PULSATORY LIPOSOMES – A POSSIBLE BIOTECHNOLOGICAL DEVICE FOR CONTROLLED DRUG DELIVERY. I. THE LIPOSOME SWELLING[#]

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Abstract. In this paper we describe only the first part of the duty cycle of a pulsatory liposome. A unilamellar lipid liposome filled with an aqueous solution of an impermeant solute is introduced into a hypotonic aqueous environment. Because of the mechanical tension induced by an osmotic flow, the vesicle swells up to a critical size, when suddenly a transbilayer pore appears. 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. In this paper we have obtained the differential equation of the swelling stage. Its analytical solution is the dependence of time on vesicle radius, which is the inverse of the direct function that would be of interest. We have also computed several parameters related to the swelling process: the critical swelling time and the duration of the last cycle of vesicle activity for some initial concentrations of solute.

Key words: osmotic gradient, swelling vesicle, swelling time, stopping time.

INTRODUCTION

The passage of molecules, especially of large ones, through cellular membranes is a very important problem for certain biotechnology applications. Formation of a pore in lipid bilayers following some controlled processes may be an adequate and interesting way of transmembrane transport.

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Some pores, named stochastic pores, can appear due to structural and dynamic properties of lipid bilayers [3–7, 9], but others may be favored by mechanical tension induced in different ways [11, 12]. 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 optically induced mechanical tension [1, 2, 12].

There are two very interesting biotechnology applications which require an increase in membrane permeability: gene therapy and targeted delivery of special compounds. In the first one, the transport of DNA fragments through cellular and nuclear membranes is requested [13]. The second application uses special molecules encapsulated in vesicles, which have to be transported to a specified target location [14]. Having reached that point, one supposes that the liposome discharges its content in the external medium by its breakdown.

In our last two papers we have considered how a lipid vesicle has to release the drug molecules in a well-controlled fashion [11, 15]. It must work as a pulsatory liposome, the energy of which is supplied by the concentration gradient across membrane of an impermeant solute. In this series of three papers, we will perform a detailed analysis of the most important stages of a cycle in the evolution of a pulsatory liposome.

In the present paper we are studying the swelling of a lipid vesicle subjected to osmotic stress. Pore opening at the end of the swelling stage as an essential event for the pulsatory liposome functioning will be analyzed in the second paper. All the processes which contribute to the vesicle relaxing are described by three differential equations in the third and last paper of the series.

We have named such a liposome a pulsatory liposome, since it performs a cyclic activity. We will demonstrate that these liposomes may be programmed to work a certain number of cycles, settled in advance. Also, we will calculate the amount of special substances delivered during each cycle.

MATERIALS AND METHODS

PHENOMENOLOGICAL BASES OF A PULSATORY LIPOSOME

Let us consider a liposome filled with aqueous solution containing an impermeant solute. The initial state of the liposome is an equilibrium one and is characterized by smooth and unstretched lipid membrane. It is considered the reference state. This liposome is inserted into a bath containing hypotonic aqueous medium. Due to osmotic pressure, created by the transmembrane gradient of solute concentration, water molecules will flow into the liposome through its membrane.

This osmotic flow of solvent determines: 1) the swelling of the liposome; 2) the stretching of liposomal membrane; 3) the dilution of the internal solution. The surface tension also increases in parallel with this liposomal expansion. The surface tension increases the pressure inside the vesicle.

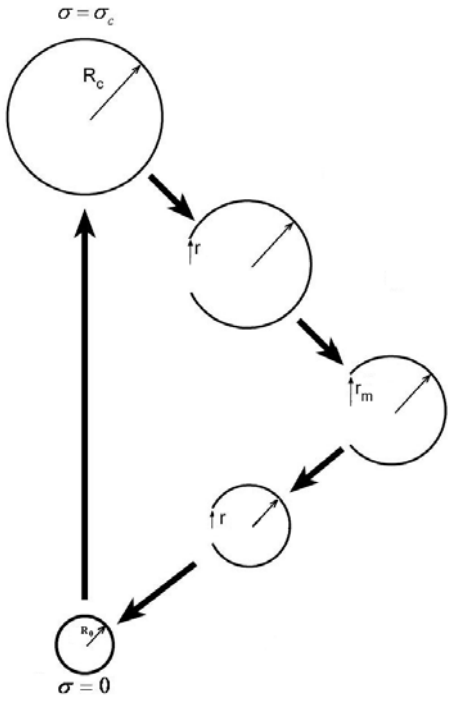

Fig. 1. A cycle of the pulsatory liposome. In the first stage, the liposome swells from the initial state of radius R_0 to the critical state of radius R_c, when a transbilayer pore appears (the upper part of the picture). In the second stage, the pore radius increases up to a maximum value, r_m; after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

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 increases up to a critical size, when a transient transmembrane pore appears. This event is followed by two simultaneous processes: the pore dynamics and the outflow of internal content of the vesicle, due to Laplace pressure.

The pore dynamics consists of two phases: 1) the pore radius increases up to the maximum value, r_m, and 2) the pore radius decreases until the closure of the pore (Fig. 1). 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 membrane tension decreases until it becomes equal to the linear tension of the membrane edge. 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 returns to its initial size. We can suppose that the dynamics of the liposome described above can restart over and over again. This cyclic process ceases when the osmotic gradient becomes smaller than a critical value, which will be discussed below.

In the present paper we will present a mathematical model of the first stage of a pulsatory liposome cycle: the liposome swelling.

THE LIPOSOME SWELLING STAGE

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

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

where the terms have the following significance: V is the liposome volume, P_{w} (measured in m/s) the water permeability through liposome membrane, $V_{\mu\mathrm{w}}$ the water molar volume (in m^3/mol), A the membrane area, ΔC_{s} (measured in mol/m^3) the transmembrane solute concentration gradient, ΔP the excess Laplace pressure, $\mathrm{N_A}$ the Avogadro number, k_{B} the Boltzmann constant, and T the absolute temperature.

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

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

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

According to Hooke's law, if the closed spherical membrane is stretched by a surface tension, 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 amount of internal solute is conserved throughout the liposome swelling stage. For the swelling of the liposome during the first cycle, 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 the solute concentration when the liposome has reached the volume V during the swelling process, and C_{fs} 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 equal to zero, then $\Delta C_s = C_s$.

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

$$\frac{dR}{dt} = P_w V_{\mu w} \left(\frac{C_{0s} R_0^3}{R^3} - \frac{2\beta E}{R_0^2} \frac{R^2 - R_0^2}{R} \right) \tag{5}$$

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

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

By integrating the equation (5) one obtains the liposome radius $R(t)$ as a function of time. The initial condition is:

$$R(0) = R_0 \tag{7}$$

The analytical solution of equation (5) is:

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

where:

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

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

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

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

The most important parameter is the initial solute concentration.

RESULTS

THE SWELLING TIME

We have considered a unilamellar liposome inserted into a large box which contains water. In the relaxed state the liposome radius is equal to 19.7 μm. The relaxed state is the initial state of each cycle of a pulsatory liposome. Such unilamellar vesicles were used in experimental studies [2].

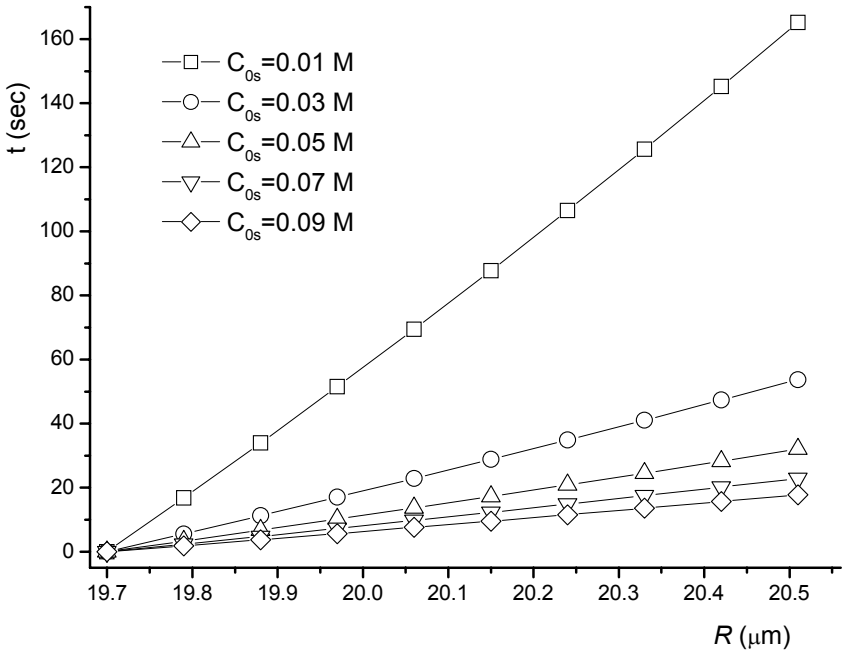

Fig. 2. The representation of time t (measured in seconds) required for the liposome to swell up to the radius R due to osmotic incoming water flow, as computed from equation (8). Five initial solute concentrations in the liposome were considered.

These vesicles were tensed by intense optical illumination in the presence of fluorescent probes embedded in the lipid bilayer. 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 (8) applied to the above-mentioned liposome for five initial concentrations of the internal aqueous solution of a non-permeating solute: C_{0s} =0.01 M, 0.03 M, 0.05 M, 0.07 M, and 0.09 M. The membrane permeability coefficient for water p_w is equal to 3×10^{-5} m/s, 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 [2].

In order to find the dependence of liposome radius R on time during the swelling process, we used an indirect method for solving equation (8). Thus, we calculated the time of liposome swelling up to a given value of liposome radius. First, we obtained the swelling time as a function of liposome radius for each solute concentration: $t = f(R)$. The results are plotted in Fig. 2.

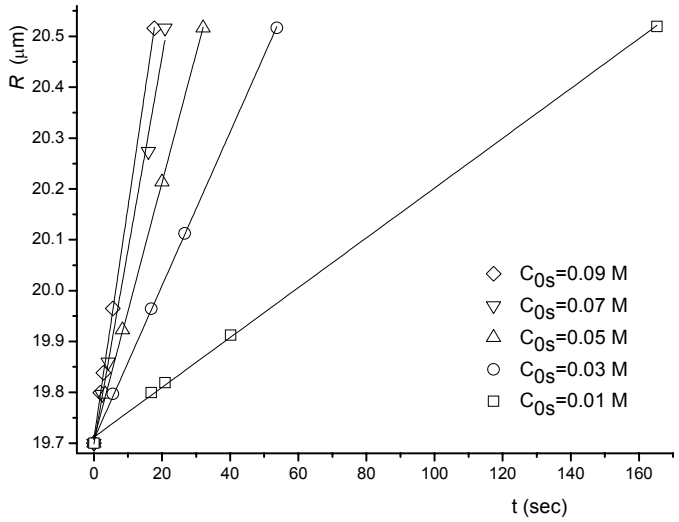

Fig. 3. The dependence of the liposome radius on time during the swelling process of a lipid liposome inserted into hypoosmotic medium for five values of the initial internal concentration of aqueous solution.

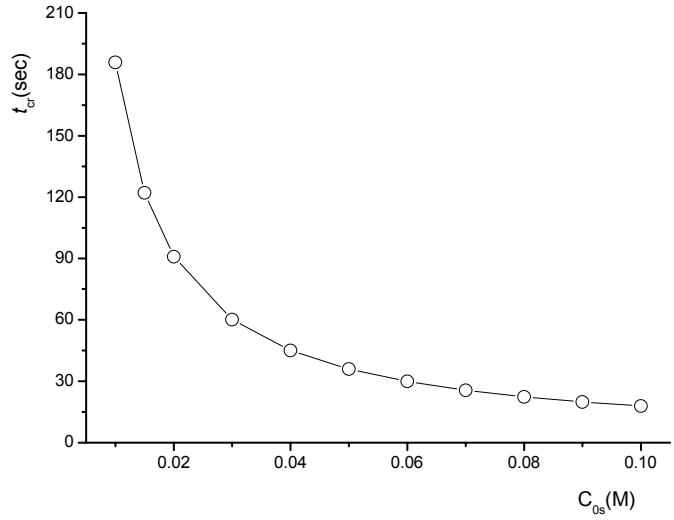

Fig. 4. The dependence of the critical time, t_{cr}, required by the liposome to reach its critical size, on the initial concentration of the osmotic solute inside the liposome.

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

In Fig. 3 we have plotted these functions for the selected concentrations listed on the graph. It is interesting that for low solute concentrations the dependence of the swelling liposome radius on time is linear. This cannot be true for greater solute concentrations. The method of numeric calculation of the swelling time at different values of liposome radius during the expanding process and the determination of the inverse function $R = f(t)$ by fitting the values computed above may be used for high solute concentration gradients.

We should remind that we considered liposomes of initial radius R_0 = 19.7 μm, and we have supposed that the liposome ruptures when it reaches the critical radius R_c = 20.6 μm.

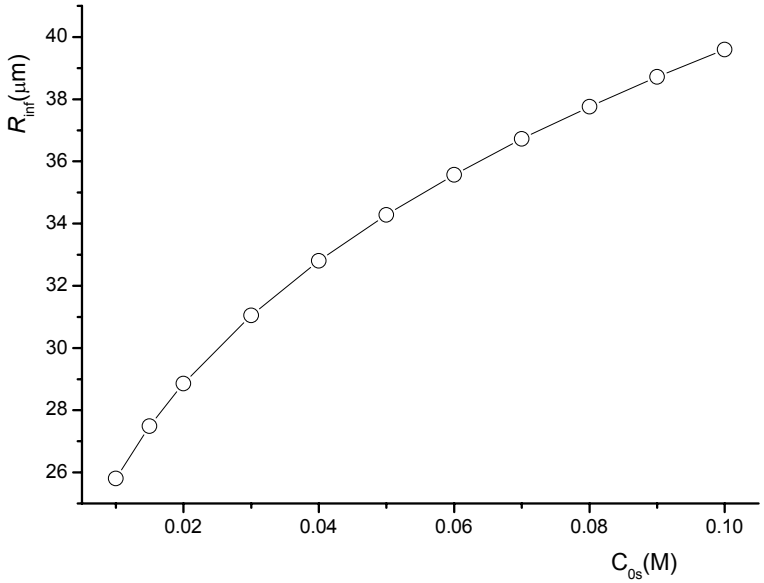

Fig. 5. The plot of liposome radius when the swelling process stops before the liposome reaches the critical radius, depending on the initial solute concentration inside the liposome.

While this is an artificial hypothesis, we have no reason to argue against this statement yet.

We computed the swelling time required by the liposome to reach the critical radius R_c, for all the selected initial solute concentrations. We named this time the critical time, t_{cr}. The dependence of the critical time on initial solute concentration is represented in Fig. 4.

If the initial solute concentration is not sufficient to reach the critical radius, the swelling process ceases, the liposome remaining an indefinite time in this state. When the swelling process ceases, the liposome has reached a certain radius. We

named this variable the radius at an infinite duration of the process, because the swelling has stopped, and denoted it $R_{inf.}$

In Fig. 5 we have drawn the graph of R_{inf} as a function of the initial solute concentration.

DISCUSSION

In this paper we have computed the inverse function of the differential equation solution given by formula (8) only for five initial concentrations of solute in the low range of values. This function, $R = f(t)$, is a linear function. The numerical inversion of function (8) for large concentrations may be computed in the same way, but the corresponding analytical formula may be more difficult to find.

In Fig. 4 we plotted the swelling time for a particular liposome having an initial radius $R_0 = 19.7$ μm and a critical radius $R_c = 20.6$ μm. This liposome was initially filled with solute in aqueous solution at different concentrations. The swelling time decreased with increasing initial solute concentration.

For example, at the initial concentration $C_{0s} = 0.01$ M the swelling time t_{sw} is equal to 180 s, but for the initial concentration $C_{0s} = 0.1$ M the swelling time is equal to 20 s.

An interesting and useful parameter is the liposome radius when it ceases to work. This parameter may be calculated from equation (8) for infinite t.

We have computed this parameter for the case in which the solute concentration in the initial state of the last cycle has values similar to those used above.

REFERENCES

1. BROCHARD, F., P.G. DE GENNES, O. SANDRE, Transient pores in stretched vesicles: role of leak-out, *Physica A*, 2000, **278**, 32–51.
2. 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.

7. 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.
8. 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.
9. 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*, vol. 3, H. Ti Tien and A. Ottova eds., Elsevier Science Publishers, Amsterdam, 2003, pp. 173–204.
10. POPESCU, D., C.N. ZAHARIA, I. STELIAN, M.L. FLONTA, Compensation of the neurotransmitters deficiency in the synaptic cleft, *Romanian J. Biophys.*, 2006, **16**, 189–204.
11. 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, pp. 275–294.
12. SANDRE, O., L. MOREAUX, F. BROCHARD-WYART, Dynamics of transient pores in stretched vesicles, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 10591–10596.
13. VERMA, I.M., M. SOMIA, Gene therapy – promises, problems and prospects, *Nature* (London), 1997, **389**, 239–242.
14. ZASADZINSKI, J.A., Novel approaches to lipid based drug delivery, *Curr. Opin. Solid State Mat. Sci.*, 1997, **2**, 345–349.
15. POPESCU, D., A.G. POPESCU, The working of a pulsatory liposome, *J. Theor. Biol*., 2008, **254**, 515–519.