# PULSATORY LIPOSOMES – A POSSIBLE BIOTECHNOLOGICAL DEVICE FOR CONTROLLED DRUG DELIVERY. III. THE LIPOSOME RELAXATION<sup>#</sup>

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Abstract. A unilamellar liposome filled with an aqueous solution of an impermeant solute was introduced into a hypotonic aqueous medium. Because of the mechanical tension induced by osmotic flow, the vesicle swells up to a critical size, when suddenly a transbilayer pore appears. A part of the intravesicular material leaks out through this pore, and the liposome membrane relaxes and eventually recovers. The swelling begins again, and the liposome experiences a cyclic process. For this reason we have named it a pulsatory liposome. In this paper we derived the differential equations of both the vesicle and the pore dynamics. We also computed all the parameters of the second part of a duty cycle (pore lifetime, number of cycles, the duration of vesicle activity, the amount of material leaked out during a cycle) for a particular case. The condition to program a vesicle to work for n consecutive cycles was deduced.

Key words: Osmotic gradient, stretched vesicle, pulsatory vesicle, drug release biocontroller.

## **INTRODUCTION**

The process of pore appearance in lipid vesicles has an important peculiarity, which consists in the possibility of existence of an energy barrier for pore closure under certain conditions [13, 14]. This property is very important from the point of view of the usefulness of pulsatory liposomes in biotechnology applications [10–12].

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The competition between the osmotic flow across the membrane and the internal solution leakage determines the pore evolution and the speed of the vesicle decrease in size. The pore appearance may be influenced by structural defects such as fluctuations in thickness [3-5, 9] or the existence of clusters [6, 7].

There are a lot of theoretical and experimental studies about pore formation in plane lipid bilayers, but there are considerably fewer publications about pore opening in lipid vesicles [1, 2, 15–17].

In this article we present a theoretical study comprising all the processes which take place along the second stage of the life cycle of a pulsatory liposome.

#### **MATERIALS AND METHODS**

#### THE LIPOSOME RELAXATION STAGE

Let us suppose that a unilamellar liposome swells (no matter what the cause is) up to a critical size, when suddenly a transbilayer pore appears. From this moment, the liposome relaxation starts. During this part of the pulsatory liposome cycle two simultaneous processes take place: the pore evolution from its birth to its disappearance, and the liposome relaxation from a critical state of radius  $R_c$  to the initial state of radius  $R_0$ .

First we will analyze the pore dynamics.

As a matter of fact, the pore dynamics is driven by the difference between the membrane tension and the edge tension due to exposure of the hydrophobic membrane core to water (Fig. 1).



Fig. 1. A cross-section through a bilayer with a pore. The opening of the pore is driven by the superficial tension force  $F_{\sigma}$  acting on the membrane, and the closure is driven by the edge tension force  $F_{\gamma}$ . Its evolution is determined by the balance of these two opposing forces.

According to Fig. 1, the pore evolution is governed by the equilibrium between the driving force for pore opening,  $F_{\sigma}$ , corresponding to membrane tension  $\sigma$ , and the driving force for pore closure,  $F_{\gamma}$ , corresponding to edge tension  $\gamma$ . The edge tension  $\gamma$  is caused by the hydrophobicity of phospholipids, and contributes to the energy barrier which hinders pore formation. The surface tension coefficient  $\sigma$  is determined by the interactions between the molecules composing the lipid bilayer and by the effects of interfacial tension. It reduces the energy barrier height for pore nucleation.

### The dynamics of the transbilayer pore

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

$$\Delta E_{\rm v} = 2\pi d\eta_{\rm b} r \frac{\partial r}{\partial t} \tag{1}$$

where *d* is the lipid bilayer thickness.

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

$$\sigma r - 2\gamma = 2\eta_{\rm b} d \, \frac{\partial r}{\partial t} \tag{2}$$

Pore opening is driven by the membrane tension  $\sigma$ , while its closure by the edge tension  $\gamma$  (Fig. 1).

The pore appearance in a lipid vesicle is more complex than in a plane lipid bilayer, because: 1. in vesicles the membrane tension changes as the pore grows, while in plane lipid bilayers the surface tension is constant inasmuch as the lipid reservoir around it; and 2. the edge tension cannot be neglected.

#### Leak-out of the internal liquid

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

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

The flow of expelled liquid per time unit is:

$$Q = \pi r^2 v \tag{3}$$

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

The incoming water flow to the liposome through its membrane due to osmotic imbalance is:

$$j_{\rm w} = P_{\rm w} V_{\mu \rm w} A \left( \Delta C_{\rm s} - \frac{\Delta P}{N_{\rm A} k_{\rm B} T} \right) \tag{4}$$

The symbols in equation (4) have the following meanings:  $P_w$  (measured in m/s) is the water permeability through the liposome membrane;  $V_{\mu w}$  is the water molar volume (in m<sup>3</sup>/mol); A is the membrane area;  $\Delta C_s$  (measured in mols/m<sup>3</sup>) is the transmembrane solute concentration gradient;  $\Delta P$  is the Laplace pressure;  $N_A$  is Avogadro's number;  $k_B$  is Boltzmann's constant, and T is the absolute temperature.

The Laplace pressure under a spherical surface is given by the formula:

$$\Delta P = \frac{2\sigma}{R} \tag{5}$$

where  $\sigma$  is the tension of the stretched membrane and *R* is the liposome radius.

The net flow over time unit has to equal the rate of decrease in vesicle volume  $V_{\text{ves}}$ :

$$\frac{\partial V_{\text{ves}}}{\partial t} = Q - j_{\text{w}} \tag{6}$$

The push-out force is:

$$F_{\rm p} = \Delta P \cdot \pi r^2 \tag{7}$$

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

$$F_{\rm v} = 3\pi\eta_{\rm l} r v \tag{8}$$

Taking into account the above relations (5), (7) and (8) the outward flow velocity of the internal liquid is:

$$v = \frac{2\sigma r}{3\eta_1 R} \tag{9}$$

Taking into account the relations (3), (4), (6) and (9), one obtains from equation (6) an equation for the vesicle radius:

$$4\pi R^2 \frac{\partial R}{\partial t} = -\frac{2\pi\sigma r^3}{3R\eta_{\rm l}} + P_{\rm w}V_{\mu\rm w} \left(4\pi R^2 - \pi r^2\right) \left(\Delta C_{\rm s} - \frac{\Delta P}{N_{\rm A}k_{\rm B}T}\right)$$
(10)

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

$$W_{\rm el}(R,r) = \frac{E}{2A_0} \left[ \left( A - A_0 \right)^2 - \pi r^2 \right]^2 + 2\pi \gamma r$$
(11)

and the bilayer surface tension is given by:

$$\sigma(R,r) = \frac{\partial W_{\rm el}}{\partial A} = \frac{E}{4\pi R_0^2} \Big[ 4\pi (R^2 - R_0^2) - \pi r^2 \Big]$$
(12)

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

$$\frac{\sigma}{\sigma_{\rm c}} = 1 - \frac{r^2}{4(R_{\rm c}^2 - R_0^2)} - \frac{R_{\rm c}^2 - R^2}{R_{\rm c}^2 - R_0^2}$$
(13)

Having formula (5) and (13) in mind, the final form of the differential equation (10) is:

$$\frac{\partial R}{\partial t} = -\frac{Er^3}{6R_0^2\eta_1} \frac{R^2 - R_0^2}{R^3} + P_w V_{\mu w} \left(1 - \frac{r^2}{4R^2}\right) \left(C_{\rm in} - \frac{2\beta E}{R_0^2} \frac{R^2 - R_0^2}{R}\right)$$
(14)

We have considered the solute concentration outside the liposome equal to zero ( $C_{out} = 0$ ). So, in the formula (14) the transmembrane solute concentration gradient is equal to the solute concentration inside the liposome ( $\Delta C_s = C_{in}$ ).

## The change in composition of the internal liquid

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

$$\frac{\mathrm{d}(C_{\mathrm{in}}V_{\mathrm{ves}})}{\mathrm{d}t} = -\pi r^2 C_{\mathrm{in}} v \tag{15}$$

which is equivalent with:

$$\frac{\mathrm{dln}(C_{\mathrm{in}}V_{\mathrm{ves}})}{\mathrm{d}t} = -\frac{3r^2v}{4R^3} \tag{16}$$

The equations (2), (14) and (16) can be solved numerically using Euler's method to obtain the time dependence of vesicle radius, pore radius, and internal solute concentration (R(t), r(t) and  $C_{in}(t)$ ) during the second stage of the life cycle of a pulsatory liposome.

The dependence of the liposome radius on time during the first stage of each cycle is obtained from equation (14). The pore lifetime, which is equal to the liposome relaxation time, can be also obtained. The most important parameters are: internal solute concentration, internal liquid viscosity, and bilayer viscosity.

## THE AMOUNT OF SOLUTE RELEASED PER CYCLE

By integrating equation (16) we can calculate the amount of drug (or any special chemical substance) released during a cycle:

$$\int_{C_{\rm fs,n}V_{\rm c}}^{C_{\rm 0s,n}V_{\rm 0}} d\left(\ln(C_{\rm in}V_{\rm lip})\right) = -\frac{\sigma_{\rm c}}{2\eta_{\rm l}\left(R_{\rm c}^2 - R_{\rm 0}^2\right)} \int_{0}^{\tau_{2,\rm n}} \frac{r^3}{R^4} \left(R^2 - \frac{r^2}{4} - R_{\rm 0}^2\right) dt$$
(17)

We have used the indices as follows: f - for the end (final stage) of the cycle;s - for solute;*n*- for the rank of the cycle; c - for the critical state reached at theend of the swelling stage; 0 - for the initial state of the liposome at the beginningof a cycle.

The conservation of solute contained inside the vesicle during the swelling stage may be written for each cycle. For the  $n^{\text{th}}$  cycle it is:

$$C_{0s,n}V_0 = C_{s,n}V = C_{fs,n}V_c$$
(18)

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

$$\ln \frac{C_{0s,n+1}}{C_{0s,n}} = -\frac{\sigma_{c}}{2\eta_{l} \left(R_{c}^{2} - R_{0}^{2}\right)} \int_{0}^{\tau_{2,n}} \left(R^{2} - \frac{r^{2}}{4} - R_{0}^{2}\right) dt$$
(19)

Let us introduce the following notation:

$$I_n = \frac{\sigma_c}{2\eta_l \left(R_c^2 - R_0^2\right)} \int_0^{\tau_{2,n}} \left(R^2 - \frac{r^2}{4} - R_0^2\right) dt$$
(20)

The amount of solute  $\Delta m_n$  released during the  $n^{\text{th}}$  cycle is equal to:

$$\Delta m_n = C_{0s,n-1} - C_{0s,n} = C_{0s,n-1} \left( 1 - e^{-I_{n-1}} \right) =$$
  
=  $C_{0s,1} \left( 1 - e^{-I_1} \right) \left( 1 - e^{-I_2} \right) \dots \left( 1 - e^{-I_{n-1}} \right)$  (21)

The integral value  $I_n$  is variable because both internal liquid viscosity  $\eta_1$  and pore lifetime  $\tau_{2n}$  decrease with rank of the cycle.

#### RESULTS

We solved the system of three differential equations using Euler's method with a step size  $\delta t = 1$  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. The initial conditions were:  $r(0) = 1.576 \ \mu\text{m}$ ;  $R(0) = 20.6 \ \mu\text{m}$ , and  $C(0) = 0.01 \ \text{M}$ .

The differential equations were integrated for a liposome size used in experimental studies. The vesicle radius in relaxed state ( $\sigma = 0$ ) was  $R_0 = 19.7 \,\mu\text{m}$ , and the critical radius was  $R_c = 20.6 \,\mu\text{m}$  [1, 2, 15]. The membrane critical surface tension was  $\sigma_c = 1.7 \times 10^{-5} \,\text{N/m}$  [2]. The edge tension was  $\gamma = 8.1 \times 10^{-12} \,\text{N}$  [2]. The lipid bilayer viscosity was  $\eta_b = 100 \,\text{N} \cdot \text{s/m}^2$  [15]. The aqueous solution viscosity was  $\eta_l = 3.2 \times 10^{-2} \,\text{N} \cdot \text{s/m}^2$  [15]. The elastic modulus for surface stretching was  $E = 0.2 \,\text{N/m}$ .



Fig. 2. The plot of the pore radius as a function of time for the expansion stage of the pore up to the maximum size of the pore.



Fig. 3. The plot of the pore radius evolution after the moment when it has reached its maximum radius.



Fig. 4. The plot of the pore radius as a function of time starting from pore nucleation up to its disappearance. This is a solution of differential equation (2).



Fig. 5. The plot of the vesicle radius as a function of time for the expansion stage of the pore up to its maximum size.



Fig. 6. The plot of the vesicle radius evolution after the moment when the pore reached its maximum radius.



Fig. 7. The plot of the vesicle radius as a function of time during the relaxing stage of a cycle of a pulsatory liposome. This is the solution of differential equation (14).

The evolution of the pore size is plotted in Figs. 2–4. We have drawn the pore evolution before reaching the maximum value of its radius (Fig. 2), and after it reached its maximum size (Fig. 3). In Fig. 4 we have plotted the pore evolution along its lifetime.

In Figs. 5–7 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 Fig. 8 we have plotted the change in solute concentration during the pore lifetime, when the aqueous solution leaks out the vesicle. It is very interesting that the solute concentration decreases linearly during vesicle relaxation.



Fig. 8. The plot of the solute concentration inside a liposome as a function of time, during the relaxing stage of the liposome. It is the solution of differential equation (16).

#### CONCLUSIONS

Pulsatory liposomes can be used for drug delivery in specified locations. In our opinion the amounts of drug should be sufficient to exert beneficial effects specifically in diseased regions, because there are no drug losses, as happens in other ways of drug delivery. The molecular mechanism of drug action should be known in detail in order to estimate the two parameters characterizing the pulsatory liposome: the time intervals between two successive pore openings, and the amount of drug released with the internal liquid leaked out during each cycle. Recently, an approximate solution for the working cycle of a pulsatory liposome was published [11, 12]. The preparation of pulsatory liposomes with such properties and their delivery at the site of action remain a biotechnology challenge. 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 [8].

Pulsatory liposomes, free or included inside other vesicles, may reach hepatocytes due to hydrodynamic effects of blood circulation [8].

The transient pores in liposomes could also be used for compensation of neurotransmitter deficiency in the synaptic cleft [10]. Finally, we consider that, in the future, pulsatory liposomes may be used as special devices for active substances controlled release.

#### REFERENCES

- BROCHARD, F., P.G. DE GENNES, O. SANDRE, Transient pores in stretched vesicles: role of leak-out, *Physica A*, 2000, 278, 32–51.
- KARATEKIN, E., O. SANDRE, H. GUITOUNI, N. BORGHI, P.-H. PUECH, F. BROCHARD-WYART, Cascade of transient pores in giant vesicles: Line tension and transport, *Biophys. J.*, 2003, 84, 1734–1749.
- 3. POPESCU, D., C. RUCAREANU, G. VICTOR, A model for the appearance of statistical pores in membranes due to selfoscillations, *Bioelectrochem. Bioenerg.*, 1991, **25**, 91–103.
- 4. POPESCU, D., G. VICTOR, The transversal diffusion coefficient of phospholipid molecules through black lipid membranes, *Bioelectrochem. Bioenerg.*, 1991, **25**, 105–108.
- 5. POPESCU, D., C. RUCAREANU, Membrane selfoscillations model for the transbilayer statistical pores and flip-flop diffusion, *Mol. Cryst. Liquid Cryst.*, 1992, **25**, 339–348.
- 6. POPESCU, D., Association probabilities between the single-chain amphiphiles into a binary mixture in plan monolayers (II), *Biochim. Biophys. Acta*, 1993, **1152**, 35–43.
- POPESCU, D., L. MOVILEANU, G. VICTOR, G. TURCU, Stability and instability properties of aggregation of single chain amphiphiles into binary mixtures, *Bull. Math. Biol.*, 1997, 59, 43– 61.
- POPESCU, D., L. MOVILEANU, S. ION, M.L. FLONTA, Hydrodynamic effects on the solutes transport across endothelial pores and hepatocytes membranes, *Phys. Med. Biol.*, 2000, 45, N157–N165.
- POPESCU, D., S. ION, A.I. POPESCU, L. MOVILEANU, Elastic properties of bilayer lipid membranes and pore formation, In: *Planar Lipid Bilayers (BLMs) and Their Applications*, H. Ti Tien, A. Ottova, eds., Elsevier Science Publishers, Amsterdam, 2003, 173–204.
- POPESCU, D., C.N. ZAHARIA, I. STELIAN, MARIA-LUISA FLONTA, Compensation of the neurotransmitters deficiency in the synaptic cleft, *Romanian J. Biophys.*, 2006, 16, 189–204.
- POPESCU, D., L. MOVILEANU, A.G. POPESCU, The behaviour of the closed lipidic bilayer under osmotic stress may be used in new biotechnological applications, In: *Mathematical Biology Research Trends*, L.B. Wilson, ed., Nova Science Publishers, New York, 2008, 275–294.
- 12. POPESCU, D., A.G. POPESCU, The working of a pulsatory liposome, *J. Theoret. Biol.*, 2008, **254**, 515–519.

- POPESCU, D., A.G. POPESCU, B. AMUZESCU, Pulsatory liposomes a possible biotechnological device for controlled drug delivery. I. The liposome swelling, *Romanian J. Biophys.*, 2010, 20, pp. 37–46.
- POPESCU, A.G., D. POPESCU, B. AMUZESCU, E. MARIES, Pulsatory liposomes a possible biotechnological device for controlled drug delivery. II. The pore appearance, *Romanian J. Biophys.*, 2010, 20, 171–181.
- 15. SANDRE, O., L. MOREAUX, F. BROCHARD-WYART, Dynamics of transient pores in stretched vesicles, *Proc. Natl. Acad. Sci.*, 1999, **96**, 10591–10596.
- 16. VERMA, I.M., M. SOMIA, Gene therapy-promises, problems and prospects, *Nature* (London), 1997, **389**, 239–242.
- 17. ZASADZINSKI, J.A., Novel approaches to lipid based drug delivery, *Curr. Opin. Solid State Mat. Sci.*, 1997, **2**, 345–349.