DETERMINATION OF THE PARAMETERS CHARACTERIZING A CYCLE OF THE PULSATORY VESICLE

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Abstract. In this paper, we have obtained the parameters characterizing the first cycle of the periodical working of a pulsatory vesicle. Under certain conditions, if a unilamellar lipid vesicle filled with aqueous solution of an osmotic substance is introduced into a hypotonic aqueous medium, it becomes a periodical dynamic system. Each cycle may be characterized by some dynamical parameters and useful quantities resulting by solving analytically the differential equation describing the swelling stage of the cycle and a numerical solution of a system of three differential equations which models the periodic stage of the cycle.

Key words: Pulsatory liposome, dynamical parameters, useful parameters.

INTRODUCTION

The pore appearance in lipid bilayers following some controlled processes may be a useful and interesting way for transmembrane transport [10, 12].

The pore appearance in plane lipid bilayer may be influenced by thickness fluctuations [11, 12, 15] or by structural defects [13, 14].

In the lipid vesicle the pore appearance may be favored by mechanical tension induced by different ways [1, 2, 4–6, 17, 27, 28]. Recently, a sequence of 30–40 pores was observed in the same giant vesicle, a pore at a time, which can appear in vesicles stretched by optical induced mechanical tension [3, 7]. There are

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two very interesting biotechnological applications which request the increase of membrane permeability: gene therapy and targeted special substances delivery [29, 30].

In our previous papers we have shown how a lipid vesicle can release the drug molecules, in a well-controlled fashion [18–20]. It must work as a pulsatory liposome. Its energy is supplied by the concentration gradient across membrane of an impermeant solute.

In this paper, we will make an analysis of the two stages of a cycle of the working pulsatory liposome: swelling and relaxation. Then, we give the dynamic parameters characterizing a cycle of the periodical activity of a pulsatory vesicle. Before the two parts highlighted before, we placed a subchapter which contains a description of the phenomenological base of the running of a pulsatory liposome. This subchapter is similar to that from a paper published before [22].

MATERIAL AND METHODS

PHENOMENOLOGICAL BASE OF A PULSATORY LIPOSOME

Let us consider a liposome filled with aqueous solution containing an osmotic solute. The initial state of the liposome is characterized by smooth and unstretched lipid membrane and by the internal solute concentration. It is considered as equilibrium reference state. This liposome is inserted into a bath with a hypotonic aqueous medium. So, the reference state becomes the initial state of the liposome dynamics. Due to the osmotic pressure, created by the transmembrane gradient of solute concentration, water molecules inflow inside to liposome, across their membrane.

The osmotic flow of solvent determines: 1) the swelling of the liposome; 2) the stretching of liposome membrane; 3) the dilution of the internal solution. Also, the surface tension increases at the same time with the liposome expansion. The surface tension increases the pressure inside the vesicle, while the osmotic pressure decreases [22, 23, 25].

Under these experimental conditions, either the liposome membrane may be ruptured and destroyed or one pore may appear through its lipid bilayer. If the swelling process is slow enough, the liposome increases up to a critical size, when a transient transmembrane pore appears. This event is followed by two simultaneous processes: 1) the pore dynamics, and 2) the leak out of the internal material of the vesicle, due to the excess Laplace pressure.

The pore dynamics consists of two successive phases: 1) first the pore radius increases up to the maximum value, r_m, and 2) followed by the decrease of the pore radius until the perfect closure of the pore [24, 25].

Both processes, the increase of the pore size and the leakage of the internal liquid, determine the membrane relaxation due to the reduction in the membrane tension.

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 the membrane tension.

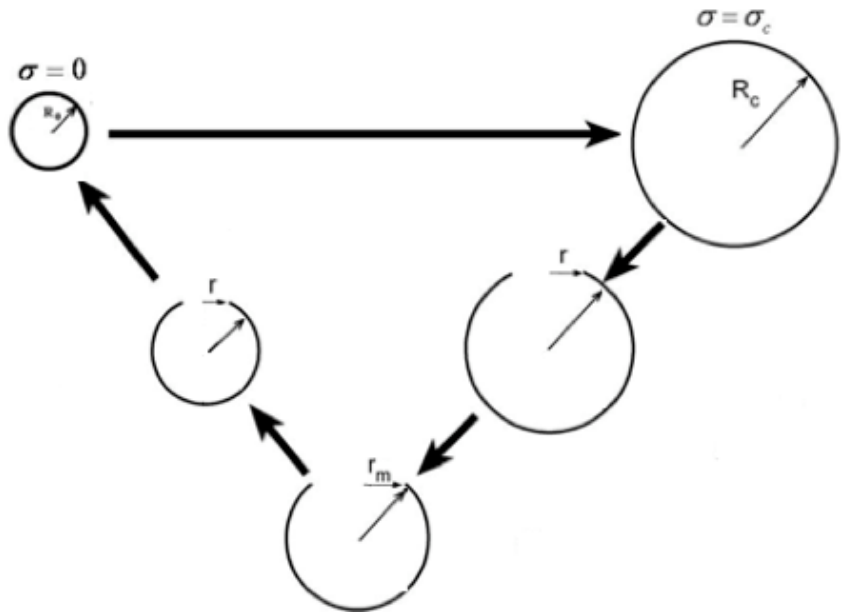

Fig. 1. A cycle of the pulsatory liposome. In the first stage, the liposome swells from the initial state of radius, R_0, to the critical state of radius, R_c, when a transbilayer pore appears (the top part of the picture). In the second stage, the pore radius increases up to a maximum value, r_m, after that the pore radius decreases up to the pore disappearance. Simultaneously with the pore evolution, the liposome relaxes up to its radius becomes equal to R_0 (the bottom part of the picture) [21].

From the moment when the line tension equals the membrane tension the second part of the pore dynamics starts, and so the pore radius reduces until the pore closes. Therefore, the liposome comes back at its initial size. We can envision that the dynamics of the liposome described above can start over. In Fig. 1 a cycle of a pulsatory liposome was drawn.

This cyclic process ceases when the osmotic pressure becomes smaller than the excess Laplace pressure.

The competition between the osmotic flow across the membrane and the internal solution leakage determines the pore evolution and the rate of the vesicle decreases.

In what it follows we will describe a mathematical modelling of the two parts of a pulsatory liposome cycle: the liposome swelling and its relaxation.

LIPOSOME SWELLING STAGE

In the initial state the liposome is characterized by its radius R_0, membrane area S_0, volume V_0, membrane tension $\sigma = 0$ and solute concentration C_0. In the swelling stage, the liposome radius increases from initial value R_0 to a critical value R_c due to water influx. The change of the liposome volume is determined by osmotic influx of water and is described by the following equation:

$$\frac{\mathrm{d}V}{\mathrm{d}t} = P_{\mathrm{w}} V_{\mu \mathrm{w}} S \left(\Delta C - \frac{\Delta P}{N_{\mathrm{A}} k_{\mathrm{B}} T} \right) \tag{1}$$

The notations from equation (1) have the following significances: V is the liposome volume; P_w (measured in m/s) is the water permeability through liposome membrane; $V_{\mu w}$ is the water molar volume (in m^3/mol); S is the membrane area; ΔC (measured in mols/m^3) is the transmembrane solute concentration gradient; ΔP is the 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 formula:

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

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

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

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

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

The internal solute amount is conserved all the time of the liposome swelling. For the swelling of the liposome during to the first cycle, we can write:

$$C_0 V_0 = CV \tag{4}$$

where, C_0 is the initial solute concentration, C is the solute concentration when the liposome has reached the volume V during the swelling process.

If one considers the external solute concentration is equal to zero, then $\Delta C = C(t)$.

With equations (2), (3), and (4) in mind, we find from equation (1) that:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = P_{\mathrm{w}} V_{\mu \mathrm{w}} \left(\frac{C_0 R_0^3}{R^3} - \frac{2\beta E}{R^2} \frac{R^2 - R_0^2}{R} \right) \tag{5}$$

In the above formula we have used the following notation:

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

The analytical solution of equation (5) is:

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

with the initial condition, $R(0) = R_0$, and the following notations:

The swelling time of the liposome can be calculated from the following equation:

$$z(t) = \frac{R^2(t)}{R_0^2} \tag{8}$$

$$\alpha = \sqrt{1 + \frac{2C_0 R_0}{\beta E}} \tag{9}$$

$$R_0 \sqrt{z(t)} = R_c \tag{10}$$

LIPOSOME RELAXATION STAGE

Leak-out of the internal liquid

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

The net flow on time unit has to be equal to the decrease rate of the vesicle volume, V:

$$4\pi R^2 \frac{\partial R}{\partial t} = -\frac{2\pi\sigma r^3}{3R\eta_l} + P_w V_{\mu w} \left(4\pi R^2 - \pi r^2\right) \left(\Delta C_s - \frac{\Delta P}{N_A k_B T}\right) \tag{11}$$

where the first term is the volume of internal liquid leaked out through the opened pore and the second term is the water volume entered inside the liposome in time unit.

Taking into account that the outward flow velocity of the internal liquid is [12]:

$$v = \frac{2\sigma r}{3R\eta_l} \tag{12}$$

and having formulae (2) and (3) in mind, the final form of the differential equation (10) is:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = -\frac{Er^3}{6R_0^2\eta_1}\frac{R^2 - R_0^2}{R^3} + P_{\mathrm{w}}V_{\mu\mathrm{w}}\left(1 - \frac{r^2}{4R^2}\right)\left(C - \frac{2\beta E}{R_0^2}\frac{R^2 - R_0^2}{R}\right) \tag{13}$$

This differential equation describes the evolution vesicle radius during the relaxation process.

The dynamics of the transbilayer pore

In order to find an equation for pore radius we have considered an energetic balance.

The surface free energy change due to the bilayer deformation following the pore appearance is equal to [7, 19, 24]:

$$\Delta E_{\mathrm{e}} = \pi r^2\sigma - 2\pi r\gamma \tag{14}$$

and is dissipated into lipid 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 of thickness 2h, is:

$$\Delta E_{\mathrm{v}} = 4\pi r\eta_{\mathrm{b}}h\frac{\mathrm{d}r}{\mathrm{d}t} \tag{15}$$

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

$$4\eta_{\mathrm{b}}h\frac{\mathrm{d}r}{\mathrm{d}t} = r\sigma - 2\gamma \tag{16}$$

Pore opening is driven by the membrane tension, σ, and its closure by the line tension, γ [8, 24].

The pore appearance in lipid vesicles is more complex than in the plane lipid bilayers, because:

1) In vesicles the membrane tension changes as the pore grows, while in the plane lipid bilayer the surface tension is constant inasmuch as there is a lipid reservoir around it;

2) The line tension cannot be neglected.

The composition change of the internal liquid

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

$$\frac{\mathrm{d}(CV)}{\mathrm{d}t} = -\pi r^2 C v \tag{17}$$

which is equivalent with:

$$\frac{\mathrm{d}\left[\ln(CV)\right]}{\mathrm{d}t} = -\frac{\sigma r^3}{2\eta_l R^4} \tag{18}$$

The equations (13), (16) and (18) form a system of three differential equations that can be solved numerically using Euler's method to obtain the time dependence of $R(t)$, $r(t)$ and $C(t)$ during the second stage of a cycle of the periodic process.

Also the pore lifetime which is equal to the liposome relaxation time can be obtained. The most important parameters are: inside solute concentration, internal liquid viscosity and bilayer viscosity.

RESULTS AND DISCUSSION

There are two types of parameters characterizing a pulsatory liposome:

- dynamic parameters: liposome radius, $R(t)$; pore radius, $r(t)$; internal solute concentration, $C(t)$; internal solute quantity, $Q(t)$; external delivered solute, $Q_{out}(t)$.
- useful parameters: number of cycles, length time of cycle, delivered solute on cycle.

The dynamical parameters regard both stages of a cycle and are obtained as a solution of the differential equations mentioned above. From this reason we will specify some details regarding the two stages of a cycle: swelling stage and relaxing stage.

SWELLING STAGE

We have considered a unilamellar liposome inserted into a large box containing water. In the relaxed state the liposome radius is equal to $R_0 = 19.7$ μm. The relaxed state is the initial state of each cycle of pulsatory liposome. In the relaxed state the liposome membrane is unstretched ($\sigma = 0$).

Such unilamellar vesicles were used in experimental studies [15]. When the vesicles reach the critical size ($R_c = 20.6$ μm) the membrane ruptures and a pore opens.

We have supposed that the liposome swells up to the critical state due to osmotic stress. The swelling time was calculated using the formula (7) applied to the above liposome for five initial concentrations of the internal aqueous solution of a non permeating solute: $C_0 = 0.01$ M. The membrane permeability coefficient

for water is equal to $p_w = 3\times10^{-5}$ m/s [7] and water molecular volume is $V_{\mu w} = 18.04\times10^{-6}$ m^3/mol. The two-dimensional stretch modulus of the lipid bilayer is $E = 0.2$ N/m [3].

The analytical solution of the differential equation (7) for swelling liposome gives the time as a function of liposome radius: $t = t(R)$.

The dependence of the liposome radius, R, on the time was easily obtained by inversion of the function $t = t(R)$.

It is interesting that for small solute concentrations the dependence of the swelling liposome radius on time is linear.

This cannot be true for greater solute concentrations. The method of the numerical calculations of the swelling time at different values of liposome radius during the expanding process and the determination of the inverse function $R = R(t)$ by fitting the above calculated values may be used for large solute concentration gradients.

THE RELAXING STAGE

We solved the system of three differential equations using Euler's method with the step size of $\delta t = 1\mu$s in order to see the dependence of $r(t)$, $R(t)$ and $c(t)$ on time. Before numerical integration all the three equations were prepared by scaling of the variables and parameters. The initial conditions were: $r(0) = 1.576$ μm; $R(0) = R_c$ and $C(0) = 0.01$M.

The differential equations (13), (16) and (18) were integrated for a liposome used in experimental studies. The vesicle radius in relaxed state ($\sigma = 0$) is $R_0 = 19.7$ μm, and the critical radius is $R_c = 20.6$ μm [3, 7, 31]. The line tension is $\gamma = 8.1\times10^{-12}$ N [7]. The membrane viscosity is $\eta_b = 100$ N·s/m^2 and the aqueous solution viscosity is $\eta_l = 3.2\times10^{-2}$ N.s/m^2 [3, 26]. The elastic module for surface stretching is $E = 0.2$ N/m. The bilayer thickness is 4 nm.

THE CYCLE OF THE PULSATORY LIPOSOME SIZE

In order to find the dependence on time of the liposome radius, R, during the swelling process, we have used an indirect method for solving the equation (7). So, we have calculated the time of liposome swelling up to some given values of liposome radius. Then, we represent the vesicle radius values for the corresponding time values. Finally, a fitting function must be found.

The evolution of the liposome radius during the relaxing stage was obtained by numerical integration of the differential equation (13) with the boundary condition $R(0) = R_c$.

In Fig. 2 we have represented the evolution of the liposome radius during the first cycle of its active life.

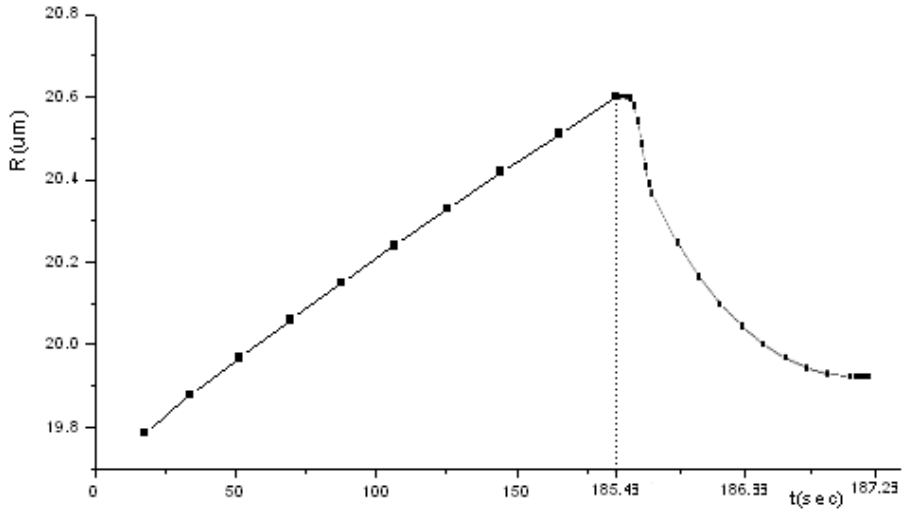

Fig. 2. The first cycle of the pulsatory liposome of initial radius $R_0 = 19.7$ μm filled with an osmotic solute concentration $C_0 = 0.01$ M. The plot of the vesicle radius, R, as a function of time during the first cycle of the liposome pulsatory running. This is the solution of the differential equations (5) and (13).

The dynamics of liposome radius is described by equation (5) along the swelling stage and equation (13) during the relaxing stage. The graph looks like a saw tooth. Probably the length will be shorter with the increasing of the cycle rank, especially due to swelling stage.

The increasing part of the graph, corresponding to the swelling stage, may be fitted by a polynomial function of two degree:

$$R(t) = 19.69513 + 0.00547\,t - 3.2285 \times 10^{-6}\,t^2 \tag{19}$$

We can see that the liposome radius at the end of the first cycle is not equal to R_0. In this case the initial liposome radius at the beginning of the second cycle is the final value at the end of the first cycle.

The evolution of the pore size during the first cycle was plotted in Fig. 3. From this picture we can see pore evolution along the first cycle. During the swelling stage the pore is closed, $r(t) = 0$. Then, suddenly, it appears. The nucleation radius is about 2 μm. The transbilayer pore increases up to its maximum radius ($r_{max} \approx 10$ μm) in 0.225 s. Then its radius decreases to zero, that is the pore closes. The length life of the first pore is 1.8 s.

In Fig. 4 we have represented the change of the solute concentration during the pore life, when the aqueous solution leaks out the vesicle. It is very interesting that the solute concentration decreases linearly during the vesicle relaxation.

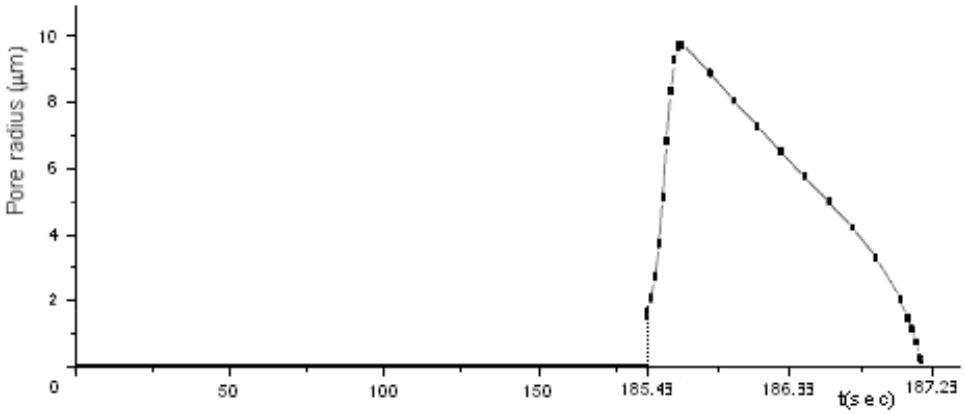

Fig. 3. The evolution of the transmembranar pore radius during the first cycle of the pulsatory liposome active life.

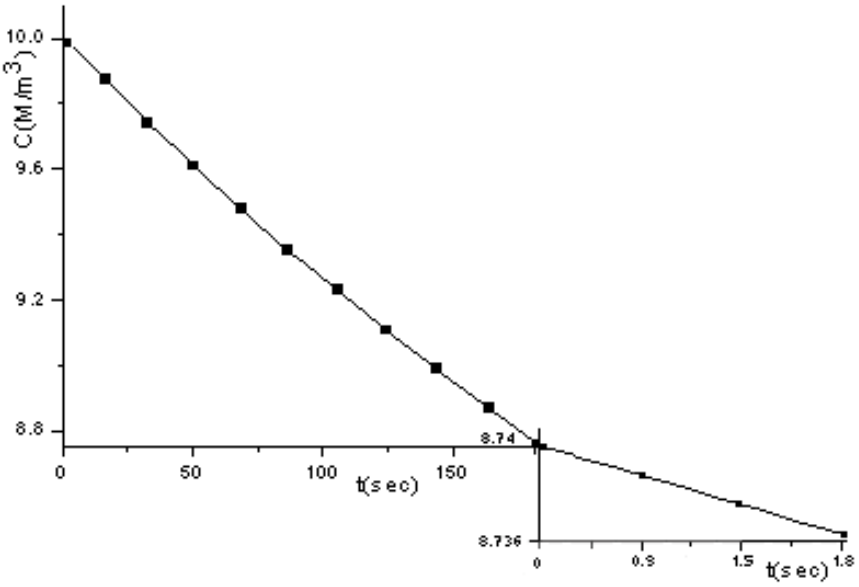

Fig. 4. The plot of the solute concentration inside of liposome as a function of time, during the first cycle of the pulsatory liposome. It is the solution of the differential equation (18).

The internal solute concentration changes due to water influx in the swelling stage and due to both the water influx and leakage of internal solutin through pore, in the relaxing stage. The internal solute concentration dependence on time is represented in orthogonal systems of different scales. The two orthogonal systems were positioned, one to another, to see the difference between the concentration change in the two stages of a cycle.

In Fig. 5 is represented the internal solute quantity along the first cycle. The initial solute quantity of osmotic solute is equal to 0.64 pmols. It remains unchanged during the swelling stage, but decreases in the relaxing stage due to the internal solution delivery in the external medium.

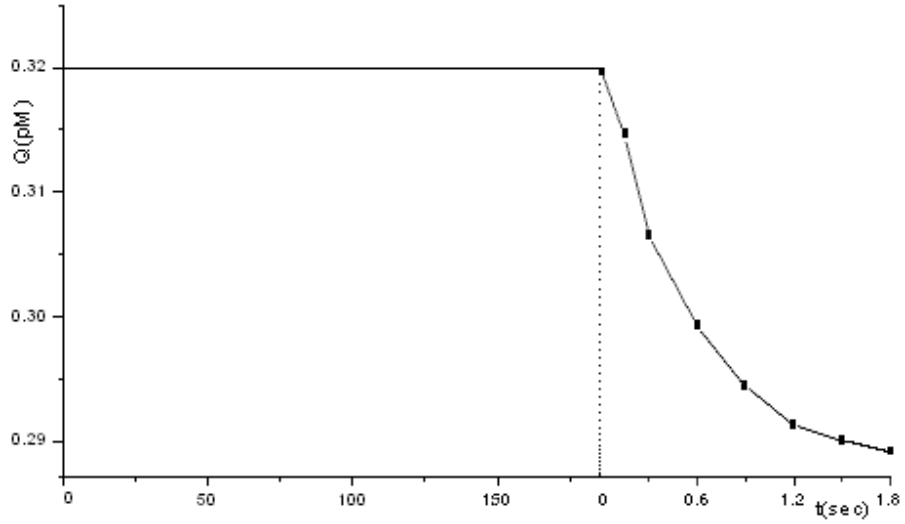

Fig. 5. The plot of the solute quantity inside of the liposome as a function of time, during the first cycle of the pulsatory liposome. This is the solution of the differential equation (18).

The solute leakage is determined by the pore size change and the excess Laplace pressure.

During first cycle the osmotic solute amount decreases only 0.03 pmols.

Using formula (7) we can calculate the swelling time for a particular liposome which has the initial radius $R_0 = 19.7$ μm and the critical radius $R_c = 20.6$ μm. The swelling time decreases with the increasing of the initial solute concentration.

For example, for initial concentration $C_0 = 0.01$ M, the swelling time is equal to $t_{sw} = 185.43$ s, but for the initial concentration $C_0 = 0.1$ M, the swelling time is equal to $t_{sw} = 20$ s.

An interesting and useful parameter is the liposome radius when it ceases to work. This parameter may be calculated from equation (7) for t is infinite.

We have calculated this parameter for the case in which the solute concentration at the initial state of the last cycle has the same value as those used above.

From this mathematical description of a cycle of the pulsatory liposome we have achieved to determine the characterising parameters of the active life of a pulsatory liposome. For example, if the initial concentration is equal to $C_0 = 0.01$ M the length time of the first cycle is:

$$T = t_{sw} + \tau_{\text{life pore}} = 185.43 + 1.8 = 187.23 \text{ seconds} \tag{20}$$

The active substance delivered in the external bath during the first cycle is:

$$q = C_0 V_0 - C_1 V_0 = 4\pi\left(C_0 - C_1\right) R_0^3 = \left(10 - 8.735\right) \times 4 \times 3.14 \times 19.7^3 \times 10^{-18} = 12.1472 \times 10^{-14} \text{ moles} = 73.1572 \times 10^9 \text{ molecules} \tag{21}$$

The number of cycles is equal to the number of the repetition of the above calculus up to when the running condition is not accomplished. Also, the length time and the amount of the delivered substances one calculates for each cycle.

The pulsatory liposome may be programmed to work a number of cycles, to deliver a certain amount of active substance a priory fixed time.

This is useful to make a medical treatment of a molecular ill place from an organism, or to deliver genetic material somewhere we have interest.

The pulsatory liposome may be regarded as a two stroke engine using the osmotic solute as a fuel. But the consumed fuel is a useful material.

In the following paper we will study the influence of the material parameters and initial condition in the pulsatory liposome activity.

The pulsatory liposome can be used for drug administration at ill places. In our opinion the drug quantities should be sufficient to have a benefit 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 [19–21].

The preparation of pulsatory liposomes with such properties and their 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 [16].

The pulsatory liposome free or included inside other vesicle may reach the hepatocyte due to hydrodynamic effects of blood circulation [16].

Finally, we think that, in the future, the pulsatory liposome may be used as a special device for active substances dosage.

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