Flux theory for Poisson distributed pores with Gaussian permeability

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ABSTRACT
The mean of the solute flux through membrane pores depends on the random distribution and permeability of the pores. Mathematical models including such randomness factors make it possible to obtain statistical parameters for pore characterization. Here, assuming that pores follow a Poisson distribution in the lipid phase and that their permeabilities follow a Gaussian distribution, a mathematical model for solute dynamics is obtained by applying a general result from a previous work regarding any number of different kinds of randomly distributed pores.

The new proposed theory is studied using experimental parameters obtained elsewhere, and a method for finding the mean single pore flux rate from liposome flux assays is suggested. This method is useful for pores without requiring studies by patch-clamp in single cells or single-channel recordings. However, it does not apply in the case of ion-selective channels, in which a more complex flux law combining the concentration and electrical gradient is required.

KEYWORDS
channel; distribution; flux; Gauss; modeling; parameter; permeability; Poisson; pore; random

Introduction
The study of the kinetic parameters of membrane channels is critical to understanding their structure-function relationship and physiological role. In such studies, the transported solute is measured, either at equilibrium or during the dynamic phase, employing one or more membranous compartments. A theoretical analysis for the design of the experiments and interpretation of their results should consider random events associated with the distribution and permeability of channels.

The mechanism of channel distribution on the lipid phase may consist of the random insertion of either preformed channels or channel-forming proteins. In both cases, it is known that membrane channels, even pores, can follow a Poisson distribution on cell membranes and vesicles. This situation occurs when channels are randomly distributed on the lipid phase, independent of the insertion of other channels, as described for liposomes, plasmatic membranes and erythrocyte ghosts (hereinafter, only vesicles will generally be mentioned, but the other membranous compartments will be represented as appropriate).1–7

Moreover, differences in total permeability may be due not only to differences in the number of channels per vesicle but also to differences in the Gaussian distributed intrinsic channel permeabilities. These effects, occurring simultaneously on a vesicle, are described by a random rate of transport. This case has been described for perforin pores in erythrocyte ghosts.7,8

Assuming that Poisson and Gaussian distributions are applied as mentioned above, the purpose of this article is to theoretically examine the global flux through pores, given that the flux follows Fick’s first law. This study would contribute to designing simple experiments to determine parameters that characterize pore-mediated transport without requiring studies by patch-clamp in single cells or single-channel recordings. In fact, a method for finding the mean single pore influx rate from liposome influx assays is suggested.

Theoretical framework and results
This theoretical approach assumes a constant external solute concentration and a similar spherical size for all the vesicles (See Appendix for the relationship between these assumptions and the range of lipid parameters). Vesicles employed in an experiment can be different sizes, but in many cases, the technique allows the size distribution to be very narrow around the mean. As a reference, in the case of vesicle size determination using dynamic light scattering, large

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unilamellar lipid vesicles have been found to show 6% deviation. Moreover, by assuming that vesicles are of equal size, the fraction of empty vesicles can be calculated as a function of the protein/liposome ratio. Indeed, the assumption that all vesicles have approximately average size and shape is needed to explain the Poisson distribution of channels or pores on vesicles because in that case, the probability of channel insertion in the lipid phase of a vesicle does not depend on the selected vesicle.

**Calculation of the transport rate in a vesicle**

Let there be a system formed by \( C \) pores randomly distributed over \( N \) vesicles of identical volume. We assume that the number \( n \) of pores per vesicle follows a Poisson distribution:

\[
P_n = \frac{e^{-\alpha} \alpha^n}{n!}
\]

in which \( \alpha \) indicates the mean of \( n \) (i.e., \( \alpha = \frac{C}{N} \)) and the single pore flux rate \( k_i \) has the mean \( \bar{k} \) and standard deviation \( \sigma \) following a Gaussian distribution:

\[
G(k_i) = \frac{1}{\sqrt{2\pi \sigma}} e^{-\frac{(k_i - \bar{k})^2}{2\sigma^2}}
\]

Therefore, given a standard normally distributed random number \( rnd_i \), \( k_i \) can be randomly generated according to

\[
k_i = \bar{k} + \sigma \cdot rnd_i
\]

Given a vesicle with \( n \) pores, let \( X_n \) and \( X_{eq} \) be the intravesicular and the extravesicular solute concentrations, respectively. We assume Fick’s first law:

\[
\frac{dX_n}{dt} = k (X_{eq} - X_n)
\]

with

\[
k \equiv \sum_{i=1}^{n} k_i
\]

and \( X_{eq} \) constant.

For the initial condition \( X_n(t=0) = X_{n0} \), the solution of Eq. 4 is

\[
X_n = X_{eq} - (X_{eq} - X_{n0}) e^{-tk}
\]

The rate of transport \( k \) is a new Gaussian distributed random variable, with mean \( n \bar{k} \), standard deviation \( n^2 \sigma \) and the probability density of \( k \) given by

\[
G_n(k) = \frac{1}{\sqrt{2\pi n \sigma}} e^{-\frac{(k - n\bar{k})^2}{2n^2\sigma^2}} (n \neq 0)
\]

These conditions imply that, given a standard normally distributed random number \( rnd \), \( k \) can be generated randomly by

\[
k = n\bar{k} + n^2 \sigma \cdot rnd
\]

Thus, in a vesicle with \( n \) Poisson distributed pores (Eq. 1), the probability density of a Gaussian distributed \( k \) (Eq. 7) is

\[
D(k) = \sum_{n=1}^{\infty} P_n G_n(k)
\]

Assuming known parameters describing both the Gaussian and Poisson distributions, the system composed by \( C \) pores and \( N \) vesicles is simulated according to the following algorithm:

1. \( N \) vesicles without pores are defined (i.e., \( n = 0 \), therefore, with transport rate \( k = 0 \)).
2. A vesicle is chosen randomly, and its number of pores is increased by 1.
3. For the chosen vesicle, a random value of \( k_i \) is obtained from Eq 3 and added to \( k \), thus generating a new value of \( k \).
4. The sequence of steps 2 and 3 is repeated \( C-1 \) times.

Using computer simulation data, Figure 1 shows a histogram with the relative frequency \( k \) (i.e., the frequency of \( k \) divided by the total number of pores) equal to the probability of obtaining any value that belongs to the corresponding interval of the histogram.

In addition, Figure 1 shows the results of calculating the integral of \( D(k) \) (Eq. 9) through the interval \([k - \delta, k + \delta]\) :

\[
I(k) = \int_{k-\delta}^{k+\delta} D(x) \, dx
\]

(like a histogram but continuously varying the position \( k \) of the center of the interval).
We can see a similarity between the proposed analytical theory and the computer simulated experiment. In addition to providing an internal control for the calculation, the purpose of this comparison is to show how Eq. 10 (a continuous theoretical model) could eventually be useful to model an experimental histogram of $k$ (a set of discrete data) with the appropriate parameters for the Gaussian and Poisson distributions.

Modeling the mean solute concentration and its influx

Below, a model function is obtained to average the solute concentration into vesicle and solute influx.

In a previous work, different kinds of pores were assumed to be distributed over the vesicles according to Poisson distributions. Let $R$ be different kinds of pores, each one named the $i$-th kind of pore ($i = 1, 2, ..., R$), with a single pore flux rate $k_i$, $n_i$ repeated pores in a vesicle and a mean of $\alpha_i$ pores per vesicle. We again assume Fick's first law for the solute flux (Eq. 4 and therefore Eq. 6), but here, instead of the formulation in Eq. 5, $k$ is given by

$$k = \sum_{i=1}^{R} n_i k_i$$

(11)

also considering that $n = \sum_{i=1}^{R} n_i$.

If $n_i$ is Poisson distributed with probability

$$P_{n_i} = \frac{e^{-\alpha_i} \alpha_i^{n_i}}{n_i!}$$

(12)

then it has been demonstrated that the mean of $X$ due to all realizations of pores over the vesicles is given by

$$\bar{X} = X_{eq} - (X_{eq} - X_0) e^{\alpha (e^{-\alpha k_t} - 1)}$$

(13)

such that

$$\langle e^{-\alpha k_t} \rangle = \sum_{i=1}^{R} e^{-k_i \alpha_i} \frac{\alpha_i}{\alpha}$$

(14)
with

$$\alpha \equiv \sum_{i=1}^{R} \alpha_i$$  \hspace{1cm} (15)

where $\alpha$ is equal to the mean of the total number of pores in the vesicles.\(^{11}\)

That is, $(e^{-k_i \, t})$ is the mean of $e^{-k_i \, t}$ weighted by $\alpha_i$, i.e., the probability of the occurrence of the $i$-th kind of pore on a vesicle.

What are the consequences if there is only one type of pore, but it has a Gaussian distributed $k_i$? In that case, we can use the above result as follows: assuming infinite types of pores $(R \to \infty)$, each with a single pore flux rate $k_i$ according to an infinitesimal probability $G(k) \, dk$. The last term is continuous and replaces $\alpha_i$ in Eq. 14, such that

$$\int_0^{+ \infty} e^{-k_i \, t} G(k_i) \, dk_i$$

is used instead of $\sum_{i=1}^{R} e^{-k_i \, t} \alpha_i$, giving

$$\langle e^{-k_i \, t} \rangle = \frac{1}{2} e^{\frac{k^2}{\sigma^2} - \bar{k} \, t} \left( \text{erf} \left( \frac{\bar{k} - \sigma^2 \, t}{\sqrt{2} \, \sigma} \right) + 1 \right)$$ \hspace{1cm} (16)

with the error function $\text{erf}(x) = \frac{2}{\sqrt{\pi}} \int_0^x e^{-u^2} \, du$

Eq. 16 is true because (from the mentioned continuous case for Eq. 14)

$$\langle e^{-k_i \, t} \rangle = \int_0^{+ \infty} e^{-k_i \, t} G(k_i) \, dk_i$$

$$= \frac{e^{\frac{k^2}{\sigma^2} - \bar{k} \, t}}{\sqrt{2\pi\sigma}} \int_0^{+ \infty} \frac{1}{\sqrt{2\sigma}} e^{-\frac{|k_i - \bar{k} - \sigma^2 \, t|^2}{2\sigma^2}} \, dk_i$$

$$= \frac{e^{\frac{k^2}{\sigma^2} - \bar{k} \, t}}{\sqrt{2\pi\sigma}} \left[ \int_0^{+ \infty} \frac{1}{\sqrt{2\sigma}} e^{-\frac{|k_i - \bar{k} - \sigma^2 \, t|^2}{2\sigma^2}} \, dk_i + \frac{1}{\sqrt{2\pi\sigma}} \int_{-\bar{k} - \sigma^2 \, t}^{+ \infty} e^{-\frac{|k_i - \bar{k} - \sigma^2 \, t|^2}{2\sigma^2}} \, dk_i \right]$$ \hspace{1cm} (17)

The change in variable $u = \frac{\bar{k} - \sigma^2 \, t - \bar{k}}{\sqrt{2} \, \sigma}$ is applied in the penultimate integral, while the last integral equals 1/2 (half of the entire area under the Gaussian curve). Thus,

$$\langle e^{-k_i \, t} \rangle = \frac{e^{\frac{k^2}{\sigma^2} - \bar{k} \, t}}{\sqrt{2\pi\sigma}} \left( \frac{1}{\sqrt{\pi}} \int_0^{+ \infty} e^{-\frac{u^2}{2}} \, du + \frac{1}{2} \right)$$

$$= \frac{1}{2} e^{\frac{k^2}{\sigma^2} - \bar{k} \, t} \left( \text{erf} \left( \frac{\sqrt{2} \, \sigma \, t}{\sqrt{2} \, \sigma} \right) + 1 \right)$$ \hspace{1cm} (18)

$$X = X_{eq} - (X_{eq} - X_0) \times e^{\frac{\sqrt{2} \, \sigma \, t}{\sqrt{2} \, \sigma} \left[ \text{erf} \left( \frac{\sqrt{2} \, \sigma \, t}{\sqrt{2} \, \sigma} \right) + 1 \right]}$$ \hspace{1cm} (19)

A simulation experiment was performed using a random distribution of pores in the vesicles, in addition to the randomly chosen $k_i$ by the Gaussian distribution, but only positive values were considered. Then, the intravesicular concentration of the solute $S$ was calculated from Eq. 3, 5 (the discrete case) and 6, and the mean of that concentration must be similar to the analytical calculation given by Eq. 19. The agreement between these 2 modes of calculation is shown in Figure 2A.

An application of the theory to determine the mean single pore flux rate

The derivative of Eq. 19 and the subsequent reordering at $t = 0$, imply

$$\frac{1}{(X_{eq} - X_0)} \frac{dX}{dt} \bigg|_{t=0} = \alpha \bar{k} \left[ \frac{\sigma \, e^{-\frac{k}{2\sigma^2}}}{2^\frac{k}{\sigma^2} \bar{k}^2} + \frac{1}{2} \left( \text{erf} \left( \frac{\bar{k}}{\sqrt{2} \, \sigma} \right) + 1 \right) \right]$$

$$\times e^{\frac{\sqrt{2} \, \sigma^2 \, t}{\sqrt{2} \, \sigma}}$$ \hspace{1cm} (20)

For the parameter space given by the intervals $0.1 \leq \alpha \leq 5$ and $2 \leq \frac{\bar{k}}{\sigma} \leq 10$, the multiplying factor of $\alpha \bar{k}$ in Eq. 20 is between 0.9148 and 1.0177. Then, the following approximation is considered:

$$\frac{1}{(X_{eq} - X_0)} \frac{dX}{dt} \bigg|_{t=0} \approx \alpha \bar{k}$$ \hspace{1cm} (21)

On the other hand, at equilibrium, $X$ equals

$$X_{eq} \equiv \lim_{t \to \infty} X = X_{eq} (1 - e^{-\alpha}) + X_0 e^{-\alpha}$$ \hspace{1cm} (22)

In this case of unselective transport, the mean steady-state concentration inside the vesicles
Eq. 22 and given that \( X_{\text{eq}} \neq X_0 \), only if \( \alpha \) tends to infinity (i.e., all vesicles having channels) will the 2 concentration parameters tend to be equal: \( \lim_{\alpha \to \infty} (X_{\text{eq}}) = X_{\text{eq}} \).

From Eq. 22, \( \alpha \) can be obtained from the experimental values for \( X_{\text{eq}} \), \( X_{\text{eq}} \), and \( X_0 \): 

\[
\alpha = \ln \left( \frac{X_{\text{eq}} - X_0}{X_{\text{eq}} - X_{\text{eq}}} \right).
\]

We assume different values of \( \alpha \) (because different Channel protein/Lipid ratios can be used in experiments), \( X_0 = 0 \), and a sufficiently small value of \( \Delta t \) at the initial influx of solute, such that \( \frac{\Delta X}{\Delta t} \bigg|_{t=0} \approx \frac{\Delta X}{\Delta t} \).

Then, also using Eq. 21, a linear plot \( \frac{1}{X_{\text{eq}}} \frac{\Delta X}{\Delta t} \) versus \( \alpha \) will have slope \( \tilde{k} \) (Fig. 2).

**Discussion**

Studies on the flux of solute through pores considering statistical fluctuations in permeability makes it possible to determine the characteristic parameters of transport by measurements during the dynamic. Such permeability fluctuations may result from a Poisson model for the distribution of pores on the lipid phase or from a Gaussian model for pore permeability. Some inexpensive techniques can obtain values only from a macroscopic sample of a mixture of vesicles. Other techniques allow repeated measurements of either fluxes or solute concentrations in a single membranous compartment, such as a single cell, vesicle or membrane patch. Specifically, Poisson distributed channels have been observed even in some patch experiments. Here, a mathematical framework has been derived for the determination of the single-pore transport rate from vesicle flux assays, useful in cases that cannot be studied by patch-clamp in single cells or even single-channel recordings.

The work of Peters et al. on perforin pore formation addressed the statistical distributions of pores in experiments at equilibrium and in a transient phase. Through fluorescence microscopic single channel recording measuring the flux of a fluorescent probe (Lucifer Yellow) through perforin pores on the membrane of resealed human erythrocyte ghosts, they determined a Poisson distribution of the “rate of transport” (\( k \)) over the vesicle and a Gaussian distribution for the pore permeability (later, the size distribution of such pores, but formed on large unilamellar vesicles, was obtained from a micrograph.)
This distribution resembled the distribution of single pore conductances\(^5\)). Their approach used 4 parameters, \(\alpha, \bar{k}, \sigma, \text{ and } \sigma_0\), such that

\[
k = n\bar{k} + \sigma_n \cdot \text{rnd},
\]

with \(\sigma_n\) the standard deviation

\[
\sigma_n = n\sigma + \sigma_0
\]

However, this notation differs from the notation used in the original article,\(^7\) in which \(\bar{\mu}\) and \(\sigma_1\) are used instead of \(\bar{k}\) and \(\sigma\), respectively. In that article, it was erroneously considered that the theoretical standard deviation is equal to \(n\sigma\), but the term \(\sigma_0\) in Eq. 25 was justified “to account also for instrumental parameters such as the reproducibility of the bleaching and measuring process.”\(^7\) A similar analysis was performed for single nuclear pores in optical single transporter recording experiments.\(^13\) In this work, although the published values for \(\bar{k}\) and \(\sigma\) have been preserved,\(^7\) the parameter \(\sigma_0\) was not necessary because a correction has been applied to the exponent of the theoretical standard deviation. The following statistical result is well known: the sum of \(n\) Gaussian variables, having mean \(\bar{k}\) and standard deviation \(\sigma\), gives a new Gaussian variable distribution with mean \(n\bar{k}\) and standard deviation \(n\sigma\) (not \(n\sigma\), as mentioned above). Thus, in our calculations, the necessary correction has been included in Eq. 8. Finally, the result of the simulation, as shown in Figure 1, is similar to the experimental results shown in Figure 2A of Peters et al.\(^7\) This similarity shows the correct performance of the algorithm used here for simulation of the experimental system of pores and vesicles. For statistical validation, we employed a large number of vesicles compared with the original experiment.

Moreover, a previous theoretical overall result\(^11\) is applied here, considering that pores with a Gaussian distributed "single pore flux rate" \((k_i)\) can be mathematically represented by infinite pores. The resulting expression for the intravesicular solute concentration, obtained by combining Poisson and Gaussian distributions, appears in the Eq. 19 and agrees with the computer simulation (Fig. 2A).

Based on these results, a protocol has been proposed to obtain the "mean of \(k_i\)" \((\bar{k})\) from either macroscopic (i.e., a sample of mixed vesicles) or microscopic (i.e., the mean of single vesicle results) solute measurements. Two factors increase the error in estimating the \(\bar{k}\): the finite size of the time interval for the calculation of the initial flux; and the approximation used for the result of, Eq. 21 regarding the linearity of the solute flux with respect to \(\alpha\bar{k}\) (a reasonable approximation for the studied range of parameter space, as shown in the results). Although \(\sigma\) could be determined from Eq. 19, fitting the parameters to experimental data, it is interesting that, in the studied parameter space, the determination of \(\bar{k}\) does not depend on the value of \(\sigma\). Finally, Eq. 21 is similar to the case in which the experiment has only a single kind of pore, randomly distributed,\(^11\) but with only one value of \(k_i\), equal to \(\bar{k}\).

To study the mentioned flux of solute through pores, the recorded signal during the transport kinetics experiment must be due to the transport of solute and not to the formation of the pore in the membrane. Fortunately, some cases of kinetics of membrane-peptide association show a transient rapid phase, even with practically instantaneous diffusion-limited times. However, there are cases such as the synthetic, amphipathic peptide GALA, whose integration over large unilamellar phosphatidylcholine vesicles is approximately 2 minutes.\(^16\) In such cases, to prevent such a problem, an environmental variable such as pH can quickly modulate channel activity, as in the case of GALA, wherein the pore transporter activity is not registered at pH 7.5 but at pH 5.0 in a transition period of less than one second. In other cases, a second solute can open a channel almost instantaneously, such as in Ca\(^{2+}\)-dependent K\(^+\) channels, in which there is a threshold for Ca\(^{2+}\) below which the K\(^+\) channels remain silent.\(^17\) In other experimental designs, a liposome with a previously integrated channel on its surface can be added to a solution containing the solute: e.g., calcium channels reconstituted in phospholipid vesicles can be added to a solution of \(^{45}\)Ca\(^{2+}\), and the uptake of this ion across the vesicular membrane can be measured.\(^18\) In any case, the appropriate protocol depends on the nature of the system.

I believe this proposed theoretical study may be particularly useful in experiments for pore characterization, employing simple techniques capable of
detecting the mean of intravesicular solute concentration. However, there are cases in which the channel distributions are not of Poisson type.\textsuperscript{14,15} Future theoretical research should consider alternative functions for pore distributions and single pore flux rates. Moreover, for the case of ion-selective channels, instead of Fick’s first law, a more complex flux law must be applied, such as the Nernst-Planck equation, which combines concentration and electrical gradient.\textsuperscript{19}

**Disclosure of potential conflicts of interest**

No potential conflicts of interest were disclosed.

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**Appendix**

Range of lipid parameters for the proposed experiments on the influx of solute into a vesicle.

Let there be a total volume $V$ containing $L$ lipid molecules forming vesicles in aqueous solution, each vesicle of volume $v$ and consisting of $L_v$ lipid
molecules. We define $V_{ex}$ and $V_{tot\ in}$ as the external volume and total internal volume, respectively; $C_{ext\ 1}$ and $C_{ext\ 2}$ as the external solute concentration at times 1 and 2, respectively; and $C_{int\ 1}$ and $C_{int\ 2}$ as the internal solute concentration at times 1 and 2. According to the conservation of solute:

$$V_{ex}C_{ext\ 1} + V_{tot\ in}C_{int\ 1} = V_{ex}C_{ext\ 2} + V_{tot\ in}C_{int\ 2}$$

(24)

and assuming that the volume of the lipid phase is negligible:

$$V = V_{tot\ in} + V_{tot\ in}$$

(25)

and

$$V_{tot\ in} = \nu \frac{L}{L_v}.$$  

(26)

($\frac{L_v}{\nu}$ is the number $N$ of vesicles)

From Eqs. 24–26 and given that $C_{int\ 1} \approx 0$ (a condition in the previously proposed experiment on the initial influxes of solute into vesicles),

$$C_{ext\ 2} = C_{ext\ 1} \left[ 1 - \frac{C_{int\ 2}}{C_{ext\ 1}} \left( \frac{L_v}{\nu} \frac{V}{L} - 1 \right) \right]$$

(27)

From Eq. 24–26, and given that $C_{ext\ 1} > C_{ext\ 2}$

$$C_{ext\ 1} > C_{ext\ 2} \geq C_{ext\ 1} \left( 1 - \frac{L_v}{\nu} \frac{V}{L} - 1 \right)$$

(28)

Typically, each unilamellar vesicle could consist of $L_v = 127,000$ lipid molecules, with a mean diameter $d = 109$ nm. Then, assuming spherical vesicles, $\nu = \frac{4}{3}\pi\left(\frac{d}{2}\right)^3$, and therefore

$$\frac{L_v}{\nu} = 311, 1 \mu\text{mol/ml}$$

(29)

On the other hand, we assume the total lipid concentration (sufficient for most experiments) is as follows:

$$\frac{L}{V} \leq 10 \mu\text{mol/ml}$$

(30)

By substituting Eq. 29 and 30 into Eq. 28, $C_{ext\ 1} > C_{ext\ 2} \geq 0.97 C_{ext\ 1}$, we can then consider

$$C_{ext\ 1} \approx C_{ext\ 2}.$$  

Thus, the external solute concentration is approximately constant for the set of experimental conditions given by Eq. 29 and 30.