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

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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 they 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].

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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 past 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 appears 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 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, determine the membrane relaxation and the decrease of the membrane mechanical tension.

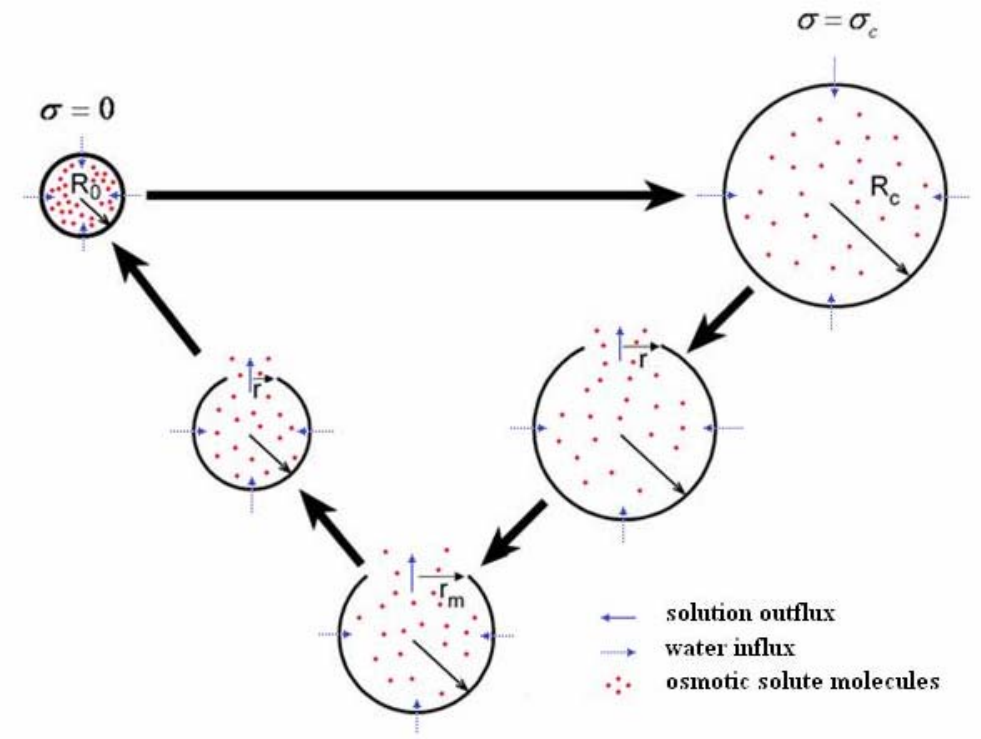

Fig. 1. Evolution of a pulsatory liposome during a cycle. Each cycle has two phases: swelling and relaxation. In the first phases, the liposome swells from the initial state (the radius is equal to R_0, the membrane is smooth and unstretched, $\sigma = 0$), up to the critical state when the pore appears (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 the 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, we have named such a vesicle, 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 until now.

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

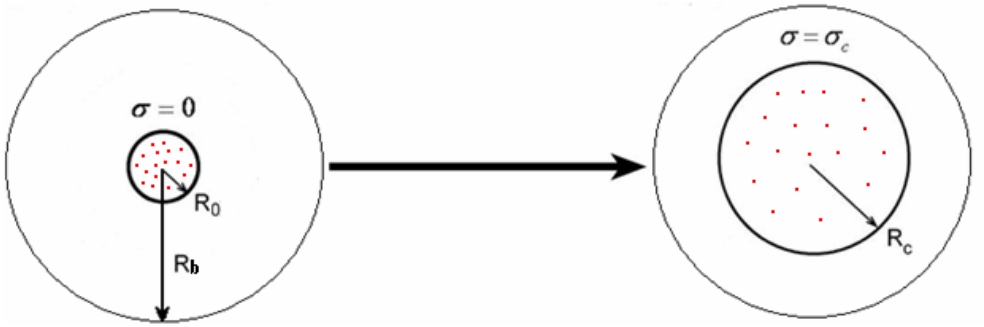

Fig. 2. The swelling phase of a pulsatory liposome, when it is introduced into a spherical closed bath filled with water. In the initial state the liposome membrane is untensed ($\sigma = 0$). In the final state just before the appearance of the pore, named the critical state, the mechanical tension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

$$\frac{\mathrm{d}V}{\mathrm{d}t} = P_{\mathrm{w}} V_{\mu\mathrm{w}} S\left(\Delta C_{\mathrm{m}} - \frac{2\beta\sigma}{R}\right) \tag{1}$$

where 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), S – the vesicle area; $\Delta C_{\mathrm{m}} = C_{\mathrm{int}} - C_{\mathrm{out}}$ 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; $\beta = 1/(\mathrm{N_A k_B} T)$; $\mathrm{N_A}$ – is the Avogadro number, $\mathrm{k_B}$ – the Boltzmann constant, and $T = 300$ 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:

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

the final form of the equation (1) is:

$$\frac{\mathrm{d}R}{\mathrm{d}t} = P_{\mathrm{w}} V_{\mu\mathrm{w}} \left[\Delta C_{\mathrm{m}} - \frac{2\beta E}{R}\left(\frac{R^2}{R_0^2} - 1\right)\right] \tag{3}$$

where R is the liposome radius during 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:

$$C_{01} V_0 = C_1 V = C_{\mathrm{c1}} V_{\mathrm{c}} \tag{4}$$

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:

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

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

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

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

The analytical solution of equation (6) is:

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

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

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 solute concentration inside the liposome is C_{0n} and inside the external bath C_{e0n}, whose calculation formula is obtained as follows:

– *The solute concentration, C_{0n}.* It 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 calculate:

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

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 solute concentration, C_{e0n}.* 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:

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

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 inside the external bath is equal to:

$$C_{e\,0n} = \frac{\Delta Q_{n-1}}{V_b - V_0} = C_{01} \frac{1 - f^{n-1}}{F - 1} \tag{11a}$$

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:

$$C_{e\,n} = \frac{\Delta Q_{n-1}}{V_b - V} = C_{01} \frac{1 - f^{n-1}}{F - x^3} \tag{11b}$$

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

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

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

$$\frac{\mathrm{d}x}{\mathrm{d}t} = \frac{P_{\mathrm{w}} V_{\mu\mathrm{w}} C_{01}}{R_0} \left[\frac{f^{n-1}}{x^3} - \frac{2\beta E}{R_0 C_{01}} \left(x - \frac{1}{x} \right) - \frac{1 - f^{n-1}}{F - x^3} \right] \tag{13}$$

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:

$$\mathrm{N_A k_B} T \Delta C_{\mathrm{N}} \leq \frac{2\sigma}{R} \tag{14}$$

RESULTS

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

The membrane permeability coefficient for water $P_w = 3\times10^{-5}$ m/s, and water molecular volume is $V_{\mu w} = 18.04\times10^{-6}$ m^3/mol [6]. The two-dimensional stretch modulus of the lipid bilayer is E = 0.2 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 lower than the Laplace pressure [19, 25].

If the same liposome is inserted into a large aqueous medium (C_{out} = 0 all the 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 an opened one (B), respectively.

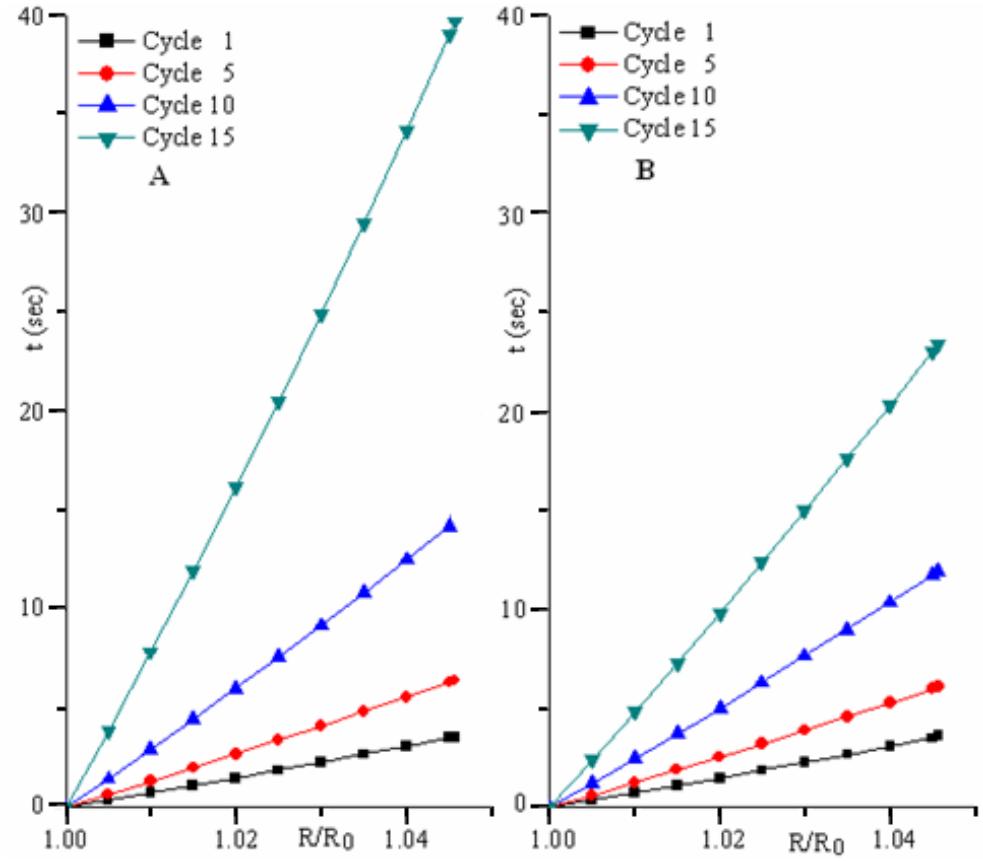

Fig. 3. Swelling time as a function of liposome radius for cycles having the order number: 1, 5, 10, 15 if the liposome is introduced into a closed bath filled with water (A) and if the same liposome is introduced into an open environment of water (B).

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 an 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).

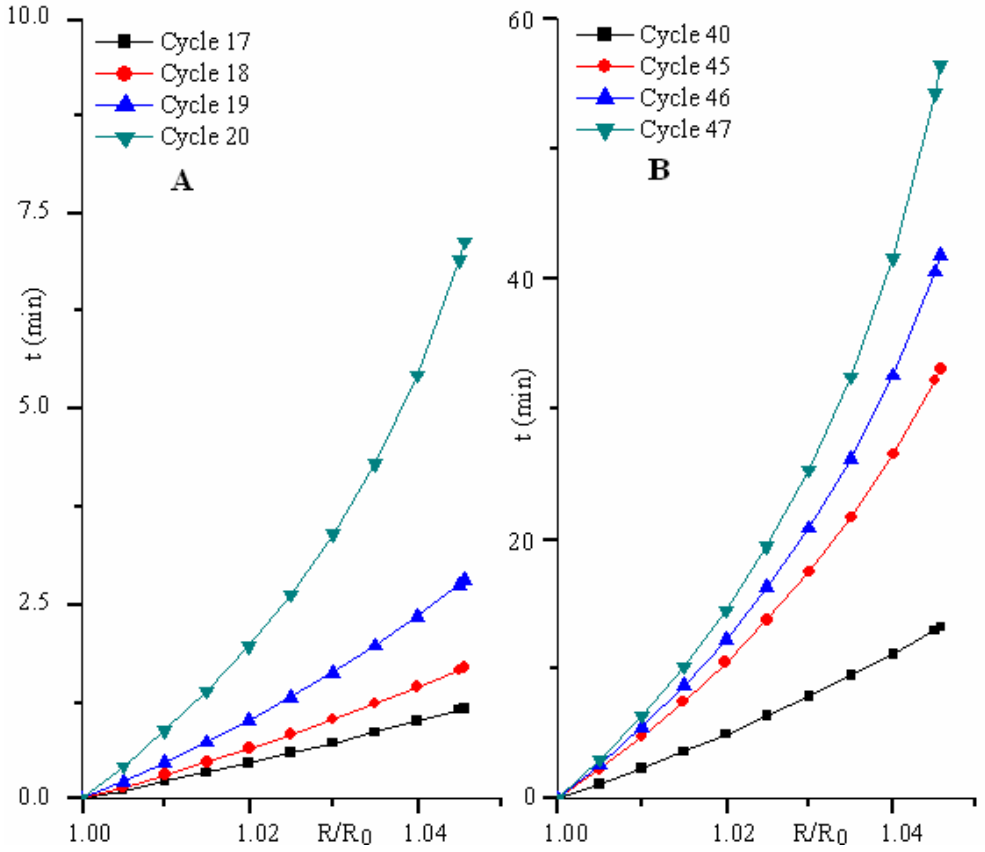

Fig. 4. Evolution of the pulsatory liposome radius during the swelling stage corresponding to the last cycles. Cycles 17, 18, 19 and 20 when the liposome is introduced into a closed medium (A) and cycles 40, 45, 46 and 47 when the liposome is in an opened hypotonic environment (B).

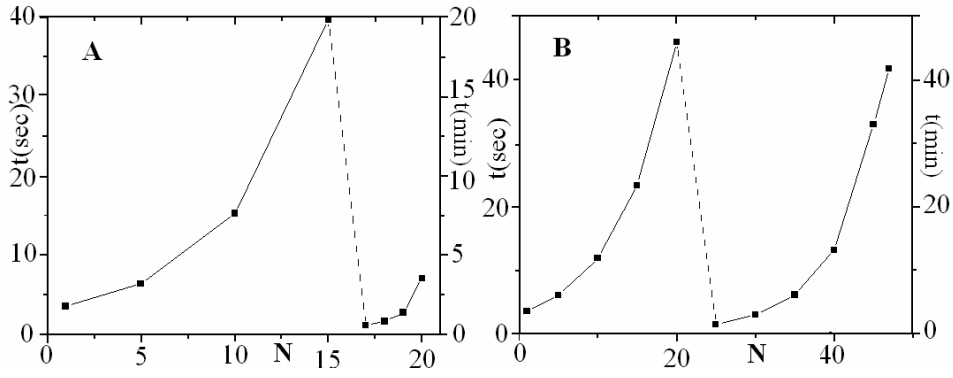

Fig. 5. The length time of swelling stage as a function of the rank cycle for the pulsatory liposome that works in a closed water medium (A) and inside an open water medium (B).

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.

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