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MATHEMATICAL MODELING OF THE PULSATORY LIPID VESICLE DYNAMICS UNDER OSMOTIC STRESS

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I consider a great lipid vesicle filled with an aqueous solution of a solute, for which its membrane is impermeable. Because of the mechanical tension induced by the osmotic flow, the vesicle swells up to a critical size, triggering a transient lipidic pore when it is introduced into a hypotonic aqueous medium. In this paper, we will obtain the differential equations of the vesicle dynamics, which is in fact a periodic process due to osmotic gradient and transbilayer pore appearance. We will also analyse the characteristic parameters of this process: swelling time, pore lifetime, number of cycles, the length time of vesicle activity, material quantity leaked out during a cycle. In the end, we present the condition to design a n-cycles working vesicle and propose some biotechnological applications.

Key words: Osmotic gradient; Stretched vesicle; Pulsatory vesicle; Drug releasing biocontroller.

1. INTRODUCTION

The passage of molecules, especially of large ones, through cellular membrane is a very important problem for some biotechnological applications. The pore appearance in lipid bilayer following some controlled mechanisms may be an adequate and interesting way of transmembrane transport.

Some pores, named stochastic pores, can appear due to structural and dynamic properties of lipid bilayer [3–7, 9], but other ones may be favored by a mechanical tension induced in different ways [11, 12]. A sequence of 30–40 pores was recently observed in the same giant vesicle stretched by an optical induced mechanical tension [1, 2, 12]. In this succession, only a single pore can appear into the vesicle membrane at a time.

There are two very interesting biotechnological applications, which require the increase of membrane permeability: gene therapy and targeted special substances delivery. In the first one, the transport of DNA fragments through cellular and nuclear membranes is requested [13]. The second application uses special substance molecules encapsulated in vesicles, which have to be transported and released to a target place [14].

Having reached that point, one supposes that the liposome discharges its content by its breakdown.

In this paper, we will explain how a lipid vesicle can release the drug molecules, in a well-controlled fashion.

Such a vesicle is called pulsatory liposome and has cyclic activity. We will demonstrate that this liposome may be scheduled to work a certain settled in advance number of cycles and we will calculate the amount of special substances delivered during each cycle.

2. PHENOMENOLOGICAL BASES OF A PULSATORY LIPOSOME

Let us consider a liposome filled with aqueous solution containing an impermeable solute. The initial state of the liposome is an equilibrium characterized by smooth and unstretched lipidic membrane; it is also considered a reference state. This liposome is inserted into a bath with a hypotonic aqueous medium. Due to osmotic pressure created by the transmembrane gradient of the solute concentration, water molecules inflow into liposome through its membrane. The osmotic flow of the solvent determines:

1) the swelling of the liposome;
2) the stretching of liposomal membrane;
3) the dilution of the internal solution.

Also, the surface tension grows in the same time with the liposome expansion and increases the pressure inside the vesicle. Under these experimental conditions, either the liposomal membrane may be ruptured and destroyed or one pore may appear through its lipid bilayer. If the swelling process is slow enough, the liposome grows up to a critical size, moment when a transient transmembrane pore appears. Two simultaneous processes follow this event: 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. In the first one, the pore radius increases up to the maximum value, r_m, and in the second one, the pore radius decreases until the closure of the pore (Fig. 1). Both phenomena, the increase of the pore size and the leakage of the internal liquid, determine the membrane relaxation due to the reduction in the mechanical tension of the membrane.

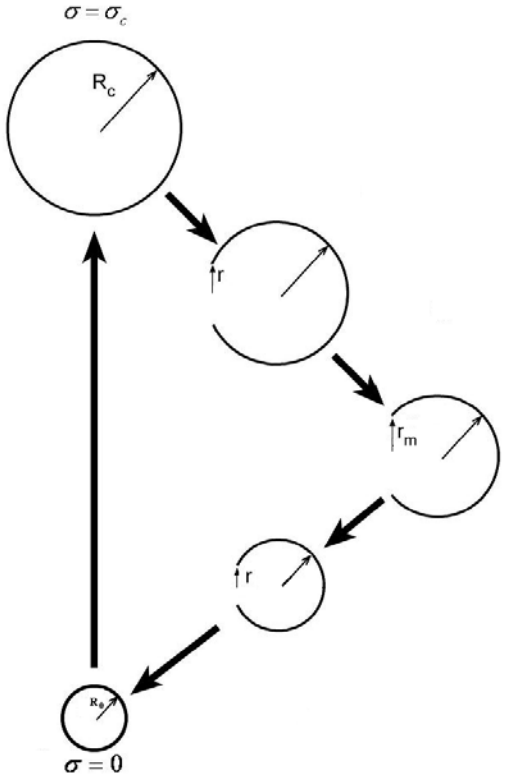

Fig. 1 – A cycle of the pulsatory liposome. In the first stage, the liposome swells from the initial state of radius, R_0, to the critical state of radius, R_c, when o transbilayer pore appears (the left part of the picture). In the second stage, the pore radius increases to a maximum value, r_m, after which it decreases to the pore disappearance. It is observed that, simultaneously with the pore evolution, the liposome relaxes until its radius equals to R_0 (the right part of the picture).

In fact, the pore dynamics is driven by the difference between the membrane tension and edge (line) tension due to water exposure of the hydrophobic core membrane (Fig. 2).

The membrane tension decreases until it reaches the line tension of the membrane edge. During and even after this, the internal liquid continues to leak outside the liposome. This moment when the two tensions equalize represents the beginning of the second part of the pore dynamics during which the pore radius reduces until the pore closes. Therefore, the liposome comes back to its initial size. We can envision now that the liposome dynamics described above starts over. This cyclic process ceases when the osmotic gradient becomes smaller than a critical value, which will be discussed below.

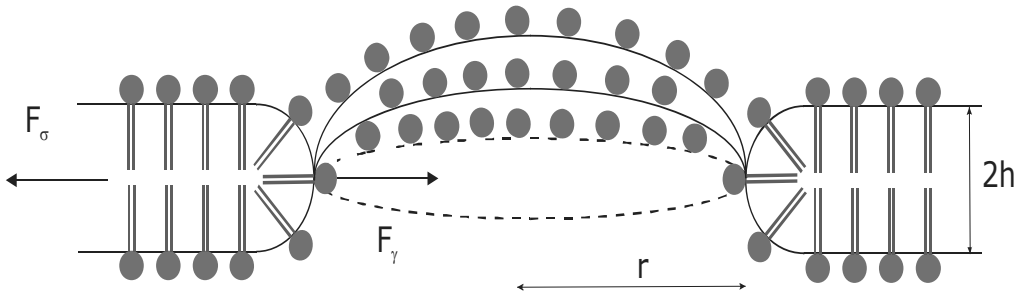

Fig. 2 – A cross section through a bilayer with a pore. The opening of the pore is driven by the force F_σ based on the membrane tension and the closure is driven by F_γ based on the line tension. Its evolution is determined by the balance of the two opposing forces.

In the next part, we will describe a mathematical model that will cover the two parts of a pulsatory liposome cycle: the liposome swelling and its relaxation.

3. THE LIPOSOME SWELLING STAGE

In the reference state, the liposome is characterised by its radius R_0, membrane area A_0, and the volume V_0. In the swelling stage, the liposome radius increases from this initial value R_0 to a critical value R_c due to water influx. The change in the liposome volume is described by the following equation:

$$\frac{\mathrm{d}V}{\mathrm{d}t} = P_w V_{\mu w} A\left(\Delta C_s - \frac{\Delta P}{N_A k_B T}\right), \tag{1}$$

where: V is the liposome volume; P_w is the water permeability through the liposome membrane (measured in m/s); $V_{\mu w}$ is the molar water volume (m^3/mol); A is the membrane area; ΔC_s is the gradient of the transmembrane solute concentration (mol/m^3); ΔP is the excess Laplace pressure; N_A is the Avogadro number; k_B is the Boltzmann constant, and T is the absolute temperature.

The Laplace pressure is given by the:

$$\Delta P = \frac{2\sigma}{R}, \tag{2}$$

where, σ is the tension of the stretched membrane and R is the liposome radius.

According to Hook's law, if the closed spherical membrane is stretched by a surface tension σ, its radius changes as:

$$R(\sigma) = R_0\sqrt{1+\frac{\sigma}{E}}, \tag{3}$$

where, E is the elastic modulus for the surface stretching or compression.

The amount of the internal solute is conserved during the liposome swelling and we can write:

$$C_{0s}V_0 = C_s V = C_{fs}V_c, \tag{4}$$

where C_{0s} is the initial solute concentration; C_s is the solute concentration when the liposome has reached the volume V during the swelling process and C_{fs} is the solute concentration at the end of swelling stage before pore nucleation when the liposome volume is V_c.

If one considers the external solute concentration to be zero, then $\Delta C_s = C_s$.

Using the equations (2), (3), (4) we rewrite (1) as:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = P_w V_{\mu w}\left(\frac{C_{0s}R_0^3}{R^3} - \frac{2\beta E}{R_0^2}\quad\frac{R^2 - R_0^2}{R}\right), \tag{5}$$

where

$$\beta = \frac{1}{N_A k_B T}. \tag{6}$$

By integrating (5), one obtains the liposome radius *R(t)* as a function of time with the initial condition

$$R(t = t_0) = R_0. \tag{7}$$

The analytical solution of the equation (5) is:

$$\frac{8\alpha\beta E P_w V_{\mu w}}{R_0^2} t = (\alpha - 1)\ln\left|\frac{\alpha + 1}{2z + \alpha - 1}\right| + (\alpha + 1)\ln\left|\frac{\alpha - 1}{2z - \alpha - 1}\right|, \tag{8}$$

where

$$z(\mathrm{t}) = \frac{R^2(t)}{R_0^2}, \tag{9}$$

$$\alpha = \sqrt{1 + \frac{2C_0 R_0}{\beta\quad E}}. \tag{10}$$

The swelling time of the liposome can be calculated by solving for *t* the following equation:

$$R(t) = R_c. \tag{11}$$

The most important parameter for the pulsatory liposome running is the initial solute concentration.

4. THE LIPOSOME RELAXATION STAGE

The liposome swells up to a critical size, when suddenly a transbilayer pore appears. This is the moment when the liposome relaxation begins. In this part of the pulsatory liposome cycle, two processes simultaneously take place: the pore evolution from its birth to its disappearance, and the liposome relaxation from the critical state of radius R_c to the initial geometric size of radius R_0.

We will firstly analyse the pore dynamics. According to fig. 2, the pore evolution is leaded by the driving force for pore opening F_σ corresponding to membrane tension σ and the driving force for pore closure F_γ corresponding to line tension γ. The line tension γ is caused by the hydrophobic property of phospholipids, and contributes to the energy barrier, which hinders the pore formation. The surface tension coefficient σ reduces the barrier height for pore nucleation.

The surface free energy change due to the bilayer deformation following the pore appearance is dissipated into lipidic bilayer volume by the intermolecular friction forces characterized by the internal viscosity η_b. The energy change due to internal viscosity of the lipid bilayer is:

$$\Delta E_v = 2\pi\quad r\eta_b d\frac{\partial r}{\partial t}, \tag{12}$$

where *d* is the thickness of the lipid bilayer.

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

$$r\sigma - 2\gamma = 2\eta_b d\,\frac{\partial r}{\partial t}\,. \tag{13}$$

Another important process which simultaneously takes place with the pore evolution is the relaxation of the liposome. This process will be analyzed in the following.

After pore appearance, the internal liquid leaks out and the vesicle decreases its size.

The flow of expelled liquid in time unit is $Q = \pi r^2 v$, where r is the pore radius and v is the mean leak-out velocity of internal liquid. The decrease rate of the vesicle volume V_{ves} is given by the difference between this Q and the osmotic flow of solvent (water) j_w:

$$\frac{\partial V_{\text{ves}}}{\partial t} = Q - j_w\,. \tag{14}$$

The pushing out force is:

$$F_p = \Delta P \cdot \pi r^2\,. \tag{15}$$

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

$$F_v = 3\pi\eta_l r\,\mathrm{v}\,. \tag{16}$$

Using the above relations (2), (15), and (16), we can rewrite the outward flow velocity of the internal liquid as

$$\mathrm{v} = \frac{2\sigma r}{3R\eta_l}\,. \tag{17}$$

Taking into account the relations (14) and (17), one obtains an equation for the vesicle radius:

$$4\pi R^2\frac{\partial R}{\partial t} = -\frac{2\pi\sigma r^3}{3R\eta_l} + P_w V_{\mu w}\left(4\pi R^2 - \pi r^2\right)\left(\Delta C_s - \frac{\Delta P}{N_A k_B T}\right). \tag{18}$$

The elastic energy of a membrane of radius R with a pore of radius r on it is

$$W_{el}(R,r) = \frac{K_s}{2}\left[\left(A - A_0\right)^2 - \pi r^2\right]^2 + 2\pi\gamma r \tag{19}$$

and the bilayer surface tension is given by

$$\sigma(R,r) = \frac{\partial W_{el}}{\partial A} = K_s\left[4\pi(R^2 - R_0^2) - \pi r^2\right]. \tag{20}$$

In order to avoid the elastic constant K_s one can use the following relation:

$$\frac{\sigma}{\sigma_c} = 1 - \frac{r^2}{4(R_c^2 - R_0^2)} - \frac{R_c^2 - R^2}{R_c^2 - R_0^2}\,. \tag{21}$$

The solute amount inside the liposome is modified by the solute efflux through the open pore according to the equation

$$\frac{\mathrm{d}\left(C_{\text{in}} V_{\text{ves}}\right)}{\mathrm{d}t} = -\pi r^2 C_{in}\,\mathrm{v}\,, \tag{22}$$

which is equivalent to:

$$\frac{\mathrm{d}\ln\left(C_{\text{in}} V_{\text{ves}}\right)}{\mathrm{d}t} = -\frac{3r^2\,\mathrm{v}}{4R^3}\,. \tag{23}$$

Having formula (2) and (21) in mind, the final form of the differential equation (18) is:

$$\frac{\partial R}{\partial t} = -\frac{Er^3}{6R_0^2\eta_l}\frac{R^2 - R_0^2}{R^3} + P_w V_{\mu w}\left(1 - \frac{r^2}{4R^2}\right)\left(C_{in} - \frac{2\beta E}{R_0^2}\frac{R^2 - R_0^2}{R}\right) \tag{24}$$

The equations (13), (22) and (24) can be solved numerically using Euler's method to obtain the time dependence of *R(t)*, *r(t)* and $C_{in}(t)$ during the second stage of a cycle of the periodic process.

The time dependence of the liposome radius in the first stage of each cycle is obtained from equation (5). The pore lifetime, which equals the liposome relaxation time, can also be obtained. The most important parameters are: the inside solute concentration, the internal liquid viscosity, and the bilayer viscosity.

5. THE SOLUTE AMOUNT RELEASED PER CYCLE

By integrating the equation (23) we can calculate the amount of the drug (or any special chemical substance) released during a cycle.

$$\int_{C_{fs,n}V_c}^{C_{0s,n}V_0} \mathrm{d}\ln(C_{in}V_{lip}) = -\frac{\sigma_c}{2\eta_l\left(R_c^2 - R_0^2\right)}\int_0^{\tau_{2,n}} \frac{r^3}{R^4}\left(R^2 - \frac{r^2}{4} - R_0^2\right)\mathrm{d}t\,. \tag{25}$$

We have used the indices as follows: f – for the end (final) of the cycle; s – for solute; *n* – for the rank of the cycle; c – for the critical state reached at the end of the swelling stage; 0 – for the initial state of liposome at the beginning of a cycle. We have noted with $\tau_{2,n}$ the length time of the second part of the nth cycle (the vesicle relaxation stage). Also, $\tau_{2,n}$ is the life time of the pore, which appears in the n^{th} cycle.

A similar relation as (4) may be written for each cycle. Thus, for the nth cycle we have

$$C_{0s,n}V_0 = C_{s,n}V = C_{fs,n}V_c\,. \tag{26}$$

Taking into account that $C_{fs,n} = C_{0s,n+1}$ and $C_{fs,n}V_c = C_{0s,n}V_0$ we obtain:

$$\ln\frac{C_{0s,n+1}}{C_{0s,n}} = -\frac{\sigma_c}{2\eta_l\left(R_c^2 - R_0^2\right)}\int_0^{\tau_{2,n}}\left(R^2 - \frac{r^2}{4} - R_0^2\right)\mathrm{d}t\,. \tag{27}$$

Let us use the following notation:

$$I_n := \frac{\sigma_c}{2\eta_l\left(R_c^2 - R_0^2\right)}\int_0^{\tau_{2,n}}\left(R^2 - \frac{r^2}{4} - R_0^2\right)\mathrm{d}t\,. \tag{28}$$

The amount of solute, Δm_n, released during the n^{th} cycle is equal to

$$\begin{aligned}\Delta m_n &= C_{0s,n-1} - C_{0s,n} = C_{0s,n-1}\left(1 - \mathrm{e}^{-I_{n-1}}\right) = \\ &= C_{0s,1}\left(1 - \mathrm{e}^{-I_1}\right)\left(1 - \mathrm{e}^{-I_2}\right).....\left(1 - \mathrm{e}^{-I_{n-1}}\right).\end{aligned} \tag{29}$$

The internal amount of solute is conserved during each vesicle swelling stage. This quantity is smaller than the amount of solute contained by vesicle in the previous cycle. Therefore, the viscosity of the internal liquid from the vesicle decreases with the growth of the cycle order. It results that the internal liquid leaks out faster from the vesicle through the increased pore and the relaxation time $\tau_{2,n}$ will also decrease. Hence, the value of the integral I_n will vary because both internal liquid viscosity η_l and the pore life $\tau_{2,n}$ decrease with the growth of the cycle rank.

6. POSSIBLE APPLICATION

A very interesting application may be the compensation of neurotransmitter deficiency into synaptic cleft.

It is known that the process leading to depression is the depletion of neurotransmitters in the synaptic cleft. This is designated as the *biogenic amine theory of depression*. There are four ways to prevent the neurotransmitter depletion by drug action: a) to increase the release of neurotransmitters from the presynaptic terminal; b) to prolong the interaction time with the postsynaptic receptors; c) to inhibit the enzymes which inactivate or destroy the neurotransmitters; d) to delay the re-uptake of neurotransmitters in the presynaptic neurons . The tricyclic amines (desipramine, imipramine and amitriptyline) which block the reuptake of noradrenalin and serotonin into the presynaptic neuron are powerful antidepressants. However, the depletion of neurotransmitter in the synaptic space may be compensated regardless of its cause, by the existence in this space of some liposomes filled with the neurotransmitter molecules, which are in deficit. It is possible to introduce liposomes filled with different types of neurotransmitters. The liposomes may deliver controlled quantities of neurotransmitter, periodically. The liposomes can contain drug molecules, such as tricyclic amines, to block the re-uptake process in the presynaptic membrane.

7. DISCUSSION

The pulsatory liposomes can be used for drug administration at ill places. In our opinion, the drug quantities should be sufficiently to have a beneficial effect on molecular ill places, because there are not drug losses as in other ways of drug administration. It must be known the molecular mechanism of action of drug in order to determine the two parameters characterizing the pulsatory liposome: the time intervals between two successive pores and the amount of drug released with the internal liquid leaked out through each pore. Recently, an approximate solution for the cyclic running of a pulsatory liposome was published [15].

The preparation of pulsatory liposomes with such properties and its transport to the action place of drug molecules is a biotechnological task. Some very interesting applications of pulsatory liposomes filled with drug are in the case of hepatic cells or of the synaptic cleft. 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 pulsatory liposomes free or included inside to other vesicle may reach to hepatocytes due to hydrodynamic effects of blood circulation [8].

Also, the transient pores in liposomes could be used for compensation of neurotransmitter deficiency in the synaptic cleft [10]. Finally, we are thinking that, in the future, the pulsatory liposome may be used as a special device for active substances dose.

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