

THE INFLUENCE OF THE EXTERNAL BATH ON THE NUMBER OF CYCLES OF A LIPID UNILAMELLAR VESICLE UNDER HYPOTONIC CONDITIONS

D. POPESCU[#], A.G. POPESCU

Department of Mathematical Modelling in Life Sciences, Institute of Mathematical Statistics and Applied Mathematics, 13, Calea 13 Septembrie, Bucharest-050711, Romania, [#]e-mail: dghpopescu@gmail.com

Abstract. In this paper we consider a unilamellar vesicle (i.e., a liposome) filled with an aqueous solution of an osmotic solute, inserted into a spherical closed box filled with water. Due to the mechanical tension induced by the osmotic flow, the lipid vesicle swells up to a critical size, when suddenly only one transient pore appears. The appearance of the pore changes the sense of the liposome evolution. A part of internal solution comes out of the liposome through transmembrane pore in the aqueous medium from closed box. The liposome relaxes up to the initial size, when the pore is closed and another cycle can begin. Therefore, the liposome performs a periodic dynamic process. The differential equation that describes the swelling stage of the pulsatory liposome during each cycle of its running time, when it is inserted in a closed environment (a closed bath) was obtained. Such liposomes may be used, in the future, in medical applications, as devices for controlled drug delivery to ill tissues. In tissues, the drug charged liposomes will work inside a closed space, where it will thus deliver the drug.

Key words: pulsatory liposome, osmotic stress, liposome swelling.

INTRODUCTION

The transport of ions and molecules through the cell membrane is very important for many biological processes. The unilamellar lipid vesicle, also named unilamellar liposome, is an artificial cell membrane model, representing its lipid component. Liposomes may be considered as efficient systems for intracellular delivery of bioactive molecules [17, 18, 21–23, 27, 28]. It is assumed that the liposomes discharge their entire contents by breaking the membrane. The transport of molecules, especially of large molecules across vesicle bilayer is a new strategy for biological material exchange between two adjacent media with very interesting and useful biotechnological applications [18, 25].

Received: September 2018;
in final form October 2018.

One of the most studied transmembrane transport pathways is the formation of lipid pores through the lipid zone of cell membranes or lipid bilayer of unilamellar liposomes [9–16, 26]. The interest in such pores grows continuously due to their biotechnological and medical applications.

There are at least two important biotechnological applications in which the increase of membrane permeability is required: gene therapy and targeted drug delivery [8, 18]. In the first application, the transport of DNA fragments through cellular and nuclear membranes is requested [27].

The second application uses encapsulated drug molecules in the vesicles, which have to be transported and released to a target place [1, 3, 28]. A third application may be considered the pulsatory liposomes.

Over the last decades, many papers have been published on pulsatory liposomes [7, 19, 20–23, 25]. If a liposome filled with aqueous solution of an osmotic solute is introduced into a hypotonic environment, it will become a dynamic system with a cyclical evolution. In each cycle, the osmotic influx of water through lipid bilayer swells the vesicle up to a critical state, when a transient pore opens somewhere in the stretched bilayer of the vesicle. A part of the liposome internal content is expelled through the pore, and the liposome decreases until it reaches its initial size. Now, a new cycle can begin. This type of liposome was named pulsatory liposome.

Such liposomes work like a two-stroke engine [12] and they could be very important, from a medical standpoint, because they can release drugs in a well-controlled fashion [23, 24]. They can be programmed in advance to deliver certain quantities of pharmacological or special substances at specified intervals at desired places.

This article is organized as follows: After a short introduction, we briefly presented how a pulsatory liposome works. Then we presented the mathematical modeling of the dynamical evolution of the pulsatory liposome. The results were included in the next chapter. The significance, importance and perspectives of this research have been emphasized in the last part of the article.

MATERIALS AND METHODS

PULSATORY LIPOSOME OPERATION

Let us consider a liposome, large enough, filled with an aqueous solution of an osmotic solute. This liposome is introduced into a sufficiently large spherical bath filled with water.

The liposome swelling due to the osmosis process is a fairly slow process, so that the liposome could not break. However, there is another possibility: the liposome grows to a critical dimension when a pore will appear somewhere into its membrane.

The appearance of the pore is an important event for the vesicle dynamics because it reverses the direction of the vesicle evolution that is it stops swelling and starts to relax and decrease of its size up to initial value.

The pore dynamics is dictated by two opposed forces: the membrane surface tension and the line tension of the pore edge. The pore evolution may be described as follows. In the first stage of the pore dynamics, the pore radius increases to a maximum value r_m , and the internal material leaks out from the vesicle through the pore, due to Laplace pressure. Both phenomena, the pore radius increase and the internal material release outside of the liposome, determines the membrane relaxation and the decrease of the membrane mechanical tension.

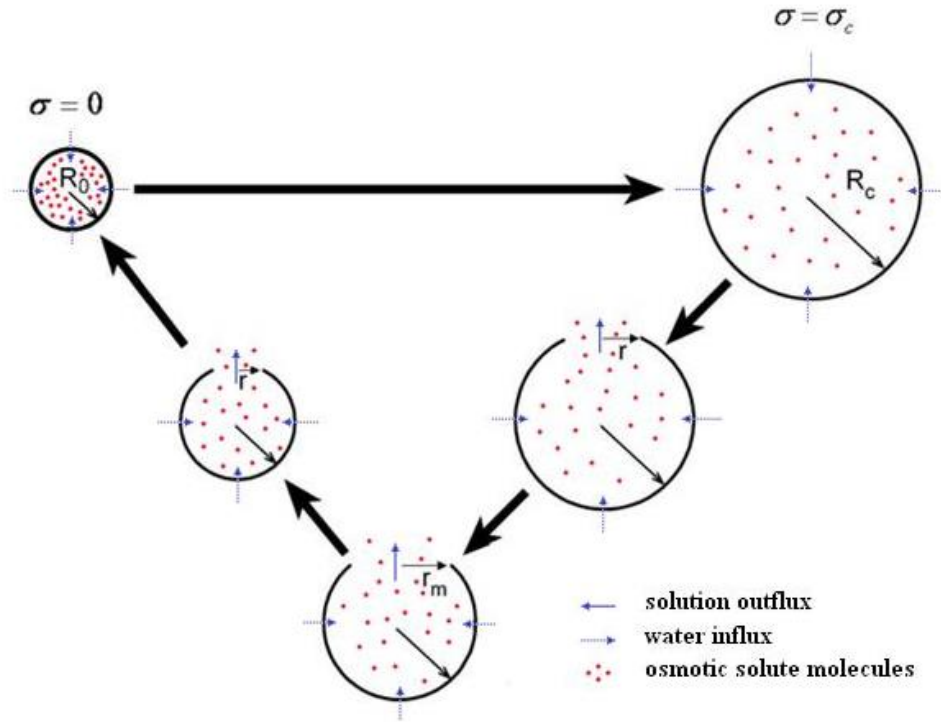


Fig. 1. Evolution of a pulsatory liposome during a cycle. Each cycle has two phases: swelling and relaxation. In the first phases, the liposome swells from the initial state (the radius is equal to R_0 , the membrane is smooth and unstretched, $\sigma = 0$), up to the critical state when the pore appears (the radius is equal to R_c , the membrane is strained, $\sigma = \sigma_c$). The second phase of the cycle is determined by the

appearance of the transmembrane pore. The pore evolution has two stages: in the first part the pore radius rises up to a maximum value (r_m), after which, in the second stage, the pore radius decreases until the pore disappears and the membrane of the liposome recovers. Through pore, some of the liposome inside content is expelled to the external environment. Finally, the pore disappears and the liposome returns to its initial state and a new cycle can start.

From the moment when the line tension equals the membrane surface tension, the second part of the dynamic pore starts, therefore the pore radius decreases until its disappearance, and the vesicle reaches both to the initial diameter and to the relaxed state ($\sigma = 0$). After that, the vesicle dynamics described above can begin to swell again by itself and a new cycle starts. 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, such vesicle, we have named it pulsatory liposome. At the end of each cycle only a single parameter was changed: the internal solute concentration. The dynamics of a pulsatory liposome during a cycle is drawn in Fig.1.

The cyclic dynamics evolution of a pulsatory liposome is described by a couple formed by a differential equation for vesicle radius during the swelling stage and a system of three differential equations for vesicle radius, pore radius and solute internal concentration for the relaxed stage.

Only the dynamic evolution of the liposome inserted into an infinite hypotonic medium has been studied up to now.

In this paper we analyze the pulsating dynamics of liposomes when these are introduced into a closed hypotonic environment (Fig. 2).

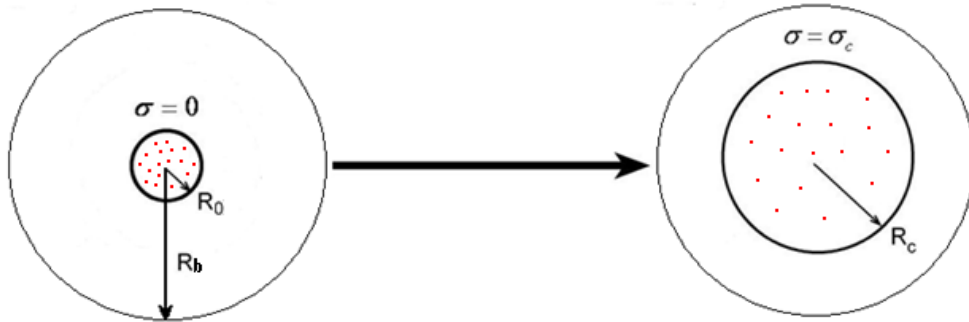


Fig. 2. The swelling phase of a pulsatory liposome, when it is introduced into a spherical closed bath filled with water. In the initial state the liposome membrane is untensed ($\sigma = 0$). In the final state just before the appearance of the pore, named the critical state, the mechanical tension reached its critical value ($\sigma = \sigma_c$).

MATHEMATICAL DESCRIPTION OF SWELLING STAGE

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 changes, because of osmotic influx. The direct consequence of volume growth is the stretch of the membrane and the appearance of Laplace pressure.

The volume variation of the liposome is described by the following equation:

$$\frac{dV}{dt} = P_w V_{\mu w} S \left(\Delta C_m - \frac{2\beta\sigma}{R} \right) \quad (1)$$

where V is the liposome volume, P_w (measured in m/s) – the water permeability through liposome membrane, $V_{\mu w}$ – the water molar volume (in m^3/mol), S – the vesicle area; $\Delta C_m = C_{\text{int}} - C_{\text{out}}$ is the transmembrane gradient of the osmotic solute concentration; C_{int} is the osmotic solute concentration inside the liposome and C_{out} is the osmotic solute concentration outside of the liposome; $\beta = 1/(N_A k_B T)$; N_A – is the Avogadro number, k_B – the Boltzmann constant, and $T = 300 \text{ K}$ – the absolute temperature; $2\sigma/R$ is the Laplace pressure; σ is the mechanical tension of the stretched membrane and R is the liposome radius.

Taking into account that for the stretched vesicle without pore the mechanical tension is equal to:

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

the final form of the equation (1) is:

$$\frac{dR}{dt} = P_w V_{\mu w} \left[\Delta C_m - \frac{2\beta E}{R} \left(\frac{R^2}{R_0^2} - 1 \right) \right] \quad (3)$$

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

In each cycle, the amount of internal solute is conserved during the liposome swelling stage. So, for the swelling of the liposome during the first cycle, we can write the mass conservation law:

$$C_{01} V_0 = C_1 V = C_{c1} V_c \quad (4)$$

where C_{01} is the initial solute concentration for the first cycle, C_1 is the solute concentration when the liposome has reached the volume V during the swelling process, and C_{c1} is the solute concentration at the end of swelling stage before pore nucleation, when the liposome volume is equal to V_c . The index “1” refers to the first cycle.

If one considers the external solute concentration equal to zero, then $\Delta C_m = C_1$, being equal to C_{01} at the beginning of the cycle and C_{c1} at the cycle end.

The final form of the differential equation which describes the swelling stage of the first cycle becomes:

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

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

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

The initial condition is: $R(0) = R_0$, that is: $x(0) = 1$.

The analytical solution of equation (6) is:

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

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

The solution of the differential equation (6) 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 until it reaches the critical state. It can be computed from the equation (6) if the variable x , is replaced by its critical value: $x = x_c = R_c/R_0$

The first cycle is a special one because the concentration of the osmotic solute in the external environment is zero.

We will now focus on the swelling stage of one cycle of order “ n ” ($n > 1$) of the pulsatory liposome activity. The solute concentration at the end of the swelling stage of the $(n-1)$ -th cycle, is equal to $C_{c(n-1)}$.

During a relaxation stage, an amount of internal solution is expelled outside the liposome in the bath. Since the relaxation stage is very short, the solute

concentration inside the liposome may be considered unchanged at the end of the relaxation phase.

So, before the start of the n -th cycle, the system bath and liposome is characterized by the following values of solute concentration:

– 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 obtaine:

$$C_{0n} = C_{0(n-1)} \frac{R_0^3}{R_c^3} = f^{n-1} C_{01} \quad (9)$$

If we define the swelling critical ratio as the ratio between the vesicle volume in the stretched state just before pore nucleation and the vesicle volume in the complete relaxed state, it results the inverse of the swelling critical ratio:

$$f = R_0^3 / R_c^3$$

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

$$\Delta Q_{n-1} = C_{01} V_0 - C_{0n} V_0 = C_{01} V_0 (1 - f^{n-1}) \quad (10)$$

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:

$$C_{e0n} = \frac{\Delta Q_{n-1}}{V_b - V_0} = C_{01} \frac{1 - f^{n-1}}{F - 1} \quad (11)$$

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:

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

$$C_{en} = \frac{\Delta Q_{n-1}}{V_b - V} = C_{01} \frac{1 - f^{n-1}}{F - x^3} \quad (11)$$

$$\Delta C_n = C_n - C_{en} = C_{01} \left(\frac{f^{n-1}}{x^3} - \frac{1 - f^{n-1}}{F - x^3} \right) \quad (12)$$

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

$$\frac{dx}{dt} = \frac{P_w V_{\mu w} C_{01}}{R_0} \left[\frac{f^{n-1}}{x^3} - \frac{2\beta E}{R_0 C_{01}} \left(x - \frac{1}{x} \right) - \frac{1 - f^{n-1}}{F - x^3} \right] \quad (13)$$

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

The number of cycles, N , that a pulsatory liposome can perform is determined by the condition that the osmotic pressure is lower than the Laplace pressure:

$$N_A k_B T \Delta C_N \leq \frac{2\sigma}{R} \quad (14)$$

RESULTS

We will apply our above theoretical results to an experimentally used lipid vesicles. Such lipid vesicles have a radius in the initial state, $R_0 = 19.7 \mu\text{m}$ and the critical radius value is $R_c = 20.6 \mu\text{m}$ [2]. This lipid vesicle was filled with an aqueous solution of osmotic substance having a concentration of $C_{01} = 0.5 \text{ M}$ and then introduced into a spherical box containing water, which has $R_b = 2.5R_0$.

The membrane permeability coefficient for water $P_w = 3 \times 10^{-5} \text{ m/s}$, and water molecular volume is $V_{\mu w} = 18.04 \times 10^{-6} \text{ m}^3/\text{mol}$ [6]. The two-dimensional stretch modulus of the lipid bilayer is $E = 0.2 \text{ N/m}$ [4].

Parameters characterizing the activity of the pulsatory liposome are: the number of cycles, the amount of dissolved substance released during each cycle, the duration of each cycle, the total amount of dissolved internal substance released during the pulsatory liposome activity and the life span of the pulsatory activity. The length of time for a cycle is equal to the sum of the swelling time and relaxation time

The size of the hypotonic environment influences directly and strongly the number of cycles and swelling time of the pulsatory liposome. The other parameters characterizing the activity of the pulsatory liposome will be less and indirectly influenced. For this reason, we wanted to study only the liposome swelling step.

The number of cycles performed by this liposome is equal to 20 and was calculated using the formula resulting from the condition that the osmotic pressure is low than the Laplace pressure [19, 25].

If the same liposome is inserted into a large aqueous medium ($C_{out} = 0$ all time), it will perform 47 cycles as it results according to the formula (14).

Figure 3 represents the time of swelling as a function of the liposome radius for the cycles having the following order numbers: 1, 5, 10 and 15 in the cases where the liposome is introduced into a closed hypotonic medium (A) and opened (B), respectively.

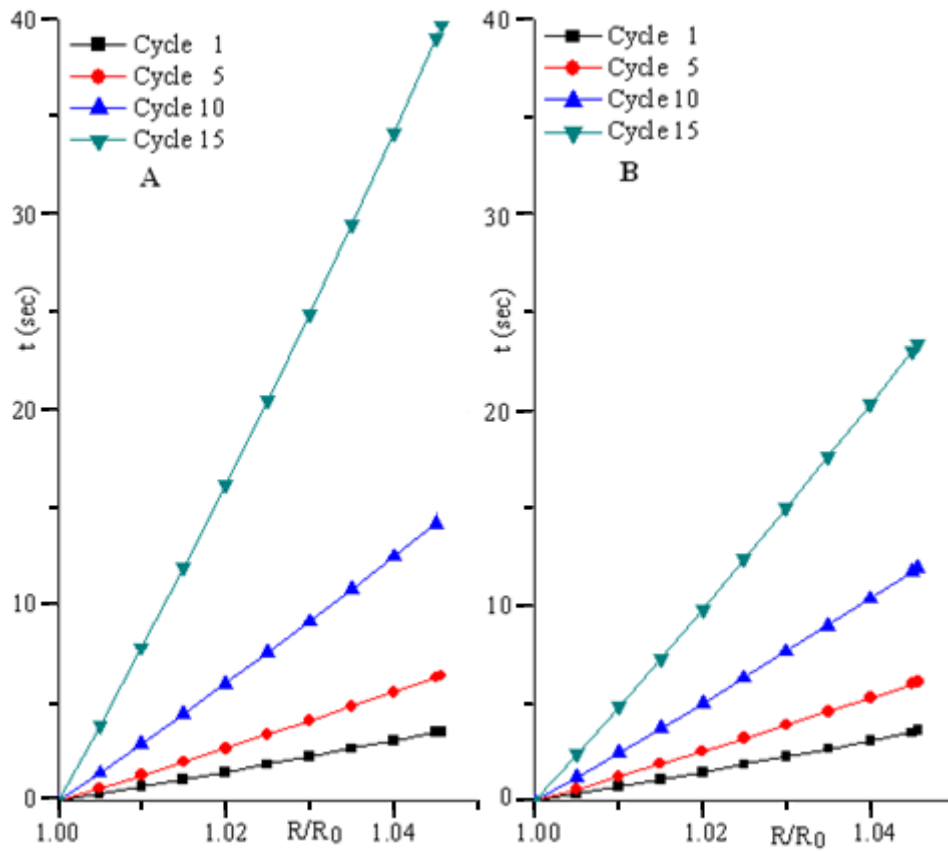


Fig. 3. Swelling time as a function of liposome radius for cycles having the order number: 1, 5, 10, 15 if the liposome is introduced into a closed bath filled with water (A) and if the same liposome is introduced into an open environment of water (B).

Figure 4 represents the time of swelling as a function of the liposome radius for the last cycles of the pulsatory liposome activity in the two cases studied here: a closed hypotonic medium (A) and opened hypotonic medium (B).

In both cases, the time-dependence of the radius of the pulsatory liposome during swelling phase is linear for all cycles except for the last 3–4 cycles.

In the two graphs of Fig. 5, the length time of swelling is measured in seconds (left vertical axis), respectively in minutes (vertical axis on the right).

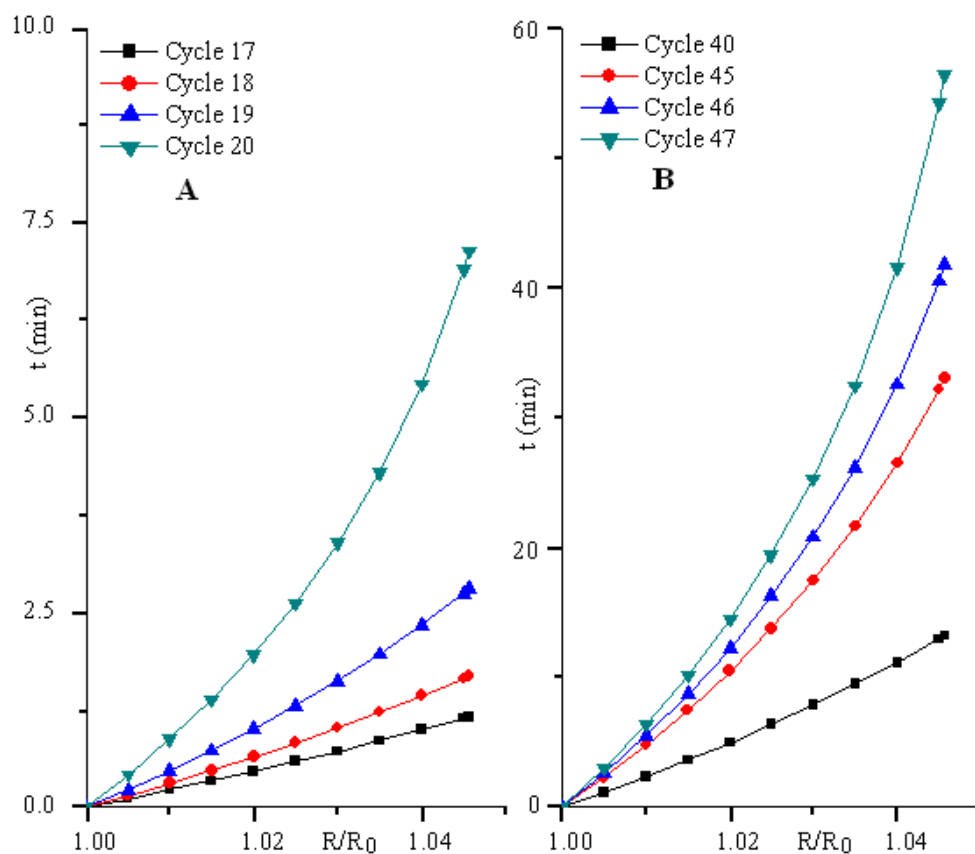


Fig. 4. Evolution of the pulsatory liposome radius during the swelling stage corresponding to the last cycles. Cycles 17, 18, 19 and 20 when the liposome is introduced into a closed (A) and cycles 40, 45, 46 and 47 when the liposome is in an opened hypotonic environment (B).

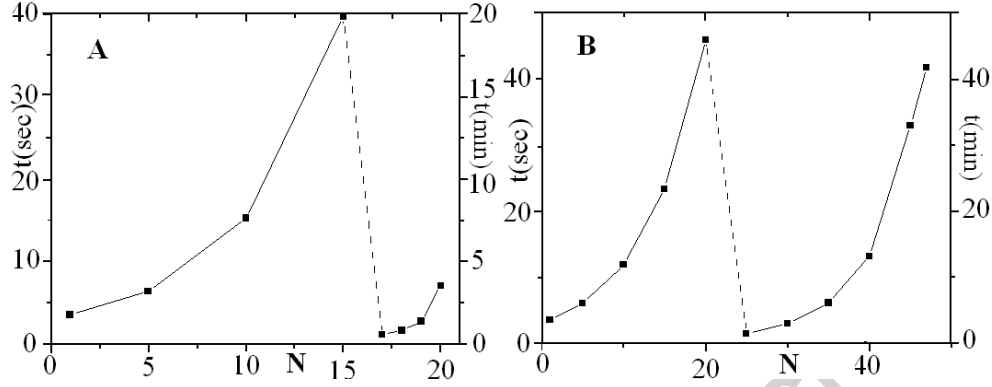


Fig. 5. The length time of swelling stage as a function of the rank cycle for the pulsatory liposome that works in a closed water medium (A) and inside an open water medium (B).

DISCUSSION AND CONCLUSION

The size of active life and, implicitly, the number of cycles performed by pulsatory liposome, depends on the initial concentration of the osmotic solute within the liposome. Each cycle has two stages: swelling and relaxation. For this reason, the pulsatory liposome can be called two-stroke biomicroengine. Until now, it was studied only the dynamics of a pulsatory liposome inserted into an infinite hypotonic environment, that is in a large bath filled with water.

It is possible that such liposomes may be used, in the future, in medical applications, as a device for controlled drug delivery to previously established tissues where the liposomes will work inside a closed space [5, 25].

For this reason, in this paper, we analyzed the swelling stage of a pulsatory liposome cycle, when the external hypotonic medium is closed.

We have focused only on the pulsatory liposome swelling stage because the external environment does not influence the parameters characterizing the pulsatory liposome relaxation phase. The relaxation stage is described by three differential equations: one for liposome radius, one for pore radius, and the third equation for solute concentration [19, 20, 25].

The amount of solution released outside during a cycle is a very important parameter, especially for biotechnological applications because the solute can be an active pharmacological substance.

REFERENCES

1. ALLEN, T.M., P.R. CULLIS, Liposomal drug delivery systems: From concept to clinical applications, *Advanced Drug Delivery Reviews*, 2013, **65**, 36–48.
2. BROCHARD, F., P.G.de GENNES, O. SANDRE, Transient pores in stretched vesicles: role of leak-out, *Physica A*, 2000, **278**, 32–51.
3. GUEDEAU-BOUDEVILLE, M.-A., L. JULLIEN, J.-M. di MEGLIO, Drug delivery piercing vesicles by their adsorption onto a porous medium, *Proc. Natl. Acad. Sci., USA* 1995, **92**, 9590–9592.
4. 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.
5. LASIC, D.D., From physics to applications, in: *Liposomes*, Elsevier, Amsterdam, 1993, pp. 318–321.
6. LAWACZECK, R., On the permeability of water molecules across vesicular lipid bilayers, *J. Membr. Biol.*, 1979, **51**, 229–261.
7. LEVIN, Y., M.A. IDIART, Pore dynamics of osmotically stressed vesicles, *Physica A*, 2004, **331**, 571–578.
8. MEYER, F., M. FINER, Gene therapy: progress and challenges, *Cell Mol. Biol.*, 2001, **47**, 1277–1294.
9. MOVILEANU, L., D. POPESCU, A theoretical model for the association probabilities of saturated phospholipids from two-component bilayer lipid membranes, *Acta Biotheoretica*, 1998, **46**(4), 347–368.
10. MOVILEANU, L., D. POPESCU, The birth, life and death of statistical pores into a bilayer membrane, in: *Recent Research Developments in Biophysics*, vol. 3, part I., Transworld Research Network, Kerala, 2004, pp. 61–86.
11. MOVILEANU, L., D. POPESCU, S. ION, A. POPESCU, Transbilayer pores induced by thickness fluctuations, *Bull. Math. Biol.*, 2006, **68**(6), 1231–1255.
12. POPESCU, D., G. VICTOR, Association probabilities between the single-chain amphiphiles into a binary mixture in plan monolayers (I), *Biochim. Biophys. Acta*, 1990, **1030**(2), 238–250.
13. POPESCU, D., CONSTANTA RUCAREANU, G. VICTOR, A model for the appearance of statistical pores in membranes due to selfoscillations, *Bioelectrochem. Bioenerg.*, 1991, **25**, 91–103.
14. POPESCU, D., CONSTANTA RUCAREANU, Membrane selfoscillations model for the transbilayer statistical pores and flip-flop diffusion, *Mol. Cryst. Liquid Cryst.*, 1992, **25**, 339–348.
15. POPESCU, D., Association probabilities between the single chain amphiphiles into a binary mixture in plan monolayers (II), *Biochim. Biophys. Acta*, 1993, **1152**, 35–43.
16. POPESCU, D., S. ION, A. 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.
17. POPESCU, D., C.N. ZAHARIA, S. ION, MARIA LUISA FLONTA, Compensation of the neurotransmitters deficiency in the synaptic cleft, *Romanian J. Biophys.*, 2006, **16**, 189–204.
18. POPESCU, D., L. MOVILEANU, A.G. POPESCU, The behavior 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.
19. POPESCU, D., A.G. POPESCU, The working of a pulsatory liposome, *J. Theoret. Biol.*, 2008, **254**, 515–519.

20. POPESCU, D., Mathematical modeling of the pulsatory lipid vesicle dynamics under osmotic stress, *Proceedings of the Romanian Academy A*, 2010, **11**(2), 108–115.
21. 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**(1), 37–46.
22. POPESCU, D., A.G. POPESCU, B. AMUZESCU, Pulsatory liposomes – a possible biotechnological device for controlled drug delivery, II. The pore appearance, *Romanian J. Biophys.*, 2010, **20**(2), 171–181.
23. POPESCU, D., A.G. POPESCU, B. AMUZESCU, Pulsatory liposomes – a possible biotechnological device for controlled drug delivery, III. The liposome swelling, *Romanian J. Biophys.*, 2010, **20**(3), 223–234.
24. POPESCU, D., D.P. IGA, Transmembrane delivery of biologically active substances by pulsatory liposomes, *Rev. Chim. (Bucharest)*, 2010, **61**(1), 78–81.
25. POPESCU, D., *The Pulsatory Lipid Vesicle Dynamics Under Osmotic Stress*, Lambert Academic Publishing, Saarbrücken, Germany, 2012.
26. SANDRE, O., L. MOREAUX, F. BROCHARD-WYART, Dynamics of transient pores in stretched vesicles, *Proc. Natl. Acad. Sci. USA*, 1999, **96**, 10591–10596.
27. VERMA, I.M., M.D. WEITZMAN, Gene therapy: twenty-first century medicine, *Annu. Rev. Biochem.*, 2005, **74**, 711–738.
28. ZASADZINSKI, J.A., Novel approaches to lipid-based drug delivery, *Curr. Opin. Solid State Mat. Sci.*, 1997, **2**, 345–349.