

Mathematical Biology

Research Trends

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Chapter 11

**THE BEHAVIOUR OF THE LIPID VESICLE
UNDER OSMOTIC STRESS**

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Abstract

There is a great interest for a mechanistic understanding of molecular transport across biological and reconstituted membranes due to its potential applications to the development of new methodologies in medical biotechnology, such as gene therapy and drug delivery. In the first part of this paper, we present the behavior of the liposomes under osmotic stress. Because of the mechanical tension induced by osmotic flow, the liposomes expand, triggering transient lipidic pores that fluctuate at the nanoscopic level until their death. We report here that this is a periodic process. Such a liposome, also called a pulsatory liposome, is characterized by the number of successive pores, the time interval between two successive pores, and the amount of exchanged material through a single transmembrane pore. The diffusion of water through the liposomal membrane is analyzed in detail. In the second part of this paper, we develop a theoretical model for analyzing experimental data, facilitating information about the diffusion and exchange through spherical interfaces. The effects of experimental parameters, including the bilayer stiffness and the viscosity of the internal fluid, are analyzed and discussed as well.

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Introduction

The transport of small molecules and macromolecules through transient transmembrane pores is a fundamental and ubiquitous process in modern cell biology. The large interest for the exploration of pores by experimental, computational and theoretical approaches is dramatically growing not only for a better understanding of molecular traffic across biomembranes, but also potential applications in medical biotechnology. In general, the transmembrane pores are either proteinaceous or lipidic. Here, our approach is devoted only for those pores that transiently appear in the lipid bilayer, and with their wall formed by phospholipids. Some pores can appear due to structural and dynamic properties of the lipid bilayer (Popescu et al., 1991; Popescu and Victor, 1991; Popescu and Rucareanu, 1992; Movileanu and Popescu, 1995, 1996, 1998; Movileanu et al., 1997, 1998; Popescu et al., 1997). These transient transmembrane pores have a stochastic nature (Popescu et al., 2003; Movileanu and Popescu, 2004; Movileanu et al., 2006). Recently, in vesicles stretched by induced tension, a single pore of several micrometers in diameter was observed (Karatekin et al., 2003). However, in the same vesicle, a few tens of transmembrane pores can appear successively. In this paper, we show a theoretical approach for demonstrating that a successive formation of pores can take place in a liposomal membrane.

There are two very interesting biotechnological applications that require the increase of membrane permeability: gene therapy and targeted drug delivery. In the first one, the transport of DNA fragments through cellular and nuclear membranes is requested (Varma and Somia, 1997). The second application uses drug molecules encapsulated in lipid vesicles, which have to be transported to a targeted place (Lasic and Needham, 1995; Zasadzinski, 1997). In the second application, the lipid vesicle has to release the drug molecules in a well-controlled and accurate fashion. The appearance of transient transmembrane pores may be stimulated using chemical and physical methods (Bar-Ziv et al., 1998; Saitoh et al., 1998; Bernard et al., 2000; Fournier et al., 2003). The chemical methods are based on the addition of an external agent (Debregeas et al., 1995; Dietrich et al., 1997; Dietrich et al., 1998). Using physical methods, including electroporation (Weaver and Chizmadzhev, 1996), osmotic shock (Dvolaitzky et al., 1993), temperature jump (Lasic, 1993), and adhesion on porous or decorated substrate (Guedeau-Boudeville et al., 1995), one can produce a stretch of the vesicle membrane, which eventually relaxes, forming transient pores. These pores may reach diameters up to 10 μm (Sandre et al., 1999).

The appearance of transient pores through the cellular membrane, which are caused by mechanical tension, is a possibility for the intracellular material to be transported outside the cell. In article, we present a formalism for analyzing the successively formed transient pores induced in a vesicle by osmotic stress, and the time interval between two successive pores. In the first part, we describe the transient pore dynamics. Then, the solute concentration inside the vesicle, depending on the time elapsed, was calculated. An interesting application in medicine is discussed: transient pores in liposomes could be used for compensation of neurotransmitter deficiency in the synaptic cleft. This article is consisted of four parts: 1) the phenomenological bases of pulsatory liposomes, 2) the internal chemical changes, 3) the pore dynamics and 4) the numerical results.

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Phenomenological Bases of a Pulsatory Liposome

Let us consider a lipid vesicle filled with aqueous solution containing an impermeable solute. The vesicle is inserted into an aqueous hypotonic medium. Initially, the vesicle membrane is smooth and unstretched. Osmotic pressure created by the gradient of solute concentration determines an influx of water molecules through liposomal membrane. The supplementary water entered inside the liposome has two consequences: the dilution of the internal solution and the swelling of the liposome. Also, the surface tension increases in the same time with liposomal expansion. The surface tension increases the pressure inside the cell. Under these experimental conditions, either the liposomal membrane may be ruptured or one pore may appear through its lipid bilayer. If the swelling process is slow enough, the liposome increases up to a critical size, in which a transient transmembrane pore forms. This event is followed by two simultaneous processes: the pore dynamics and the leak out of the internal material of the vesicle, due to Laplace pressure. The pore dynamics consists of two phases: 1) the pore diameter increases up to the maximum radius, r_m , and 2) the pore diameter decreases until the closure of the pore (Fig. 1). Both phenomena, the increase in the pore diameter and the leakage of the internal liquid, determine the membrane relaxation due to the reduction in the mechanical tension of the membrane. As a matter of fact, the pore dynamics is driven by the difference between the membrane tension and line tension (Fig. 2).

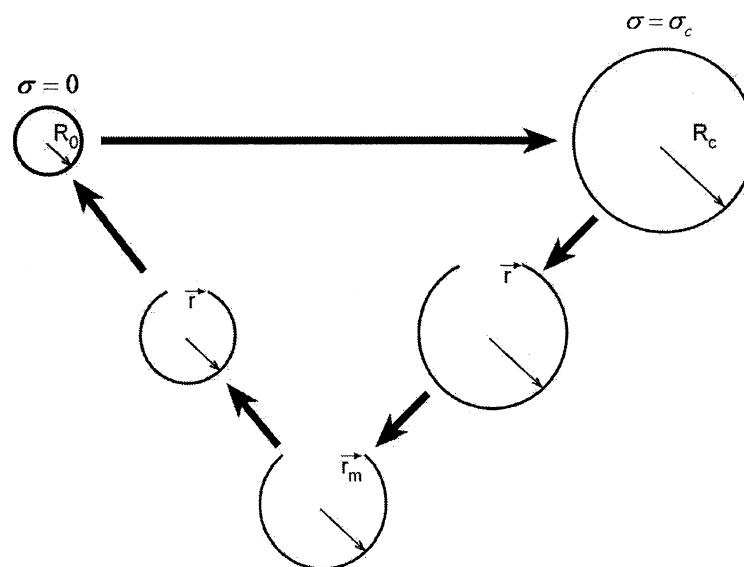


Figure 1. The evolution of a pulsatory liposome during a single cycle.

The membrane tension decreases until it becomes equal to the line tension of the membrane edge. The internal liquid continues to leak outside the liposome, even after the line tension equals to the membrane tension. From the time when the line tension equals to the membrane tension the second part of the pore dynamics starts, and so the pore radius reduces until the the closure of the pore. Therefore, the liposomes is in its initial size. We can envision that the dynamics of the vesicle described above can start over. This cyclic process ceases

when the osmotic gradient becomes smaller than a critical value, which will be discussed below.

The Change of the Chemical Composition of Internal Solution

Let us suppose at the beginning of its activity the lipid vesicle contains solute and water, with the molar concentrations c_{s0} , c_{w0} , respectively. Due to the influx of water, the vesicle swells itself and its radius increases from R_0 (the smooth and relaxed state) to R_c (just before the appearance of the transient pore) (Fig. 1). The quantity of water entered a lipid vesicle in each cycle is given by the following expression:

$$N^+ = \frac{4\pi(R_c^3 - R_0^3)}{3V_{\mu w}} = \frac{4\pi R_c^3}{3V_{\mu w}} \left(1 - \frac{R_0^3}{R_c^3}\right) = N(1 - f) \quad (1)$$

Where the molar volume of water is noted as $V_{\mu w}$. In the formula presented above, we introduced the following notations:

$$N = \frac{V_c}{V_{\mu w}} = \frac{4\pi R_c^3}{3V_{\mu w}} \quad (2)$$

which is the number of moles of water that would fill the stretched vesicle just before the appearance of the pore, if only water would be present.

Here,

$$\frac{1}{f} = \frac{V_c}{V_0} = \frac{R_c^3}{R_0^3} \quad (3)$$

is the reversal of swelling ratio, that is the ratio between the vesicle volumes in the stretched state (V_c), just before the pore formation, and in the relaxed state (V_0).

The Internal Liquid Composition after Each Cycle

So far, we noticed that in each cycle the liposome supports a swelling process followed by a relaxation process. Let us analyze the first cycle in both phases.

The swelling stage

At the beginning of the first cycle, the lipid vesicle contains $N_{s1} = c_{s0}V_0$ moles of solute and $N_{w1} = c_{w0}V_0$ moles of water. At the end of the swelling stage, just before the opening of

the pore, the same amount of solute is present in the lipid vesicle, but this contains a larger amount of water:

$$N_{w1} = N_{w1} + N^+ = c_{w0}V_0 + N(1-f) \quad (4)$$

The new molar concentrations at the end of the first cycle are given by the following expressions:

$$c_{s1} = \frac{N_{s1}}{V_c} = \frac{c_{s0}V_0}{V_c} = fc_{s0} \quad (5)$$

$$c_{w1} = \frac{N_{w1}}{V_c} = \frac{(c_{w0}V_0 + N(1-f))}{V_c} = fc_{w0} + \frac{1-f}{V_{\mu w}} \quad (6)$$

Relaxation stage

After the opening of the pore, the pore radius increases up to a maximum value, then it decreases, and eventually the pore closes. During this pore dynamics, an amount of internal liquid leaks out. At the beginning of the second cycle, which is the same with the end of the first cycle and, the lipid vesicle is in a relaxed state. In this state, the lipid vesicle contains the following amounts of solute and water, measured in moles:

$$N_{s2} = V_0c_{s1} = fV_0c_{s0} \quad (7)$$

$$N_{w2} = V_0c_{w1} = fV_0c_{w0} + \frac{V_0(1-f)}{V_{\mu w}} \quad (8)$$

Making the same reasoning as for the first cycle, one can find the following recurrent formula for characterizing the internal composition of the lipid vesicle at the end of the n^{th} cycle:

$$N_{sn} = f^n V_0 c_{s0}; N_{wn} = f^n V_0 c_{w0} + \frac{V_0(1-f^n)}{V_{\mu w}} \quad (9)$$

$$c_{sn} = f^n c_{s0}; c_{wn} = f^n c_{w0} + \frac{1-f^n}{V_{\mu w}} \quad (10)$$

The n cycles liposome programming

The driving force of a pulsatory liposome is generated by the osmotic gradient through the lipid bilayer. The internal concentration of the solute decreases along a cycle and with the cycle rank in sequence. Therefore, the osmotic pressure decreases as well. The lipid vesicle will swell up to its critical radius, only if the osmotic pressure at the end of the cycle is greater

than the excess Laplace pressure. Starting with this condition, we can programme a pulsatory liposome to have n cycles in its life activity by the following condition:

$$\sigma_0 \left(\frac{1}{R-h} + \frac{1}{R+h} \right) \leq \mathfrak{R}T (c_{sn}^{in} + c_{sn}^{out}) \quad (11)$$

where, c_{sn}^{in} and c_{sn}^{out} are the solute concentrations at the end of the swelling stage of the n -th cycle, inside and outside the liposome. Considering that at the beginning of the cycle, the external concentration of the solute is equal to zero, and the composition of the external medium is not affected by the vesicle running, we can take $c_{sn}^{out} = 0$. Taking into account that c_{sn}^{in} is equal to c_{son} , the condition mentioned in equation (11) becomes:

$$2\sigma_0 R / (R^2 - h^2) \leq \mathfrak{R}T f^n c_{son} \quad (12)$$

where R is the radius of the sphere between the two monolayers of the liposomal bilayer, σ_0 is the surface tension of the monolayer at the end of the cycle, and $2h$ is the hydrophobic thickness of the bilayer. \mathfrak{R} is the universal gas constant, and T is the absolute temperature. For the symmetry of the above formula, we take $R = R_c + h$. Therefore, the initial concentration of solute inside the liposome, c_{son} is equal to

$$c_{son} = \frac{2\sigma_0 R}{\mathfrak{R}T f^n (R^2 - h^2)} \quad (13)$$

If the initial concentration is known, then we can calculate the number of cycles of liposome activity from formula (12).

The solute content of the pulse

We have named the quantity of internal material leaked out in a cycle as material pulse, or simply pulse. Achieving the difference between the solute contained inside the lipid vesicle after two successive cycles, we obtain the quantity of solute contained in the pulse of internal solution delivered between the two cycles. Assuming a liposome programmed for n cycles, the solute content of the p -th puls is:

$$\Delta N_{sp} = N_{s(p-1)} - N_{sp} = f^{p-1} (1-f) V_0 c_{son} = \frac{2\sigma_0 R V_0 (1-f)}{\mathfrak{R}T f^{n-p+1} (R^2 - h^2)} \quad (14)$$

The Swelling Time of the Vesicle

The length of a cycle is equal to the sum of the pore lifetime and the swelling time. Here, we calculate the swelling time of the lipid vesicle. Due to the tonicity difference between the two

adjacent media separated by the lipid bilayer, the water will diffuse through the lipid bilayer into the lipid vesicle, which swells up to a critical diameter. The increase of the volume of the lipid vesicle in a dt time interval is determined by the water molecules that entered the lipid vesicle. Therefore,

$$dV = J_w A V_{\mu w} dt \quad (15)$$

where, V is the internal volume of the lipid vesicle, J_w is the water flow through the internal surface of the lipid bilayer with area, A , $V_{\mu w}$ is the molar volume of water. The relation (15) may be rewritten as:

$$4\pi R^2 dR = J_w 4\pi R^2 V_{\mu w} dt \quad (16)$$

which gives, after simplification, the differential equation for the radius of the lipid vesicle:

$$dR = J_w V_{\mu w} dt \quad (17)$$

Integrating the differential equation mentioned above from R_0 (when the lipid bilayer is not stretched, $\sigma_0 = 0$) to R_c (when the liposome is stretched, just before the pore formation) one can obtain:

$$R_c - R_0 = J_w V_{\mu w} \tau \quad (18)$$

where τ is the swelling time of the liposome, which is the time needed by the liposome to reach its critical state starting from the initial relaxed state. Now, we introduce a mean concentration of water molecules in the lipid bilayer after the p -th cycle as:

$$\bar{c}_w = \kappa (c_w^{out} + c_w^{in}) / 2 \quad (19)$$

where c_w^{out} and c_w^{in} are the water concentration outside and inside the lipid vesicle at the beginning of the p -th cycle, respectively. The constant κ is the partition coefficient of water within the lipid domain of the vesicle. The flow of water across the lipid bilayer is equal to:

$$J_w = \bar{c}_w \mathbf{v} \quad (20)$$

where \mathbf{v} is the mean transport velocity of water molecules through the lipid bilayer. Taking into account the relations (18), (19), and (20), the swelling time in the n -th cycle is given by:

$$\tau_n = \frac{2(R_c - R_0)}{\kappa \mathbf{v} (c_w^{out} + c_w^{in}) V_{\mu w}} \quad (21)$$

But the c_w^{out} and c_{wn}^{in} are c_{we} and c_{wn} , respectively. Taking into account that:

$$c_{we} V_{\mu w} = 1 \quad (22)$$

and

$$c_{wn} V_{\mu w} + c_{sn} V_{\mu s} = 1 \quad (23)$$

and plugging them into the formula (21), one can obtain the expression for the swelling time in the n-th cycle of the dynamics of the lipid vesicle:

$$\tau_n = \frac{2(R_c - R_0)}{\kappa v (2 - c_{sn} V_{\mu s})} \quad (24)$$

The swelling time from the period of pulsatory liposome

Introducing the c_{son} formula in the relation (24), one can obtain the formula for swelling time in the p-th cycle of a lipid vesicle programmed to evolve over n cycles:

$$\tau_p = \frac{R_c - R_0}{\kappa v \left[1 - \frac{\sigma_0 R V_{\mu s}}{\mathfrak{R} T f^{n-p} (R^2 - h^2)} \right]} \quad (25)$$

The Pore Dynamics

Energetical Conditions for Pore Appearance

Most of the models describing the formation of a single transient pore in membranes are based on a simple hypothesis proposed three decades ago by Litster (Litster, 1975). According to this hypothesis, the membrane free energy change due to the formation of a transmembrane pore is given by the Litster relation:

$$\Delta E_p = 2\pi r \gamma - \pi r^2 \sigma \quad (26)$$

A stochastic pore may tend to open or close, depending on the forces acting on its boundary (Fig.2). The appearance of a circular pore of radius r , in a membrane with the surface tension coefficient σ , is determined by the presence of two competing energetic terms: a reduction in the free energy by a surface tension component ($-\pi r^2 \sigma$), and an increase in free energy by a line tension component ($+2\pi r \gamma$). Here, γ is the line tension.

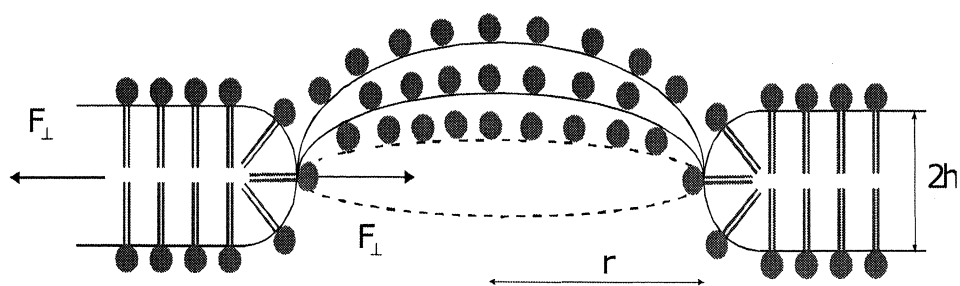


Figure 2. A cross-sectional view through a lipid bilayer containing a single transmembrane pore. Its dynamics is determined by the balance of two opposing forces. The opening of the pore is driven by the force F_σ generated by the membrane tension, whereas the closure of the pore is driven by the force F_γ due to the line tension.

The height of the free energy barrier is equal to:

$$\Delta E_{\max} = \pi \frac{\gamma^2}{\sigma} \quad (27)$$

and this is fulfilled for a critical pore radius,

$$r_0 = \frac{\gamma}{\sigma} \quad (28)$$

The line tension, γ , is caused by the hydrophobic property of phospholipids, and contributes to the free energy barrier that hinders pore formation. This component is favourable to the closing of the pore. The surface tension coefficient, σ , reduces the free energy barrier for the formation of the pore. This component drives an increase in the pore diameter.

The free energy change due to the bilayer deformation following the appearance of the pore is lost due to the internal viscosity of the lipid bilayer. Therefore, the free energy change due to the internal viscosity of the lipid bilayer is given by the following expression:

$$\Delta E_v = 4\pi r \eta_m h \frac{dr}{dt} \quad (29)$$

Equating the two energy changes of the lipid bilayer, one can obtain a differential equation for the pore radius:

$$\pi r^2 \sigma - 2\pi r \gamma = 2\pi r \eta_s \frac{dr}{dt} \quad (30)$$

$$r\sigma - 2\gamma = 2\eta_s \frac{dr}{dt} \quad (31)$$

Here, we introduce the surface viscosity, $\eta_s = 2h\eta_m$.

The equation (31) describes the pore dynamics. Unfortunately, it is difficult to solve this equation, because the membrane tension σ is not a constant. We consider a lipid vesicle in a relaxed state, when the membrane tension is nil ($\sigma = 0$), and its radius is R_0 . If this lipid vesicle experiences a membrane tension, the radius will be dependent on the surface tension coefficient σ :

$$R(\sigma) = R_0 \sqrt{1 + \frac{\sigma}{E}} \quad (32)$$

where E is the elastic modulus for surface stretching or compression, and is equal to:

$$E = \frac{48\pi K_H^2}{R_0^2 kT} \quad (33)$$

In this formula, K_H is the Helfrich bending constant and kT is the thermal energy (Brochard et al., 1976; Brochard et al., 2000).

An analytical expression for the surface tension coefficient σ

Let us suppose that from the initial state with the surface tension coefficient $\sigma = 0$ and the radius R_0 , the vesicle is stretched up to a critical state, just before the appearance of the pore. In this critical state, the lipid vesicle has the radius R_c , and the membrane tension is equal to σ_c . In any state between these ones, the vesicle is characterized by a radius R ($R_0 < R < R_c$), and a membrane tension σ ($0 < \sigma < \sigma_c$), which are related by the equation (32). The surface area in each of the three states is given by the following formulas:

$$A_0 = 4\pi R_0^2; \quad A = 4\pi R^2 = 4\pi R_0^2 \left(1 + \frac{\sigma}{E}\right); \quad A_c = 4\pi R_c^2 = 4\pi R_0^2 \left(1 + \frac{\sigma_c}{E}\right) \quad (34)$$

After its formation, the pore expands in the first part of its lifetime, then it decreases, and finally it closes. In the same time, membrane tension decreases from σ_c to zero. The membrane tension decreases due to two factors: 1) the growth of the pore and 2) the leakage of internal liquid due to the excess in Laplace pressure. We assume that the lipidic mass from the membrane is conserved during the pore lifetime. We can imagine a vesicle state at a given moment from its dynamics, when the pore radius is equal to r , and the lipid bilayer tension is σ . The following relation may be written:

$$4\pi R_c^2 = 4\pi R_0^2 \left(1 + \frac{\sigma}{E}\right) + \pi r^2 \quad (35)$$

If one considers the case which internal liquid is gelified, therefore zero leakage, the pore reaches its maximum radius, named critical pore radius and marked as r_c . In this case, the lipid membrane is in a relaxed state ($\sigma = 0$), and the following relation is available:

$$4\pi R_c^2 = 4\pi R_0^2 + \pi r_c^2 \quad (36)$$

Combining the relations (34), (35), (36), an analytical formula for the membrane tension can be obtained:

$$\frac{\sigma}{\sigma_c} = 1 - \frac{r^2}{r_c^2} - \frac{R_c^2 - R^2}{R_c^2 - R_0^2} \quad (37)$$

The pore hydrodynamics

After the appearance of the pore, the internal liquid comes out and the vesicle decreases in its size. The flow of the expelled liquid is: $Q = \pi r^2 v$, where r is the radius of the pore, and v is the mean leak-out velocity of the internal liquid. The flow divided by the time has to be equal to the rate of change of the volume of the lipid vesicle:

$$Q = \frac{\partial V_{\text{vez}}}{\partial R} \quad (38)$$

The internal liquid is pushed out through the transient pore by a Laplace pressure ΔP :

$$\Delta P = \frac{2\sigma}{R} \quad (39)$$

The force for pushing out is given by the following expression:

$$F_p = \Delta P \cdot \pi r^2 \quad (40)$$

This force may be equal to the shear viscosity force, which is involved in the outward flow:

$$F_v = 3\pi\eta_l r v \quad (41)$$

Taking into account the above relations (39), (40) and (41), the outward flow velocity of the internal liquid is:

$$v = \frac{2\sigma r}{3R\eta_l} \quad (42)$$

Introducing the formula (42) in the relation (38), one can obtain an exact equation for the radius of the lipid vesicle:

$$\frac{2\pi\sigma r^3}{3R\eta_l} = 4\pi R^2 \frac{dR}{dt} \quad (43)$$

The pore dynamics is described by equations (31), (37) and (43). Hence, we will name them as dynamics pore equations. Their solutions are $r(t)$, $\sigma(t)$ and $R(t)$.

Lifetime of the Pore

The lifetime of the pore is strongly dependent on the viscosity of the internal liquid of the lipid vesicle. If the viscosity of the internal liquid is low, then both the radius of the pore and the lifetime of the pore are small because the liquid is squeezed out very rapidly when the pore just opens. More interesting is the case when the internal liquid has a slow leakage. In the slow leak-out regime, both experimental results and theoretical predictions point out a dependence of the radius of the pore on time as that drawn in Fig. 3.

In this paper, we will use the dynamics pore equations for the calculation of the lifetime of the pore. The lifetime of the pore is equal with sum of time for pore expansion up to maximum value of its radius, and the time for its decrease up to its closure.

The expansion time

As one can see, the expansion time is very short. We assume that the internal liquid does not leak out from the vesicle in this short time. It results that the approximation $R \approx R_c$ is good and the bilayer tension is given by:

$$\frac{\sigma}{\sigma_c} \approx 1 - \frac{r^2}{r_c^2} \quad (44)$$

So the equation (31) becomes:

$$r \left(1 - \frac{r^2}{r_c^2} \right) = 2\eta_s \frac{dr}{dt} \quad (45)$$

Integrating this differential equation when the radius of the pore runs in the range $[r_0, r_m]$, one can find the expansion time t_e :

$$t_e = \tau \left(\ln \frac{r_m}{r_0} - \frac{1}{2} \ln \frac{r_c^2 - r_m^2}{r_c^2 - r_0^2} \right) \quad (46)$$

We used the following notations:

$$\tau = \frac{2\eta_s}{\sigma_0} \quad (47)$$

with the initial pore radius

$$r_0 = \frac{\gamma}{\sigma_c} \quad (48)$$

and the maximum radius of the pore r_m

When the radius of the pore attains its maximum, $\sigma_m r_m = \gamma$, because $dr/dt = 0$.

It results an equation for r_m :

$$\frac{\gamma}{\sigma_c} \approx r_m \left(1 - \frac{r_m^2}{r_c^2} \right) \quad (49)$$

The solution of this equation is:

$$r_m \approx r_c - \frac{\gamma}{2\sigma_c} \quad (50)$$

The pore decrease time

After the radius of the pore reached its maximum, the liquid continues to leak out slowly and the driving force changes its direction: $\sigma r \leq \gamma$. The liquid leaking out reduces the membrane tension, whereas the radius of the pore increases. This is the reason for which the driving force closing the pore is nearly equal to zero, and the membrane tension increases slowly:

$$\sigma \approx \frac{\gamma}{r} \quad (51)$$

Derivating the equation (37), and taking into account that $d\sigma/dt \approx 0$, one can obtain:

$$4R \frac{dR}{dt} = r \frac{dr}{dt} \quad (52)$$

Taking into account the relations (51) and (52), then the equation (31) for the pore dynamics becomes:

$$\frac{dr}{dt} \cong \frac{-2\gamma}{3\pi\eta_l} \frac{r}{R^2} \quad (53)$$

We integrate the equation (53):

$$\int_{r_m}^{r_0} \frac{dr}{r} = \frac{-2\gamma}{3\pi\eta_l R_0^2} \int_0^{t_d} dt \quad (54)$$

and we obtain the decrease time, for the second stage of its dynamics, noted with t_d :

$$t_d = \frac{3\pi\eta_l R_0^2}{2\gamma} \ln \frac{r_m}{r_0} \quad (55)$$

The closing time

When the radius of the pore has reached its initial value, the pore closes very quickly. In this state, the membrane tension is nearly equal to zero. The closing state is described by the equation (31), if we put $\sigma = 0$:

$$\frac{dr}{dt} \cong \frac{-\gamma}{2\eta_s} \quad (56)$$

The closing time results from the integration of the equation (56):

$$\int_{r_0}^0 dr = \frac{-\gamma}{2\eta_s} \int_0^{t_c} dt \quad (57)$$

Therefore, the closing time t_c is:

$$t_c = \frac{2\eta_s r_0}{\gamma} \quad (58)$$

The Time of the N-Cycle of the Pulsatory Lipid Vesicle

It is easy to see that the periode of the cyclic process experienced by the lipid vesicle under osmotic stress is composed by the sum of the lifetime of the pore and the swelling time of the lipid vesicle. Therefore, a cycle with duration T_n is given by:

$$T_n = t_n + t_e + t_d + t_c \quad (59)$$

Replacing all the time intervals with their corresponding expressions, we obtain the time of the n-th cycle:

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$$(54) \quad T_n = \frac{2(R_c - R_0)}{\kappa v(2 - c_{sn} V_{\mu s})} + \tau \left(\ln \frac{r_m}{r_0} - \frac{1}{2} \ln \frac{r_c^2 - r_m^2}{r_c^2 - r_0^2} \right) + \frac{3\pi\eta_l R_0^2}{2\gamma} \ln \frac{r_m}{r_0} + \frac{2\eta_s r_0}{\gamma} \quad (60)$$

$$(55) \quad T_n = \frac{2(R_c - R_0)}{\kappa v(2 - c_{sn} V_{\mu s})} - \frac{\tau}{2} \ln \frac{r_c^2 - r_m^2}{r_c^2 - r_0^2} + \left(\tau + \frac{3\pi\eta_l R_0^2}{2\gamma} \right) \ln \frac{r_m}{r_0} + \frac{2\eta_s r_0}{\gamma} \quad (61)$$

If the pore was programmed a priori to run n cycles, then the period of the p -th cycle of n -cycles programmed vesicle is:

$$(56) \quad T_{pn} = \frac{R_c - R_0}{\kappa v \left[1 - \frac{\sigma_0 R V_{\mu s}}{\mathfrak{R} T f^{n-p} (R^2 - h^2)} \right]} - \frac{\tau}{2} \ln \frac{r_c^2 - r_m^2}{r_c^2 - r_0^2} + \left(\tau + \frac{3\pi\eta_l R_0^2}{2\gamma} \right) \ln \frac{r_m}{r_0} + \frac{2\eta_s r_0}{\gamma} \quad (63)$$

Numerical Results and Discussion

We apply our theoretical results mentioned above to the giant vesicles obtained experimentally (Karatekin et al., 2003). Such giant vesicle has the radius in relaxed state, $R_0 = 19.7 \mu\text{m}$ and the value of the critical radius is $R_c = 20.6 \mu\text{m}$. The thickness of the lipid bilayer is of 5 nm (Karatekin et al., 2003). Let us consider a vesicle in a closed chamber that contains water. The transport velocity through the lipid bilayer is $v = 10^5 \text{ \AA/s}$ (Sackmann et al., 1978). The partition coefficient of water in the lipid bilayer is $\kappa = 64 \cdot 10^{-6}$ (Lawaczeck 1979). In fact, κ must represent the partition coefficient of water in the hydrophobic core of the lipid bilayer. Also, we consider the maximum value of the bilayer tension, just before the formation of the pore $\sigma_0 = 10^{-5} \text{ N/m}$ (Nardi et al., 1998).

The initial quantity of solute. If one introduces the above values in the formula (13), the initial concentration of solute inside the vesicle, measured in nM, if this runs over n cycles is:

$$(57) \quad N_{s0n} = \frac{12.45}{f^n} \quad (64)$$

The calculated value for the reversal swelling coefficient, f , is equal to 0.8746 for the considered vesicle. The dependence of the initial solute concentration on the programmed activity life, measured in cycles number is represented in Fig. 3.

(59) Because the fraction $\frac{\sigma_0}{\mathfrak{R} T}$ appears in all formulae, we have calculated it separately and its value is equal to $4 \cdot 10^{-9} \text{ mol.m}^{-2}$.

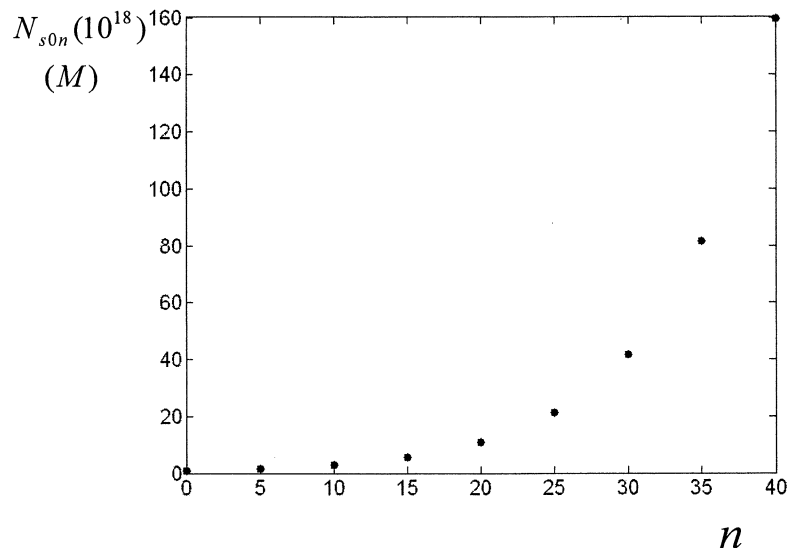


Figure 3. The dependence of the initial solute concentration on the programmed activity life, measured in cycles number.

The amount of solute delivered through a single transient pore

We think that it is useful to express the amount of solute delivered through a single pore in number of molecules than in mols. Introducing all data in formula (14) one obtain:

$$\Delta N_{sp} = \frac{425(1-f)10^4}{f^{n-p}} \quad (65)$$

We chose a liposome armed to work n cycles. The number of solute molecules leaked out from liposome through the p^{th} pore of n -sequence is given by formula (65). For a pulsatory liposome programmed to have 40 cycles, the solute quantity delivered versus p is represented in Fig. 4.

The activity life of a pulsatory liposome

It is obviously that the pulsatory liposome life with the sum of all time length cycles. Therefore, for an n -cycle liposome its life, t_n is equal to:

$$t_n = \sum_{p=1}^n T_{np} \quad (66)$$

We assume that the pore life time does not depend on the pore rank in the sequence, although the pore lifetime is influenced by the viscosity of the internal liquid that changes with the solute dilution. The pore life time is up to 10 sec. For the pulsatory liposome selected here, the time, measured in minutes, of the p -th cycle is:

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$$\tau_{np} = \frac{375}{16 \left(1 - \frac{2 * 10^{-4} V_{\mu s}}{f^{n-p+1}} \right)} \quad (67)$$

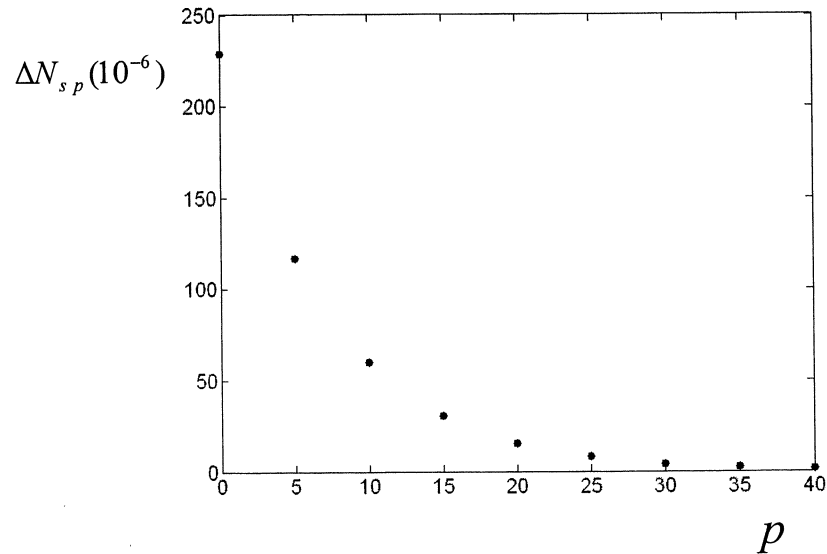


Figure 4. For a pulsatory liposome programmed to have 40 cycles, the solute quantity delivered versus p .

(65) It is known that the water pass through the lipid bilayer with a very low probability due to a high free energy barrier for crossing the membrane. We find this situation at the beginning of the each individual cycle, but more than likely that the water molecules pass through lipid bilayer the reason that the bilayer is composed from a mixture of lipid molecules. For this reason, the bilayer is heterogeneous in its own nature, containing microclusters determined by the selective dynamic association of lipid molecules (Popescu and Victor 1990; Popescu 1993; Movileanu et al., 1997, 1998). The bilayer is neither smooth, nor static, and has a heterogeneous thickness. As a consequence, the vesicle is deformable in the relaxed state from the beginning of each cycle. Therefore, it is very possible that very small structural defects appear in the membrane, and these are used by the water molecules to come into the lipid vesicle. On the other hand, the pores are very large, up to 10 μm (Karatekin et al., 2003). Larger molecules, or greater amount of the internal liquid can leak out the lipid vesicle. A very interesting application of the pulsatory liposomes filled with drugs is in the case of hepatic cells. The endothelial pores (also known as fenestrae) control the exchange of fluids, solutes and particles between the sinusoidal blood and the space of Disse. The free pulsatory liposomes or those included in other lipid vesicles may reach the hepatocyte due to hydrodynamic effects of blood circulation (Popescu et al., 2000). The transient pores in liposomes could be used for compensation of neurotransmitter deficiency in the synaptic cleft as well (Popescu et al., 2006).

(66) quence, changes selected

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