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Pulsatory Liposome: A Possible Biotechnological Device

Dumitru Popescu and Alin Gabriel Popescu

Abstract

A unilamellar liposome filled with an osmotic solution is introduced into a hypotonic aqueous environment. Because of the mechanical tension induced by the osmotic flow, the vesicle swells up to a critical size, when suddenly a transbilayer pore appears and the vesicle relaxing stage starts. A part of the intracellular material leaks out through this pore, and the liposome membrane relaxes and finally recovers. The swelling begins again and the liposome experiences a periodical process. For this reason, we have named it a pulsatory liposome. The swelling of the liposome is described by a differential equation. All the processes which contribute to the vesicle relaxing and its coming back to the initial size are described by three differential equations. The pulsatory liposome can be programmed to work a number of cycles, established before. The activity of a pulsatory liposome can be characterized by the following parameters: (a) number of cycles, the length time of each cycle, and liposome activity life; (b) the length time of the swelling stage and the relaxation stage for each cycle; (c) the amount of solute leaked out through the pore in each cycle. The pulsatory liposome may be regarded as a two-stroke engine.

Keywords: osmotic gradient, two-stroke engine, biotechnological device

1. Introduction

The transport of ions and molecules across cellular membranes is very important for many biological processes.

The pore appearance in lipid bilayers following some controlled processes may be an interesting way for transmembrane transport of molecules, especially large ones, with usefulness in some biotechnological applications [1].

There is a type of pore, called a stochastic pore, which can occur due to the structural and dynamic properties of the lipid bilayer [2–7]. On the other hand, the mechanical stretching induced in various ways in the lipid vesicle membrane may favor the appearance of transmembrane pores [8–13]. There are two interesting biotechnological applications that request the increase of membrane permeability: gene therapy and targeted special substances delivery. In the first application, the transport of DNA fragments through cellular and nuclear membranes is requested. In the second application, one uses special substances molecules

encapsulated in vesicles, which must be transported to a previously established target location [14, 15].

In this chapter, we are writing about the dynamics of a pulsatory liposome. Such liposome makes a cyclic activity that may be described by a differential equation (the swelling stage) and a system of three-differential equations (the relaxing stage). This liposome may be programmed to work a certain number of cycles, settled in advance, and can release the drug molecules, in a well-controlled fashion. Also, we will calculate the amount of osmotic solute delivered during each cycle.

2. Phenomenological bases of a pulsatory liposome

Let us consider a unilamellar liposome filled with an aqueous solution of an osmotic solute. A solute for which the liposome membrane is impermeable is named osmotic solute.

This liposome is placed into a bath containing a hypotonic aqueous solution.

The osmotic flow of solvent determines three simultaneous processes: (1) the swelling of the liposome; (2) the dilution of the internal solution; (3) the stretching of liposomal membrane. The surface tension also increases at the same time as this liposomal expansion.

The swelling process is slow enough. The liposome increases up to a critical size when a transmembrane pore appears. This event is very important for liposome's life because it changes the sense of their evolution. The pore appearance is followed by two simultaneous processes: the pore dynamics and the outflow of internal solution from vesicle [16–19].

Figure 1 represents 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. 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 releases solution outside and relaxes until its radius becomes equal to R_0 .

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

The internal liquid continues to leak outside the liposome, even after the edge tension equals the membrane tension. From the moment when the edge tension equals the membrane tension, the second part of the pore dynamics starts, and the pore radius reduces until the pore closes. Therefore, the liposome comes back to its initial size. In this new state, the liposome membrane is untensed but the solute concentration is less than in the preceding initial state. The dynamics of the liposome described above can restart over and over again. This cyclic process ceases when the osmotic gradient becomes equal to a critical value, which will be discussed below. In what follows we will describe a mathematical modeling of the two parts of a pulsatory liposome cycle: the liposome swelling and its relaxation.

3. The liposome swelling

Due to the influx of water, the liposome swells and its radius increases from an initial value of R_0 to a critical value of R_c . The liposome volume change is described by the following equation [20–22]:

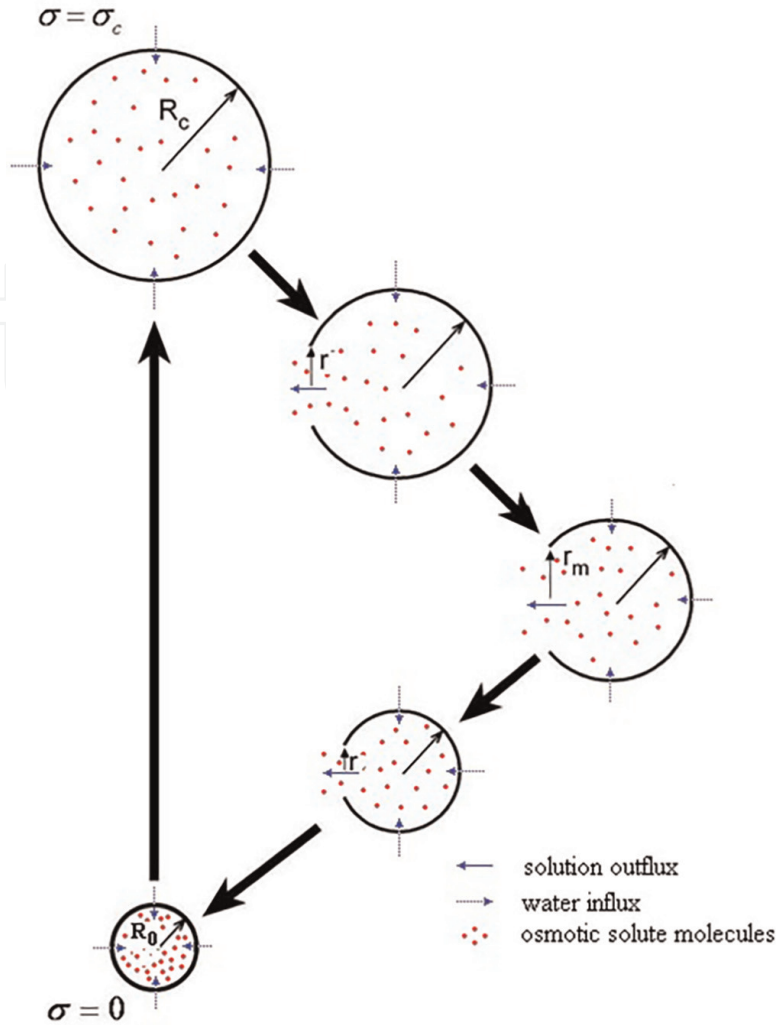


Figure 1.
 A cycle of the pulsatory liposome.

$$\frac{dV}{dt} = \frac{P_w V_{\mu w} A}{\mathcal{R}T} (\Delta P_{\text{osm}} - \Delta P_L) \quad (1)$$

The notations from Eq. (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), A is the membrane surface area, \mathcal{R} is the universal gas constant, and T is the absolute temperature.

The osmotic pressure, ΔP_{osm} , is equal to:

$$\Delta P_{\text{osm}} = \mathcal{R}T\Delta C_s \quad (2)$$

where ΔC_s (measured in mol/m^3) is the transmembrane solute concentration gradient.

The Laplace pressure is given by the formulae:

$$\Delta P_L = \sigma \left(\frac{1}{R-h} + \frac{1}{R+h} \right) \quad (3)$$

where σ is the tension of the stretched membrane, h is the hydrophobic core thickness, and R is the average radius of the liposome. Taking into account that the vesicle considered here is sufficiently large, we cut h , for now on.

According to Hooke law, if the radius of a spherical membrane increases from R_0 to R , the surface tension σ is:

$$\sigma(R) = E \left(\frac{R^2}{R_0^2} - 1 \right) \quad (4)$$

where E is the elastic modulus for surface stretching.

During the swelling stage of the liposome, the solute amount does not change. Therefore, we can write:

$$C_{0s} V_0 = C_s V = C_{fs} V_c \quad (5)$$

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 is equal to zero, then $\Delta C_s = C_{0s}$.

With Laplace pressure formula and Eqs. (4) and (5) in mind we find from Eq. (1):

$$\frac{dR}{dt} = P_w V_{\mu w} \left(\frac{C_{0s} R_0^3}{R^3} - \frac{2\beta E}{R_0^2} \frac{R^2 - R_0^2}{R} \right) \quad (6)$$

In the above-written equation we have used the following notation:

$$\beta = \frac{1}{RT} \quad (7)$$

By integrating the Eq. (6) one obtains the liposome radius $R(t)$ as a function of time. The initial condition is: $R(0) = R_0$

The analytical solution to the Eq. (6) is:

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

where

$$z(t) = \frac{R^2(t)}{R_0^2} \quad (9)$$

$$\alpha = \sqrt{1 + \frac{2C_{0s} R_0}{\beta E}} \quad (10)$$

4. The liposome relaxation

The liposome swells up to when suddenly a transbilayer pore appears when the liposome reaches its critical size [23]. From this moment the liposome relaxation starts.

During this stage of the cycle, two simultaneous processes take place: the evolution of the pore from birth to its disappearance and the relaxation of the liposome from the critical state with the radius, R_c , to the initial state with the radius, R_0 .

4.1 The differential equation for transbilayer pore dynamics

The change of the surface free energy 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 parameter η_m [24–26]. Equating the two energy changes for the lipid bilayer, one obtains a differential equation for the dynamics of the pore radius [22, 27]:

$$2\pi r\eta_m 2h \frac{\partial r}{\partial t} = \pi r^2 \sigma - 2\pi r \gamma \quad (11)$$

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

According to the Hooke law, the membrane tension is equal to:

$$\sigma(R, r) = \frac{E}{4\pi R_0^2} [4\pi(R^2 - R_0^2) - \pi r^2] \quad (12)$$

The final form of Eq. (11) is:

$$2h\eta_m \frac{\partial r}{\partial t} = \frac{Er^2}{2} \left(\frac{R^2}{R_0^2} - 1 - \frac{r^2}{2R_0^2} \right) - \gamma \quad (13)$$

4.2 The differential equation of the internal liquid leak

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

The amount of expelled liquid in time unit is:

$$Q = \pi r^2 v \quad (14)$$

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

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

$$\frac{\partial V_{lip}}{\partial t} = Q - j_w \quad (15)$$

The outward flow velocity of the internal liquid one obtains by equating the pushing out force, $F_p = \Delta P_L \pi r^2$, with the shear viscosity force involved in the outward flow, $F_v = \pi \eta_l r v$.

Taking into account, the formula of the Laplace pressure, 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 imbalance is:

$$j_w = P_w V_{\mu w} A (\Delta C_s - \beta \Delta P_L) \quad (16)$$

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

Taking into account the above equation, from Eq. (15) one obtains an equation for the vesicle radius:

$$4\pi R^2 \frac{\partial R}{\partial t} = \frac{2\pi\sigma r^3}{3R\eta_l} + P_w V_{\mu w} (4\pi R^2 - \pi r^2) (\Delta C_s - \beta \Delta P_L) \quad (17)$$

Given both Eq. (12) and the expression of Laplace pressure (12), the final form of the differential Eq. (17) is:

$$\frac{\partial R}{\partial t} = \frac{Er^3}{6\eta_l R^3} \left(\frac{R^2}{R_0^2} - \frac{r^2}{4R^2} - 1 \right) + P_w V_{\mu w} \left(1 - \frac{r^2}{4R^2} \right) \left[C - \frac{2\beta E}{R} \left(\frac{R^2}{R_0^2} - \frac{r^2}{4R^2} - 1 \right) \right] \quad (18)$$

4.3 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:

$$\frac{d(CV_{lip})}{dt} = -\pi r^2 C_v \quad (19)$$

which is equivalent with:

$$\frac{d[\ln(CV_{lip})]}{dt} = -\frac{Er^3}{2\eta_l R^4} \left(\frac{R^2}{R_0^2} - \frac{r^2}{4R^2} - 1 \right) \quad (20)$$

The system of differential Eq. (13), (18), and (20) can be solved numerically using Euler's method to obtain the time dependence of $R(t)$, $r(t)$, and $C(t)$ for the relaxing stage of a cycle. The time dependence of the liposome radius for the first stage of each cycle is obtained from Eq. (6). Also, the pore lifetime which is equal to the liposome relaxation time can be obtained.

5. The parameters characterizing a pulsatory liposome

The parameters that characterize the activity of the pulsating liposome are: (a) the length time of the swelling stage and the length time of the relaxation stage for each cycle, the length time of each cycle, and the lifetime of the liposome activity; (b) the quantity of solute leaked out through the pore in each cycle; (c) the number of cycles.

All these parameters can be obtained by solving Eq. (6) coupled with the system formed by differential Eqs. (13), (18), and (20) as a series of recurring systems of differential equations.

We have considered a unilamellar liposome inserted into a large box that contains water. In the initial state, the liposome radius is equal to $R_0 = 19.7 \mu\text{m}$ [12]. R_0 is the initial value of the liposome radius for each cycle.

The liposome swells up to the critical state due to osmotic stress. When the vesicle reaches the critical size ($R_c = 20.6 \mu\text{m}$ [12]), a pore suddenly opens. For the relaxing stage of each cycle R_c is the initial value of the liposome radius and σ_c is the initial value for membrane tension σ .

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).

The initial concentrations of the internal aqueous solution of a non-permeating solute were $C_{0s} = 11.5 \text{ mol/m}^3$.

The swelling time of the pulsatory liposome was calculated using the Eq. (8) in which $R(t) = R_c$. The membrane permeability coefficient for water p_w is equal to $3 \times 10^{-5} \text{ m/s}$, and water molar volume is $V_{\mu w} = 18.04 \times 10^{-6} \text{ m}^3/\text{mol}$. The two-dimensional stretch modulus of the lipid bilayer is $E = 0.2 \text{ N/m}$ [11]. Such unilamellar vesicles were used in experimental studies [11, 12].

The swelling time during the first cycle is $\tau_1 = 161.36 \text{ sec}$. The time dependence of the liposome radius is represented in **Figure 2**. The initial internal concentration solute of aqueous solution is equal to 11.4 mol/m^3 .

The radius of the pulsatory liposome has a nearly linear dependence on time during swelling stage (**Figure 2**).

For the study of the relaxing stage of the first cycle of the pulsatory liposome working, we solved the system of three differential equations (13), (18), and (20) using Euler's method with a step size $\delta t = 1 \text{ ms}$ in order to see the time dependence of $r(t)$, $R(t)$, and $C(t)$. Before numerical integration, all three equations were prepared by scaling the variables and parameters.

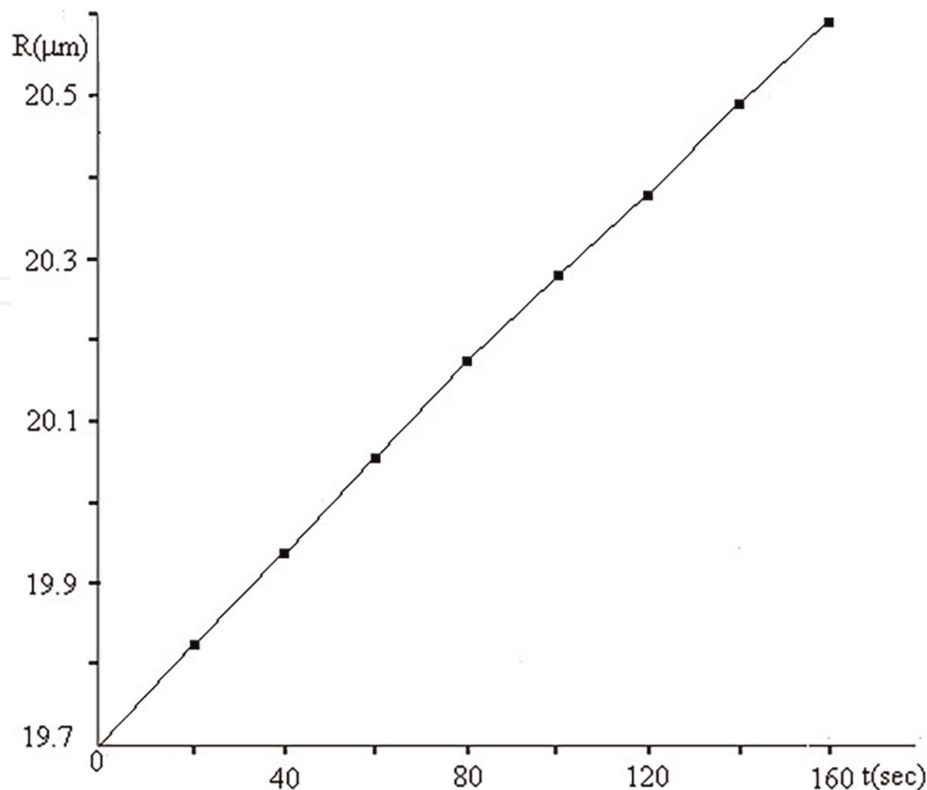


Figure 2.
The dependence of the liposome radius on time during the swelling process of a liposome inserted into water medium.

The initial conditions were: $r(0) = 1.576 \mu\text{m}$, $R(0) = 20.6 \mu\text{m}$, and $C(0) = 10.04 \text{ mol/m}^3$ [23].

The liposome radius $R(0)$ is equal to critical radius at the end of swelling stage. The initial solute concentration is equal to the solute concentration at the end of swelling stage ($C(0) = C_{0s}R_0^3/R_c^3$).

The edge tension was $\gamma = 8.1 \times 10^{-12} \text{ N}$ [11]. The lipid bilayer viscosity was $\eta_b = 100 \text{ N}\cdot\text{s/m}^2$ [10]. The aqueous solution viscosity was $\eta_l = 3.2 \times 10^{-2} \text{ N}\cdot\text{s/m}^2$ [10].

The pore evolution along its lifetime has drawn in **Figure 3**. In the first part of relaxing stage the pore radius increased up to $r_m = 9.78 \mu\text{m}$ during $t = 225 \text{ s}$, then its radius decreased until the pore disappeared in 1520 s.

The evolution of the pore size is plotted in **Figures 2–4**. We have drawn the pore evolution before reaching the maximum value of its radius (**Figure 2**), and after it reached its maximum size (**Figure 3**).

In **Figure 4** we have plotted the evolution of the vesicle size during the second stage of a cycle, that is during the relaxing of the vesicle.

For a more detailed image, we have drawn the vesicle radius evolution before and after reaching the maximum pore radius.

In **Figure 5** we have plotted the change in solute concentration during the pore lifetime when the aqueous solution leaks out of the vesicle. It is very interesting that the solute concentration decreases linearly during vesicle relaxation.

6. Programming the working of a pulsatory liposome

The internal solute concentration decreases along a cycle and with the cycle rank in sequence, and as a consequence, the osmotic pressure decreases too (**Figure 6**) [23, 28].

The liposome will swell up to its critical radius only if the osmotic pressure during the cycle is greater than excess Laplace pressure.

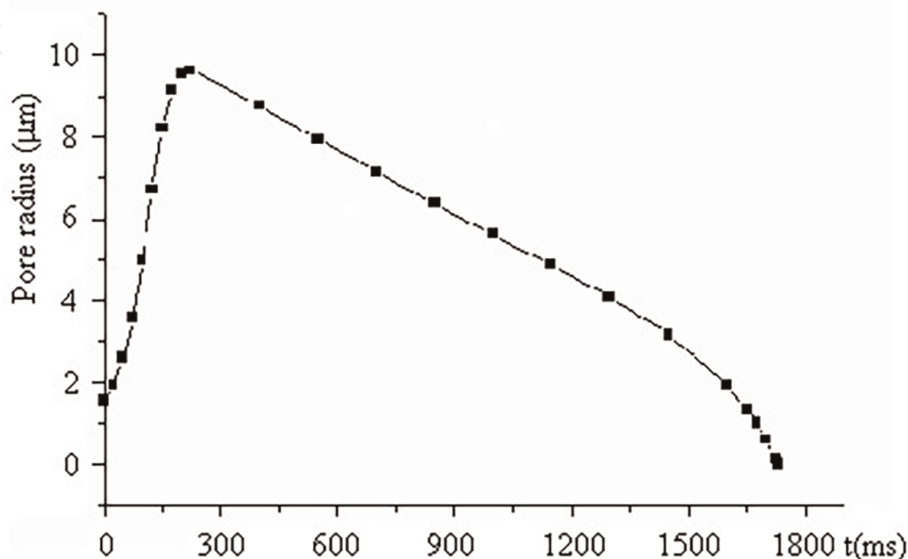


Figure 3.
The pore radius as a function of time for relaxing stage of a pulsatory liposome.

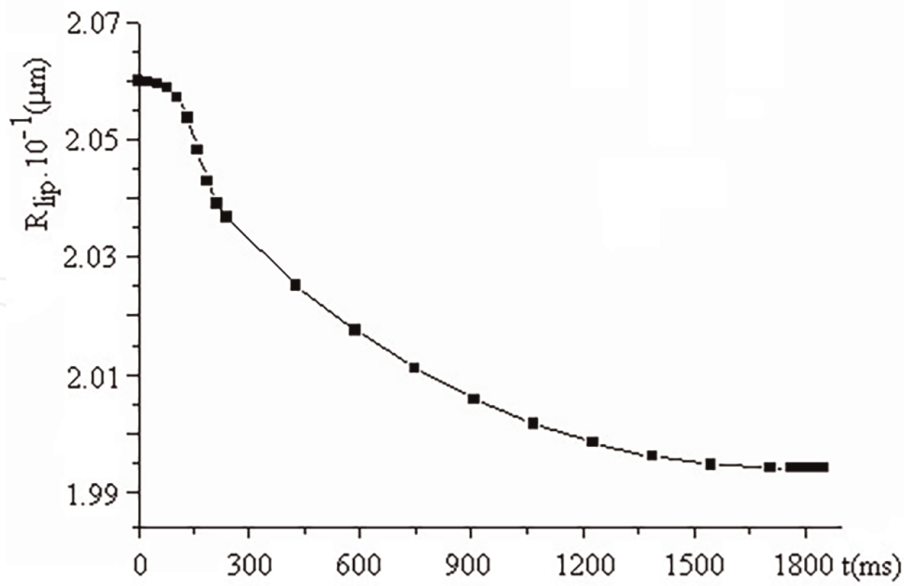


Figure 4.
 The vesicle radius as a function of time during the relaxing stage of the liposome pulsatory.

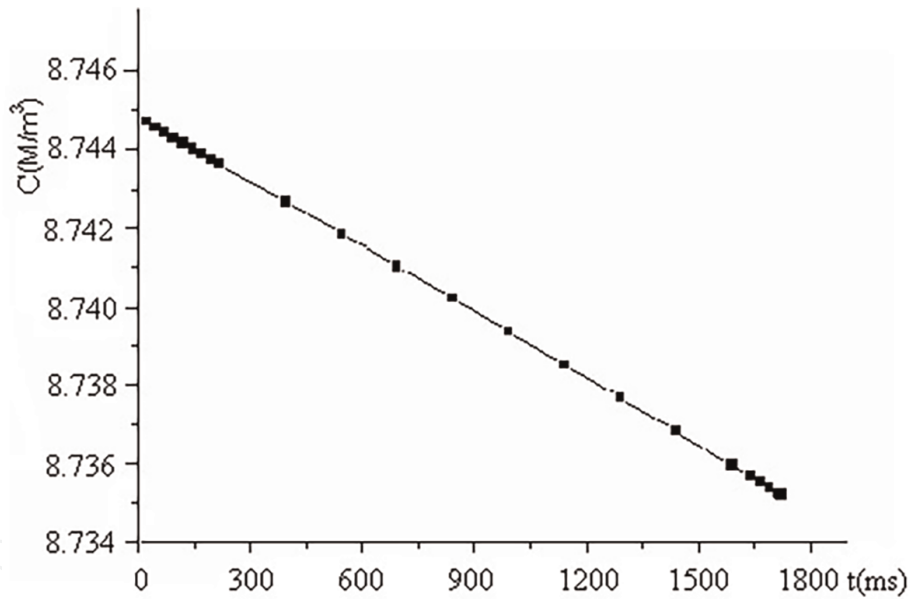


Figure 5.
 The plot of the solute concentration inside of a liposome as a function of time, during the relaxing stage of the liposome.

$$\mathcal{R}T\Delta C_s \geq \sigma \left(\frac{1}{R-h} + \frac{1}{R+h} \right) \quad (21)$$

Given the condition (21) we can program a pulsatory liposome to work n cycles:

$$\mathcal{R}T(C_{sn}^{\text{in}} - C_{sn}^{\text{out}}) = \sigma \left(\frac{1}{R-h} + \frac{1}{R+h} \right) \quad (22)$$

where C_{sn}^{in} and C_{sn}^{out} are the solute concentrations at the end of swelling stage of the n-th cycle, inside and outside of liposome, respectively. Considering that at the beginning the solute external concentration is equal to zero, and the external medium

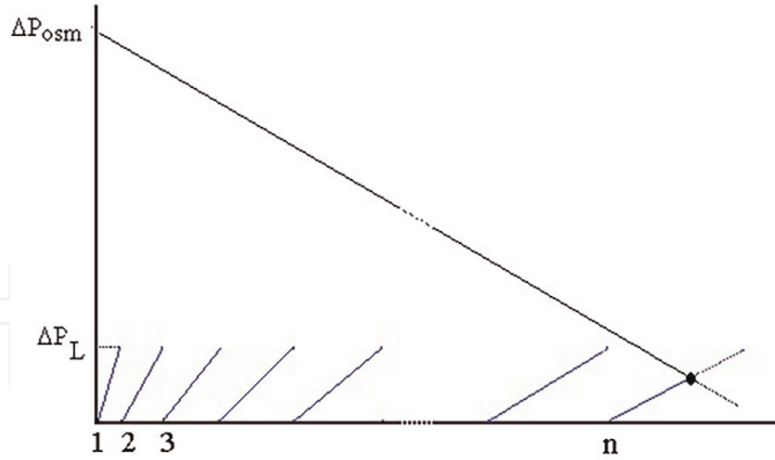


Figure 6.

The evolution of osmotic pressure and Laplace pressure during the working of the pulsatory liposome. The liposome stops working when the two pressures become equal (the black point on the figure).

composition is too less influenced by the vesicle running, we can take $C_{sn}^{out} = 0$. Taking into account that:

$$C_{sn}^{in} = f^n C_{so} \quad (23)$$

the condition (22) becomes:

$$\mathcal{R}Tf^n C_{so} = \frac{2\sigma R}{R^2 - h^2} = \frac{2ER}{R^2 - h^2} \left(\frac{R^2}{R_0^2} - 1 \right) \quad (24)$$

where f is the reversal of swelling ratio ($f = V_0/V_c = R_0^3/R_c^3$), R is the radius of the sphere between the two monolayers of the liposome bilayer, σ is the monolayer surface tension, $2h$ is the hydrophobic core thickness, \mathcal{R} is the universal gas constant, and T is the absolute temperature. Taking into account that the vesicle considered here is sufficiently large, we cut h , in order to obtain simpler formulae. R will be replaced by R_c and $\sigma = \sigma_c$. Therefore, it results from (24) that the initial solute concentration inside liposome, such as this liposome to produce n cycles, noted with c_{s0n} is equal to:

$$C_{s0n} = \frac{2\sigma_c}{\mathcal{R}T R_c f^n} = \frac{2E}{\mathcal{R}T R_c f^n} \left(\frac{R_c^2}{R_0^2} - 1 \right) \quad (25)$$

The liposome studied in this chapter, filled with a solution with a concentration equal to 10.5 M/m^3 can work 20 cycles. If the internal solute concentration C_{s0n} of the solvate meets the condition $10.5 \text{ M/m}^3 \leq C_{s0n} < 12.14 \text{ M/m}^3$, then the pulsatory liposome will stop during the swelling of the twenty-first cycle.

The length time of a cycle is equal to the sum of swelling time and pore lifetime. The solute amount delivered through pore during a cycle may be calculated from the formulae:

$$q(n) = V_0 (C_{sp} - C_{s(p+1)}) \quad (26)$$

where C_{sp} and $C_{s(p+1)}$ are the initial solute concentration before starting the p -th and $(p+1)$ -th cycle.

7. Concluding remarks

The functioning of the pulsatory liposome is determined by the transmembrane concentration gradient of the osmotic solute and by the appearance of the pore through the liposome membrane. The transmembrane osmotic gradient is the motrice force, which causes swelling of the liposome. The pore changes the direction of the liposome evolution, bringing it back to its original geometric size.

Therefore, the pulsatory liposome can be seen as a two-stroke engine. The operating energy is ensured by the transmembrane concentration gradient of the solute. The solute is the fuel of the pulsatory liposome.

The number of cycles of the pulsatory liposome can be established according to the initial solute concentration. In other words, the pulsatory liposome is a programmable biodevice.

The solute (the fuel) may be a pharmacological substance or any other special substance.

The preparation of pulsatory liposomes with such properties and their delivery at a site of action remains a biotechnology challenge [29]. Some very interesting applications of pulsatory liposomes filled with drugs have been devised for targeting hepatic cells or the synaptic cleft. Endothelial pores (also known as fenestrae) control the exchange of fluids, solutes, and particles between the sinusoid blood capillaries and the space of Disse [23, 30].

Pulsatory liposomes, free or included inside other vesicles, may reach hepatocytes due to hydrodynamic effects of blood circulation [30].

The transient pores in liposomes could also be used for compensation of neurotransmitter deficiency in the synaptic cleft [31]. The pulsatory unilamellar liposome is an example of a bionic microengine, with potential applications in chemotherapy [32].

If the liposomal membrane is endowed with protein receptors of specific recognition, pulsatory liposomes can be used in chemotherapy as carriers and deliverers of drugs to certain sites in the body [32].

The range of systems and approaches that can be used to deliver therapeutics is advancing at an incredible rate. The advances in drug delivery have important implications for anyone working in healthcare. Therefore, we consider that, in the future, pulsatory liposomes may be used as special biotechnological devices for active substance controlled release [32, 33].

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