Mathematical Description of the Functioning of the Pulsatory Liposome

Dumitru POPESCU*,[1], Dumitru Petru IGA[2], Alin Gabriel POPESCU[3], Valentin I. R. NICULESCU[4]

*Corresponding author
*,[1]Department of Mathematical Modeling in Life Sciences,
"Gh.Mihoc-Caius Iacob" Institute of Mathematical Statistics and Applied Mathematics of RomanianAcademy,
13 Septembrie street, 050911, Bucharest, Romania,
dghpopescu@gmail.com
[2]University of Bucharest,
Bulevardul Regina Elisabeta Nr. 4-12, București 030018, Romania
[3]Department of Computer Sciences, IT CORE SRL,
10, Garoafei Street, 051235, Bucharest, Romania
[4]National Institute for Laser, Plasma and Radiation Physics,
Atomistilor street, 409, Magurele, Ilfov, Romania

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Abstract: *We consider a liposome filled with the aqueous solution of an osmotic solute enclosed in a large water bath. Due to osmotic pressure, it works according to a sequence of cycles with two stages. In the first stage, the lipid vesicle with a certain input concentration of the osmotic substance is swelling up in an osmotic process until it reaches a size, when one pore is formed on its surface. The second stage follows, where the vesicle content is partially eliminated through the pore to induce a decrease in the vesicle volume up to its original size. If we take into account that the pore has two stages in its evolution, then the relaxing stage may be divided into two new stages. The swelling of the liposome is described by a differential equation. All the processes which contribute to the vesicle relaxing and its return to the initial size are described by three differential equations.*

Key Words: *mathematical description, pulsatory liposome, biotechnological applications, transmembrane pore, lipid bilayer.*

1. INTRODUCTION

The liposome is a closed lipid bilayer and is used as an artificial model of cell membrane in biophysical cellular membrane researches. Liposomes can be used as carriers of special substances. Lipophilic substances may be incorporated into the lipid bilayer, and hydrophilic substances are included inside the liposome, in the aqueous compartment. Liposomes can transport and release active pharmacological substances at the site of action.

To release the pharmacologically active substances at the site of action, the lipid bilayer of the liposome fuses with the lipidic bilayer of the target cell membrane, and releases the

entire contented at once. In this paper, we propose another way to release of specific action substances, which does not destroy the liposome: release through transient transmembrane pores.

The appearance of pores in lipid bilayer of liposomes, following some controlled processes, may be an interesting way to release molecules, especially of large ones, with utility in some biotechnological applications [1, 2]. The transmembrane stochastic pores, can occur due to the structural and dynamic properties of the lipid bilayer [3–9]. On the other hand, the mechanical stretching induced in various ways in the lipid vesicle membrane may favor the appearance of transmembrane pores [10-15]. In this paper, we will present a mathematical description of the functioning of the pulsatory liposome. Also, we will write about how a lipid vesicle releases the drug molecules, in a well-controlled manner. Such liposome is named pulsatory liposome and has a cyclic activity.

2. THETHEORETICAL STUDY

We consider a lipid vesicle filled with an aqueous solution of an osmotic solute. This lipid vesicle is placed in a sufficiently large spherical bath filled with water. In the initial state, the liposome has a radius equal to R_0 and its membrane is smooth and unstretched ($\sigma = 0$). Due to the osmosis process, the liposome swells from the initial state to the critical state reached when a pore suddenly appears (the critical radius of the liposome is equal to R_c) [2, 16, 17]. The appearance of the pore causes the deflation of the liposome to its initial state.

The pore evolution has two stages: in the first part the pore radius increases up to a maximum value (r_m), after which, in the second part, the pore radius decreases until the pore disappears and the membrane of the liposome recovers.

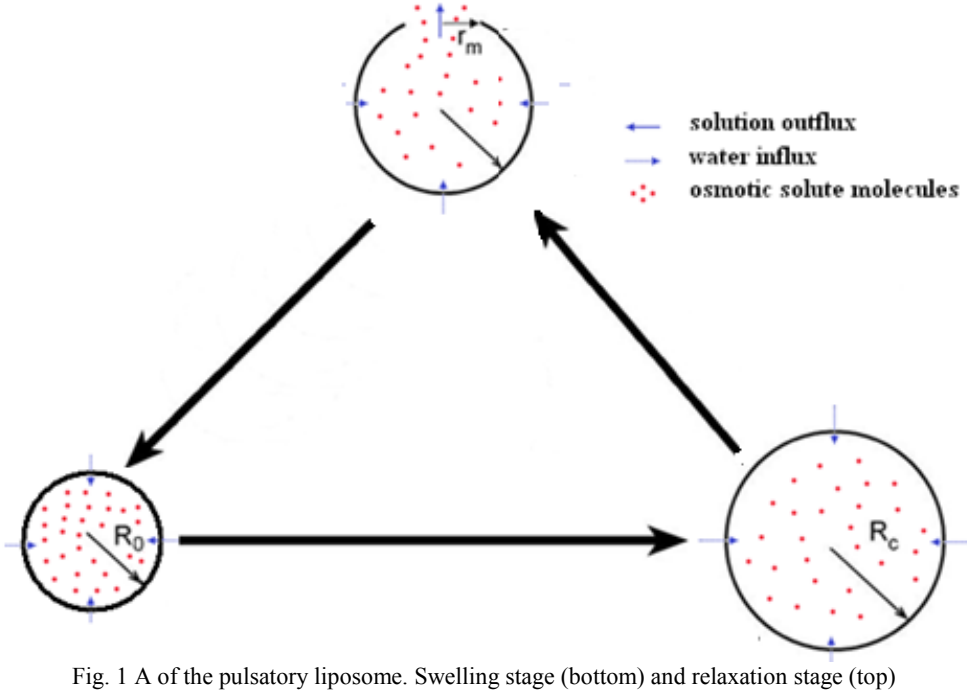

Fig. 1 A of the pulsatory liposome. Swelling stage (bottom) and relaxation stage (top)

Through the pore, some of the content inside the liposome is expelled to the external environment. Finally, the pore disappears and the liposome returns to its initial size and a new

cycle can starts [9, 16, 17-22]. At the end of each cycle, only the internal concentration of the osmotic solution is changed. It is easy to see that the liposome swelling is performed by osmotic pressure and the Laplace pressure. In conclusion, a vesicle filled with the solution of an osmotic solute and introduced into a hypotonic medium, can perform a periodic dynamic activity under the simultaneous and opposed actions of osmotic pressure and Laplace pressure. For this reason, we call such a vesicle a pulsatory liposome. The dynamics evolution of a pulsatory liposome during a cycle is drawn in Fig. 1.

3. MATHEMATICAL DESCRIPTION

The initial state of the vesicle is characterized by the radius R_0, the area A_0, the volume V_0, the osmotic solute concentration, C_{01} and mechanical tension, $\sigma = 0$ (the vesicle membrane is smooth and untensed). The vesicle volume increases, because of osmotic pressure. The direct consequence of volume growth is the stretch of the membrane and the appearance of Laplace pressure. As mentioned above, the liposome swelling occurs due to two pressures acting in opposite directions: osmotic pressure and Laplace pressure.

The volume variation of the liposome is described by the following equation [23-25]:

$$\frac{\mathrm{d}V}{\mathrm{d}t} = \mathrm{P_w V_{\mu w}} \beta A (\Delta P_{osm} - \Delta P_L) \tag{1}$$

Notations from the above equation have the following meaning: V is the liposome volume, Pw – the water permeability through liposome membrane, Vμw – the water molar volume, A –the vesicle area; $\beta = 1/(N_A k_B T)$; N_A– the Avogadro number, k_B – the Boltzmann constant, and T – the absolute temperature; $\Delta P_{osm} = N_A k_B T \Delta C_m$ – osmotic pressure; $\Delta P_L = 2\sigma/R$ – Laplace pressure.

For the stretched vesicle without pore the mechanical tension, σ, is equal to:

$$\sigma = \mathrm{E}\frac{\mathrm{R}^2 - \mathrm{R}_0^2}{\mathrm{R}_0^2} \tag{2}$$

where, R is the liposome radius during of the swelling, R_0 is the liposome radius in initial untensed state, E is the elastic modulus for surface stretching or compression.

The useful form of the equation (1) is:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = \mathrm{P_w V_{\mu w}}\left[\Delta C_m - \frac{2\beta \mathrm{E}}{\mathrm{R}}\left(\frac{\mathrm{R}^2}{\mathrm{R}_0^2} - 1\right)\right] \tag{3}$$

where, $\Delta C_m = C_{int} - C_{out}$ is the transmembrane gradient of the osmotic solute concentration, C_{int} is the osmotic solute concentration inside the liposome, C_{out} is the osmotic solute concentration outside of the liposome.

The internal solute concentration, C_{int}, decreases during liposome running. If the liposome is found in an infinite water environment, $C_{out} = 0$ all time. If the liposome is found in a closed hypotonic environment, C_{out} increases during liposome running.

The quantity of the osmotic solute inside the liposome is conserved during the liposome swelling stage of each cycle.

So, for the swelling of the liposome during the first cycle, we can write the mass conservation law:

$$\mathrm{C_{01} V_0} = \mathrm{C_1} V \tag{4}$$

where, C_{01} is the initial solute concentration, C_1 is the solute concentration when the liposome has reached the volume V during the swelling process. For the first cycle the external solute concentration, $C_{out} = 0$, then $\Delta C_m = C_1$.

With equations (2), (3), and (4) in mind, we find from equation (1) the final form of the differential equation which describes the swelling stage of the first cycle:

$$\frac{dR}{dt} = P_w V_{\mu w}\left[\frac{C_{01}R_0^3}{R^3} - \frac{2\beta E}{R}\left(\frac{R^2}{R_0^2} - 1\right)\right] \tag{5}$$

Introducing a new variable $x(t) = R(t)/R_0$ it results:

$$\frac{dx}{dt} = -\frac{2\beta E P_w V_{\mu w}}{R_0^2 x^3}\left(x^4 - x^2 - \frac{C_{01}R_0}{2\beta E}\right) \tag{6}$$

The initial condition is: x(0) = 1.

The analytical solution of equation A6 is:

$$\frac{8\alpha\beta E P_w V_{\mu w}}{R_0^2}t = (\alpha+1)\ln\left|\frac{\alpha-1}{2x^2-\alpha-1}\right| + (\alpha-1)\ln\left|\frac{\alpha+1}{2x^2+\alpha-1}\right| \tag{7}$$

$$\alpha = \sqrt{1 + \frac{2C_{01}R_0}{\beta E}} \tag{8}$$

The solution of differential equation (7) is the bijective function t(x): t = t(x), $x \in [1, R_c/R_0]$ and $t \geq 0$. If it is necessary one can calculate the inverse function R = R(t). The swelling time of the pulsatory liposome is the time which is necessary so the liposome to reach from the initial state to the final state, known as the critical state. It can be computed from the equation (7) if variable x is replaced by its critical value:

$$x = x_c = \frac{R_c}{R_0} \tag{9}$$

3.1 The swelling stage of the n-th cycle of a pulsatory liposome

The solute concentration at the end of the swelling stage of the $(n-1)^{th}$ cycle, is equal to $C_{c(n-1)}$.

I suppose that during a relaxation stage, an amount of internal solution is expelled outside the liposome in the bath, but the solute concentration inside the liposome remains unchanged, because the relaxing stage is very short.

So, before the start of the n-th cycle liposome is characterized by the following parameters:

– The solute concentration, C_{0n}, which is equal to $C_{c(n-1)}$. Taking into account that the solute mass is conserved for each cycle during the swelling stage, one may easily obtain:

$$C_{0n} = C_{0(n-1)}\, R_0^3/R_c^3 \tag{10}$$

– The amount of solute expelled by the vesicle in the all previous cycles which is found in the volume of water from (bath) outside the liposome is equal to:

$$\Delta Q_{n-1} = C_{01}V_0 - C_{0n}V_0 = C_{01}V_0(1 - f^{n-1}) \tag{11}$$

where f is the inverse of the swelling critical ratio: $f = R_0^3/R_c^3$.

Starting with the second cycle, the solute concentration in the external environment is no longer zero. For the n-th cycle, the initial solute concentration, outside the liposome is equal to:

$$C_{e0n} = \frac{\Delta Q_{n-1}}{V_b - V_0} = C_{01}\frac{1 - f^{n-1}}{F - 1} \tag{12}$$

where, F is the ratio of the volume of the bath, V_b, and the initial volume of the liposome, V_0: $F = R_b^3/R_0^3$

During the liposome swelling, the external concentration, C_{en}, increases. It may be calculated using the following formula:

$$C_{en} = \frac{\Delta Q_{n-1}}{V_b - V} = C_{01}\frac{1 - f^{n-1}}{F - x^3} \tag{13}$$

The transmembrane gradient of solute concentration available during the swelling process of liposome in the n-th cycle is:

$$\Delta C_n = C_n - C_{en} = C_{01}\left(\frac{f^{n-1}}{x^3} - \frac{1 - f^{n-1}}{F - x^3}\right) \tag{14}$$

Now, we can write the differential equation describing the swelling stage of the pulsatory liposome in the n-th cycle:

$$\frac{dx}{dt} = \frac{P_w V_{\mu w} C_{01}}{R_0}\left[\frac{f^{n-1}}{x^3} - \frac{2\beta E}{R_0 C_{01}}\left(x - \frac{1}{x}\right) - \frac{1 - f^{n-1}}{F - x^3}\right] \tag{15}$$

The differential equations which describe the swelling stages of pulsatory liposome, excepting the equation for the first cycle, may be solved using numerical methods.

4. THE LIPOSOME RELAXATION

4.1 The dynamics of the trans bilayer pore

The membrane free energy change due to pore appearance ($\Delta E_m = \pi r^2\sigma - 2\pi r\gamma$) is dissipated into lipidic bilayer volume do to its internal viscosity ($\Delta E_v = 2\pi r\eta_m 2h\frac{\partial r}{\partial t}$)

Equaling the two energy changes for the lipid bilayer, one obtains a differential equation for the dynamics of the pore radius [25-28]:

$$2\pi r\eta_m 2h\frac{\partial r}{\partial t} = \pi r^2\sigma - 2\pi r\gamma \tag{16}$$

The dynamics of the pore is determined by the membrane tension, σ, which acts to open it, and the line tension, γ, which acts to close it.

$$\sigma(R, r) = \frac{E}{4\pi R_0^2}[4\pi(R^2 - R_0^2) - \pi r^2] \tag{17}$$

The final form of equation (16) is:

$$2h\eta_m\frac{\partial r}{\partial t} = \frac{Er^2}{2}\left(\frac{R^2}{R_0^2} - 1 - \frac{r^2}{2R_0^2}\right) - \gamma \tag{18}$$

4.2 The outflux of the internal solution

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

The decrease rate of the liposome volume, V_{lip} is :

$$\frac{\partial V_{lip}}{\partial t} = Q - j_w \tag{19}$$

The flow of expelled liquid in time unit is:

$$Q = \pi r^2 v \tag{20}$$

where v is the mean leak-out velocity of internal liquid and *r* is the pore radius.

The flow on time unit has to be equal to the decrease rate of the liposome volume, V_{lip}:

The outward flow velocity of the internal liquid is obtained by equaling the pushing out force, $F_p = \Delta P_L \pi r^2$, with the shear viscosity force involved in the outward flow, $F_v = 3\pi\eta_l r v$. Given the Laplace pressure, the flow rate is the flow velocity is:$v = 2\sigma r/(3R\eta_l)$.

Here, η_l is the viscosity of aqueous solution. The incoming water flow to the liposome through its membrane due to osmotic pressure is:

$$j_w = P_w V_{\mu w} A(\Delta C_s - \beta \Delta P_L) \tag{21}$$

where, $A = 4\pi R^2 - \pi r^2$ is the membrane surface area.

Taking into account equations (20) and (21), from equation (19) the differential equation for the lipid vesicle radius is obtained:

$$4\pi R^2 \frac{\partial R}{\partial t} = \frac{2\pi\sigma r^2}{3R\eta_l} + P_w V_{\mu w}(4\pi R^2 - \pi r^2)(\Delta C_s - \beta \Delta P_L) \tag{22}$$

Given both equation (12) and the expression of Laplace pressure, the final form of the differential equation (22) is:

$$\frac{\partial R}{\partial t} = \frac{Er^2}{6\eta_l R^2}\left(\frac{R^2}{R_0^2} - \frac{r^2}{4R^2} - 1\right) + P_w V_{\mu w}\left(1 - \frac{r^2}{4R^2}\right)\left[C - \frac{2\beta E}{R}\left(\frac{R^2}{R_0^2} - \frac{r^2}{4R^2} - 1\right)\right] \tag{23}$$

4.3 The change of the internal solute concentration

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

$$\frac{d(CV_{lip})}{dt} = -\pi r^2 C v \tag{24}$$

which is equivalent with:

$$\frac{d\left[ln(CV_{lip})\right]}{dt} = -\frac{Er^2}{2\eta_l R^4}\left(\frac{R^2}{R_0^2} - \frac{r^2}{4R^2} - 1\right) \tag{25}$$

The time dependence of R(t), r(t) and C(t) during the second stage of a cycle can be obtained by solving equations (18), (23) and (25). They can be solved numerically using Euler's method.

The time dependence of the liposome radius during the swelling stage of each cycle is obtained from equation (6). Also, the pore lifetime which is equal with the liposome relaxation time can be obtained.

5. PULSATORY LIPOSOME PARAMETERS

The pulsatory liposome activity is a defined by a successive cycles of unequal length time. Each cycle is characterized by the following parameters: the length time of swelling stage, the length time of the relaxing stage, the length time of the entire cycle and the solute quantity delivered. Also, there are two important parameters that characterize the activity of a pulsating liposome: the lifetime of active liposome and the number of cycles.

The differential equation (6) and the three differential equations (18), (23) and (25) describe the dynamics of a pulsatory liposome during a cycle.

By solving this coupled system of differential equations, the time dependence of the liposome radius, R(t), pore radius, r(t), concentration of the solute, C(t) for an operating cycle is determined.

In this article, the system of differential equations (6) and {(18), (23), (25)} was solved for the first cycle of functioning of a pulsating liposome using the Euler method. The step size for numerical integration was δt = 1ms.

The initial conditions for the differential equation (6) were: the liposome radius $R(0) = R_0$ = 19.7 μm and the concentration of the osmotic solute $C(0) = C_{0s}$=11.5 mol/m^3 [14].

The initial conditions for the system of differential equations which describe the relaxing stage [(8), (23), (25)] were: the liposome radius $R(0) = R_c$ = 20.6 μm, the pore radius r(0) = 1.576 μm, the concentration of the osmotic solute is equal to the solute concentration at the end of swelling stage ($C(0) = C_{0s}R_0^3/R_c^3$), 10.04 mol/m^3 [24].

The values of the physical constants that occur in differential equations are: the permeability coefficient of the liposome membrane for water, $p_w = 3\times10^{-5}$ m/s and molar volume of water, $V_{\mu w}=18.04\times10^{-6}$m^3/mol, the stretch modulus of the lipid bilayer, E= 0.2 N/m [13], the edge tension, $\gamma = 8.1\times10^{-12}$ N [13], the lipid bilayer viscosity, η_b = 100 N·s/m^2 [12] and the aqueous solution viscosity, $\eta_l = 3.2\times10^{-2}$ N·s/m^2 [12].

The obtained results are represented in figures 2-4.

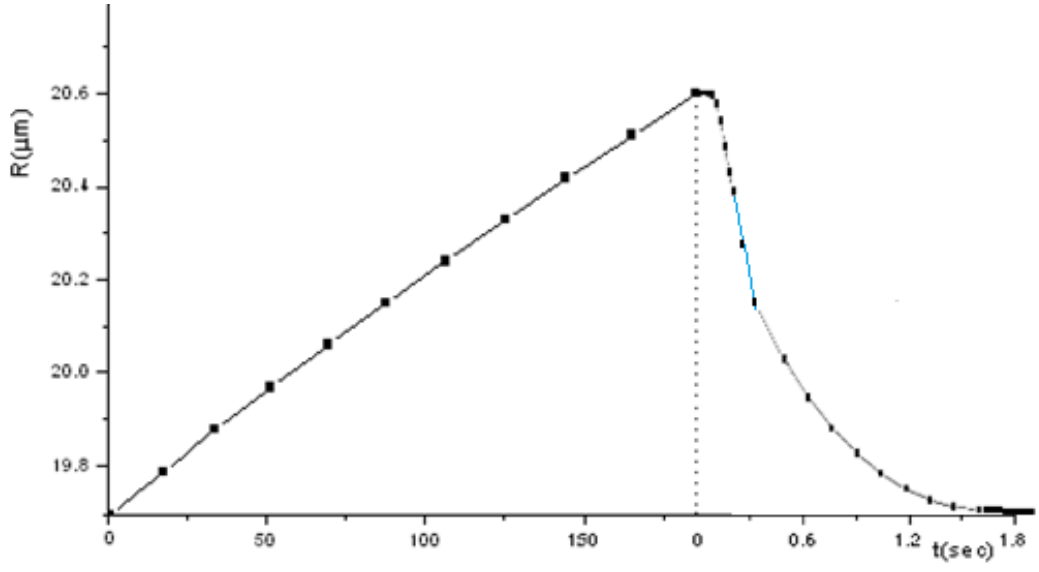

Fig. 2 The plot of the vesicle radius, R, as a function of time during to the first cycle of the pulsatory liposome

If in equation (7), $x = x_c$, the time of swelling of the pulsating liposome is obtained. In this case, the swelling time equal to τ_{sw} = 195 sec. The relaxing time is τ_{rl} = 1.8 sec.

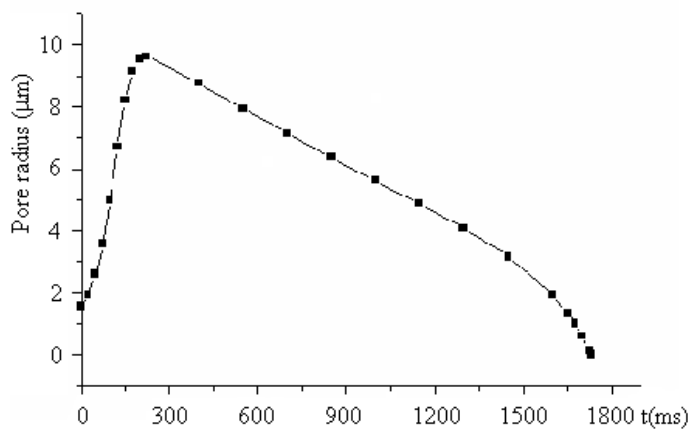

Fig. 3 The plot of the radius as a function of time

The pore radius increased up to $r_m = 9.78$ μm during $\tau_{pi} = 195$ ms, then its radius decreased until the pore disappeared in $\tau_{pd} = 1.8$ s.

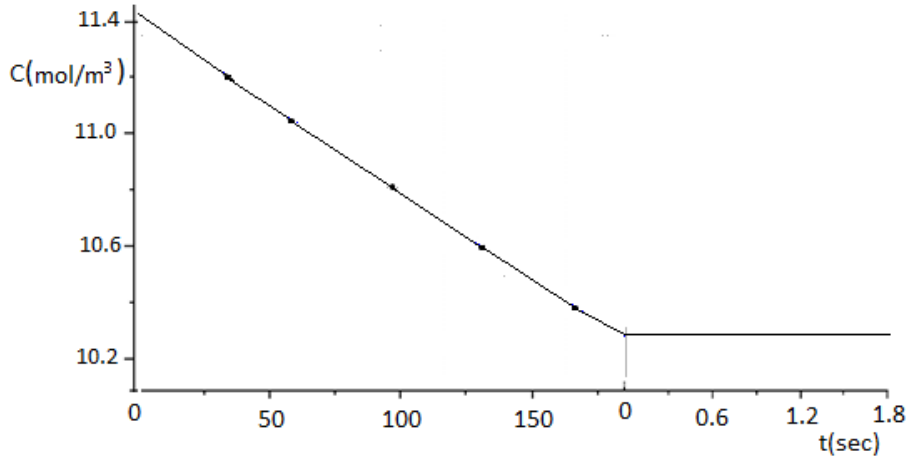

Fig. 4 The plot of the solute concentration as a function of time, during the first cycle

Solving the system of differential equations {(6), (18), (23), (25)} can be applied to the other cycles of pulsating liposome.

The initial conditions remain unchanged, except for the concentration of osmotic solvit. The value of the solute concentration at the beginning of a stage (swelling or relaxing) in the evolution of the liposome is equal to the concentration of the solute at the end of the previous stage (relaxing or swelling).

6. CONCLUDING REMARQUES

The transmembrane osmotic gradient is the driving force that causes the liposomes to swell. Osmotic pressure generates the force that causes the liposomes to swell. Eventually, liposomes can be destroyed. The appearance of a pore in the membrane of the liposome stops its swelling and changes the direction of its evolution producing its deflation. During the relaxation stage of liposomes, the radius of the pores increases to a maximum value, then decreases until the pores disappear, when the liposome reaches its original size, R_0, and a new cycle may begin.

Pulsatory liposome can be seen as a three-stroke motor: swelling stage (first stage), the liposome relaxation and growth of pore radius (the second stage) and the liposome relaxation and decrease of the pore radius (the third stage) engine. The transmembrane concentration gradient of the osmotic solute provides the functioning energy of the pulsatory liposome. The osmotic solute is the "fuel" of the pulsatory liposome, which is consumed due to its delivery into external medium. The consumed fuel may be a pharmacological substance, or any other special substances.

Pulsatory liposomes, free or included inside other vesicles, may reach hepatocytes due to hydrodynamic effects of blood circulation [28].

The transient pores in liposomes could also be used for compensation of neurotransmitter deficiency in the synaptic cleft [29, 30].

The pulsatory unilamellar liposome is an example of a bionic micro engine, with potential applications in chemotherapy [31].

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