

Table 1. Notation and parameter values.

Parameter	Values	
T, membrane temperature [a]	295 K	
r_{min}, hydrophilic pore radius [a]	0.84 nm (eq. (11))	
h, membrane thickness	5 nm [16]	
σ_{phil}, interphase tension of the hydrophilic pore surface between the phospholipid heads and water	1.9 mN m^{-1} [14]	
σ_∞, interphase tension between hydrophobic lipid tails and water	50 mN m^{-1} [16]	
D, diffusion coefficient in pore radius space	50–1100 nm^2 ms^{-1} [9,20,22,23]	
γ, pore edge energy density	22.7 pN [a]	9.2 pN [14]
E_{max}, energy of the hydrophobic pore/hydrophilic pore barrier [b]	44–48 kT	24–28 kT
r_{phob}, characteristic pore radius determining the degree of its hydrophobicity [b]	0.387–0.375 nm	0.35–0.34 nm
ρ, hydrophobic pore characteristic radius [b]	0.310–0.257 nm	0.415–0.335 nm
n_0, constant determining the stationary number of pores [b]	(6.4–6.0) $\times 10^{13}$ nm^{-1}	(7.5–7.4) $\times 10^4$ nm^{-1}

[a] Parameters obtained using experimental data.
[b] Fitted parameters.

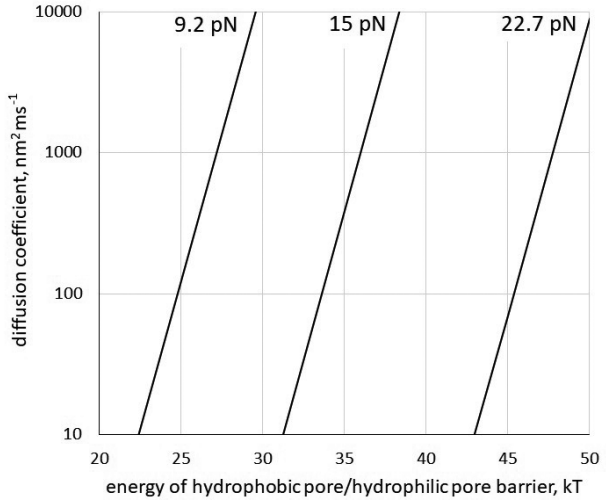

Fig. 4. Dependences of the diffusion coefficient on the magnitude of the energy barrier, under the best approximation of experimental data, for three values of the edge tension, 9.2, 15 and 22.7 pN.

the number n_0 of pores, the stationary distribution $n_{st}(r)$ was determined according to eq. (6). After that, the initial conditions (9) were set.

The diffusion coefficient D was selected so that the solutions of the Smoluchowski equation best approximated the experimental data both for the "membranes" that initially had a pore and for the membranes that initially had no pore. Computational curves are presented in fig. 3. Also, fig. 3 shows the results of the computation by eq. (10) (dotted curves). The curves thus obtained, as well as the computation results for the Smoluchowski equation approximate accurately enough the experimental data, which confirms our assumption concerning stationary Poisson processes describing the emergence and vanishing of hydrophilic pores.

Note that the approximation is not achieved with a unique selection of parameters; rather, under given γ for any energy barrier E_{max} there exists a value of D, connected to E_{max} by an exponential relationship, such that the solutions of the Smoluchowski equation approximate both experimental dependences. The results of computing the dependences $D(E_{max})$ are presented in fig. 4.

4 Discussion

In electroporation, the Smoluchowski equation was used to describe the variation in the membrane current after the application of high voltage. The emergence of considerable membrane current was associated with the occurrence of a large number of hydrophilic pores. The variation in the number of pores and their sizes can be described by the Smoluchowski equation. Under the conditions considered here, at any given moment there is a single pore, or no pores, in the membrane. Nevertheless, we use this equation to describe the kinetics of hydrophilic pores. Because the experiment did not use high voltage, we can obtain the parameters of the Smoluchowski equation that are independent of the membrane voltage: pore edge tension, energy of the hydrophobic pore/hydrophilic pore barrier, coefficient of the diffusion of pores in the radius space, initial distribution density of the number of pores, and the attempt rate density for the lipids in a membrane.

In consideration of the Smoluchowski equation, note that it describes the variation in the pore radius but does

not account for the diffusion of a pore as a whole over the membrane. The diffusion must be accounted for if we mean to cover the interaction between pores. However, in our case (see fig. 2), there were no pores or a single pore on a membrane. For that reason, we neglected the interaction of pores, and accordingly, the diffusion of pores over the membrane.

The estimate of edge tension, 22.7 pN, obtained from (13), is greater than the value, 9.2 ± 0.5 pN, obtained by Chernomordik *et al.* [14] for azolectin membranes in decane, and closer to the estimate, 30.5 ± 1.2 pN, obtained in [17] for membranes of stearoyloleoylphosphatidylcholine with 50 mol% cholesterol. It should be noted that the value 20 pN has been used in a number of studies of electroporation [9,20,31].

This estimate is also higher than the one we gave in an earlier study [4], 9.5 pN, for which we used only eq. (10a) and the literary data from [9], $E_{max} = 50\ kT$ and $\nu = 2 \times 10^{42}\,\mathrm{s^{-1}\,m^{-3}}$. Here, we use only the experimental values r_{min}, τ, Θ, obtained in this work.

The two values of γ —the one calculated by eq. (13) and the one adopted from the literature— differ by a factor of over 2. According to the model described by eqs. (12) and (13), a pore can occur anywhere in the membrane. According to [4], the emergence of pores is caused by the interaction of SDS molecules with an azolectin membrane. Assume that, as a result of the interaction of the SDS molecules with the membrane, a region is formed in the membrane whose structure favors the emergence of pores. Assuming that the absorption of SDS molecules in a membrane results in the emergence of clusters which contain or do not contain SDS molecules, a pore can occur on the boundary of those clusters. From fig. 2(a), it can be suggested that such a region, characterized by an increased productivity, emerged after the switch of the voltage on the membrane. Then the parameter S in eq. (13) is not the area of the entire membrane, but rather is determined by the size of that region. If the value $\gamma = 9.2$ pN is substituted into eq. (13), then the area of the region is $S = 0.09\,\mu\mathrm{m}^2$. Experimental data do not provide for a unique choice in favor of one of the models; therefore, in fig. 4, we present both versions of the linear tension.

In [9] (Freeman *et al.* (1994)), several values of the diffusion coefficient, from 50 to $1100\,\mathrm{nm^2\,ms^{-1}}$, are cited. In this range, with $\gamma = 9.2$ pN, the dependence $D(E_{max})$ corresponding to experimental data, is contained in the interval $E_{max} \in [24, 28]\ kT$. With $\gamma = 22.7$ pN, the dependence $D(E_{max})$ is contained in the interval $E_{max} \in [44, 48]\ kT$. For comparison, fig. 4 shows the dependence $D(E_{max})$ for $\gamma = 15$ pN.

The dependences $\log D(E_{max})$ presented in fig. 4 are linear. By analogy with eq. (12a), a relation with the diffusion coefficient can be written:

$$\frac{1}{\tau} \sim D \exp\left(-\frac{E_{max} - 2\pi\gamma r_{min}}{kT}\right). \qquad (14)$$

Here, the diffusion coefficient D plays the same role as the attempt rate density ν in eq. (12a). Equation (14) has the following physical meaning: the characteristic lifetime of a pore is determined by the diffusion of the pore in the radius space but it grows due to the presence of the energy barrier. On the other hand, as follows from eqs. (10), the characteristic time of the change of "membrane" ensemble state is approximately determined by the pore lifetime $\tau\Theta/\tau + \Theta \approx \tau$, since $\tau \ll \Theta$. In accordance with eq. (14), the dependences $\log D(E_{max})$ in fig. 4 have the required linear shape.

The set of parameters used in electroporation computations [9,20] (Barnett and Weaver (1991); Freeman *et al.* (1994)), $\gamma = 20$ pN, $D = 50\,\mathrm{nm^2\,ms^{-1}}$, with a barrier energy of $E_{max} = 45\ kT$, does not permit approximating our experimental data. According to eq. (14), the relation obtained from experiment describes a plane in the space γ, $\log D$, E_{max}. In that space, the point with coordinates (20.8 pN, $\log(763\,\mathrm{nm^2\,ms^{-1}})$, 44.5 kT) corresponds to the experimental data and is the closest to the point (20 pN, $\log(50\,\mathrm{nm^2\,ms^{-1}})$, 45 kT). The maximum discrepancy between the respective coordinates of those points falls on the diffusion coefficient: 50 and $763\,\mathrm{nm^2\,ms^{-1}}$, $763 \in [50, 1100]\,\mathrm{nm^2\,ms^{-1}}$.

In earlier work, [13], it was claimed that the parameter D might be close to the lateral diffusion coefficient for lipids. For instance, in [32], the coefficient of lateral diffusion for dipalmitoylphosphatidylcholine molecules was measured and found to be $12000\,\mathrm{nm^2\,ms^{-1}}$ at 45 °C. However, in later studies, [1,9], it was suggested that the parameter D is primarily determined by the motion of water in the pore and amounts to $50\,\mathrm{nm^2\,ms^{-1}}$, as stated above. In that case, a variation in the lipid composition of a membrane will have a considerable effect on the magnitude of the parameter D.

The variation range for the fitted parameter r_{phob} was determined from [27], where the method of molecular dynamics was used to compute the dependence of the hydrophobicity of the pore boundary on the pore lumen radius. Note that the boundary conditions can have a great effect on the results of molecular dynamics simulation. Figure 2C in [27] indicates that the characteristic radius at which the hydrophobicity of the pore boundary changes lies within the range $[0.2, 0.4]$ nm. The choice of the parameters, $r_{phob} \in [0.34, 0.39]$ nm and $\rho \in [0.26, 0.42]$ nm permitted the calculation of the barrier magnitude, $E_{max} \in [24, 48]\ kT$ at $r_{min} = 0.84$ nm. Note that in [16], the estimates were cited: $\rho = 1$ nm for the energy barrier 45 kT, the interphase tension between hydrophobic lipid tails and water $\sigma_\infty = 50$ pN, the pore radius $r_{min} \approx 1$ nm.

Equations (12) also permit estimating the attempt rate density of lipids in a membrane. In the literature, the following estimates for this parameter have been proposed: $2 \times 10^{38}\,\mathrm{s^{-1}\,m^{-3}}$ [15], $5 \times 10^{32}\,\mathrm{s^{-1}\,m^{-3}}$ [23], $2 \times 10^{42}\,\mathrm{s^{-1}\,m^{-3}}$ [9]. The authors of [9] complain that "a rigorous basis [for this estimation] is still lacking", while expressing the hope that the huge spread "is not actually significant, because it multiplies a Boltzmann factor". Adopting the values, $E_{max} = 45\ kT$ and $\gamma = 22.7$ pN, we obtain from (12a): $\nu \approx 5.6 \times 10^{33}\,\mathrm{s^{-1}\,m^{-3}}$. This estimate falls within the range of the estimates presented in the literature.

The initial distribution density of the number of pores n_0 derived from experimental data with the use of eq. (8), depends on the barrier energy $n_0 \sim \exp E_{max}/kT$ and therefore varies in the range from 7.4×10^4 to $6.4 \times 10^{13}\,\mathrm{nm}^{-1}$ when the barrier energy E_{max} varies from 24 to 48 kT. In [9], the estimate $n_0 = 1.2 \times 10^{15}\,\mathrm{nm}^{-1}$ is given which we consider overstated, as the authors use it to give the following estimate of the mean number of hydrophilic pores in a lipid membrane with area $1.45\,\mathrm{mm}^2$, under zero voltage: $N_{phil} = 7$. In experiment, under the addition of SDS molecules to the ambient solution, the mean number of hydrophilic pores was considerably less: $N_{phil} = 0.136$.

According to the Smoluchowski equation, the stationary number of pores in a membrane is determined by eq. (6). Meanwhile, under usual conditions, the number of hydrophilic pores in a membrane is small: $N_{phil} \ll 1$. In the theory of electroporation, it is accepted that the application of voltage to a membrane reduces the energy barrier to the formation of hydrophilic pores, and their number increases sharply [1]. Under the phase transition of lipids from liquid crystalline state to gel phase, the restructuring of the lipid bilayer produces a lot of structural defects. This can be accounted for by the introduction of a source of hydrophobic pores to the Smoluchowski equation [12]. The growth of the number of hydrophobic pores increases the probability of the occurrence of hydrophilic pores, and under phase transition, solitary hydrophilic pores occur. What goes on in an azolectin membrane with the addition of SDS molecules —reduction of the energy barrier, or the emergence of additional structural defects, or else the joint action of both factors— is hard to say unequivocally basing on the available experimental data.

The length of the record was about 45 s (see fig. 2). There were no pores either before or after that. This suggests that at least two processes are under way: one results in the development of conditions for the emergence of pores while the other determines the kinetics of the occurrence of pores once the conditions for their emergence have materialized. In this work, a detailed treatment of the second process is carried out. An analysis of the first process can be carried out in the framework of the phase-ordering kinetics model [33]. The theory of phase ordering kinetics is concerned with the dynamical evolution of the system from the initial disordered state to the final equilibrium state. The addition of SDS does not cause a change of the phase state of membrane lipids. However, under phase transitions and with the addition of detergent, the lateral diffusion of lipids becomes restricted. In [34], the effect of SDS on the bilayer structure was investigated by the molecular dynamics methods. It was shown that the introduction of SDS causes a decrease in the bilayer area and increase in the bilayer thickness and lipid tail order, *i.e.* changes similar to those that occur at the liquid-gel phase transition. The emergence in a membrane of clusters with different properties restricts lateral diffusion, which facilitates the occurrence of conductive pores whose lifetime exceeds greatly the molecular temporal scale.

The electroporation estimates with which we compare our results are obtained through the extrapolation of the data obtained under high voltages to the case of zero voltage, for an intact membrane. In our study, the membrane interacts with SDS molecules. Thus, our approach involves a systematic error but it appears to be admissible due to the small number of hydrophilic pores in a membrane.

In this paper, to analyze the conducting pores that appear in BLM, we used the model of Weaver, Krassowska, Chizmadzhev, Smith, Neu, etc. [1,9,26], which is based on solving the Smoluchowski equation. However, electroporation investigations are not limited to this model. In recent articles by Mir's group [35–37], close to Bray's work on the kinetics of phase ordering [33], models of electroporation are considered, in which the authors separate the conductivity that exists only during a pulse, associated with conducting pores, and the permeability in the period between pulses.

In these studies of cell electroporation, the authors distinguish between short-term (effect duration: several microseconds) increase in electrical conductivity (poration) and long-term (within several minutes) increase in membrane permeability (permealization) for various molecules and numerically simulate these effects [35]. This explains the processes of the internalization of extracellular molecules into the cells during electroporation.

In [36], the authors investigate the effect of the conductivity of the buffer on the electrical permeability and cell death. They concluded that the voltage across the cell membrane can be sufficient to cause reversible electrical permeability or cell death, only in a highly conductive environment. They also point to the important role of lateral diffusion of lipid defects.

In [37], the possible chemical consequences of the membrane electropulsation are investigated on giant unilamellar vesicles. The authors conclude that the electropulsation induces the oxidation of unsaturated phospholipids by the reactive oxygen species already presented in the solution before pulsing.

Thus, in these works, the long-term effects of electric pulses on membrane permeability, conductivity and chemical properties of cells or tissues are investigated. According to the models the most changes in physical or chemical properties can be initiated by the formation of pores (defects) in the lipid part of membranes under the action of an electric field, as well as by diffusion of lipid defects in the membrane plane.

5 Conclusion

Thus, on the basis of experimental data with the use of the Smoluchowski equation, we obtained estimates of the parameters of hydrophilic pores in a membrane: the pore edge tension, the energy of the hydrophobic pore/hydrophilic pore barrier, the diffusion coefficient for pores in the radius space, the initial distribution density of the number of pores. An estimate of the attempt rate density of the lipids in a membrane was also obtained. The obtained estimates (except for the initial distribution density of the number of pores) fall within the ranges of the data used in electroporation studies.

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Author contribution statement

All the authors contributed to the study conception and design. Experiment: A.A. Anosov, E.A. Korepanova; calculation: A.A. Anosov, E.Yu. Smirnova, E.D. Ryleeva, I.A. Gligonov, A.A. Sharakshane; writing the paper: A.A. Anosov, E.Yu. Smirnova, E.A. Korepanova, A.A. Sharakshane.

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