Transmembrane Delivery of Biologically Active Substances by Pulsatory Liposomes

DUMITRU POPESCU[1]* , DUMITRU PETRU IGA[2]

[1] Institute of Mathematical Statistics and Applied Mathematics, Department of Mathematical Modelling in Life Sciences, Romanian Academy, 13 Calea 13 Septembrie, Bucharest, Romania

[2] University of Bucharest, Faculty for Biology, Chemistry Department, 91-95 Spl. Independentei, 050095, Bucharest, Romania

The running of a biological device, named pulsatory liposome is described. In a hypotonic external medium a liposome is swelling up to a critical size, and then suddenly one transmembrane pore is formed. A part of the internal constituents, genetic material, drugs or other biologically active compounds, comes out of the liposome through this transmembrane pore. In the first part of its evolution, the pore increases, then decreases, and finally it is closed. The kinetic process described above is resumed, becoming a cyclic one. The amount of substance delivered in each cycle, the number of cycles per time unit and their duration have been calculated.

Keywords: pulsatory liposome, transmembrane transport, transient pore, drug delivery

The transport across lipid bilayer through transbilayer pores is a new strategy for biological material exchange between the two adjacent media [1, 2].

There are at least two interesting and important biotechnological applications in which the increase of membrane permeability is needed: gene therapy and targeted drug delivery. In the first one, the transport of DNA fragments through cellular and nuclear membranes is accomplished [3]. The second application uses drug molecules encapsulated in vesicles, which have to be transported to a target tissue [4]. Once it reached the cell, the vesicle has to release the drug molecules, in a well-controlled fashion. The formation of transbilayer pores can be stimulated by using chemical and/or physical methods [5-13]. By using the osmotic shock method one can produce a stretch of the vesicle membrane, which then relaxes by forming a transient pore. Such a pore may reach diameter up to 10 μm. The appearance of pores through the cellular membrane, caused by mechanical tension, constitutes a possibility for the transport of intracellular material outside the cell. Recently, in vesicles stretched by optical induced tension, a single pore, of several micrometers in size, was observed periodically in a given vesicle [14]. However, in the same vesicle, 30 – 40 pores appear successively [15,16].

In this paper, we have focused our attention on the successively formed transient pores induced in a vesicle by osmotic stress and on the time interval between two successive pore formation.

Firstly, the transient pore dynamics is described. Then, the solute concentration inside the vesicle, depending on the time elapsed, is calculated. The time period between two successive pores has been also calculated. The active life time of pulsatory liposome has been determined and some interesting applications in biology and medicine are advanced.

Theoretical foundation

Let us imagine an unillamelar liposome filled with a watery solution of a solute. The lipid bilayer normally is impermeable to the solute. At the same time, the discussed liposome is a giant liposome, called vesicle. It is introduced into a medium presenting a concentration gradient of solute between external medium and internal medium. More precisely, solute concentration outside liposome is smaller (or even zero) in comparison with inside solute concentration. Osmotic pressure created by the transmembrane gradient of solute concentration determines an influx of water molecules through liposome membrane.

Due to the supplementary water entered inside, the liposome increases up to a critical size. In this stage his membrane is submitted to a maximal mechanical tension, and one pore has to appear (fig.1). This event is followed by two simultaneous proceses: the pore radius increases and the internal material leaks out the vesicle through the pore, due to excess Laplace pressure. Both these phenomena, pore increase and internal liquid leakage determine the membrane relaxation, due to reducing of the membrane mechanical tension.

The membrane tension decreases till it becomes equal to line tension of the membrane edge. In fact, the pore dynamics is led by the difference between the membrane tension and line tension[17]. The internal liquid continues to leak out the liposome, even after the line tension equals the membrane tension. In the moment when the line tension equals the membrane tension, the second part of dynamic pore starts, therefore the pore radius decreases up to its disappearance. Now, the vesicle dynamics described above, begins again to swell and a new cycle is resumed. This cyclic process ceases when the excess Laplace pressure is higher than osmotic pressure.

The appearance and disappearance of the successive pores endow the liposome with pulsatory property. His energy is supplied by transmembranar osmotic gradient.
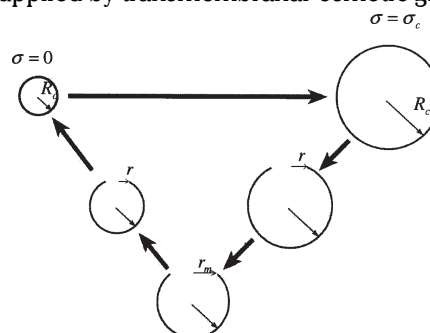

Fig.1. The evolution of a pulsatory liposome during a cycle

Theoretical results
The inside concentration in the swelling stage

Let us analyze a lipid vesicle containing a solution whose solvent is water, and the solute to which the lipid bilayer is impermeable. The solute may be: genetic material, drugs or any other special substances.

This liposome is introduced into a bath filled with water. At the beginning of its activity, the liposome contains solute, with molar concentration C_{s0}, and water, with molar concentration C_{w0}. Let suppose that the process is at the initial state of liposome evolution, before swelling starts. Initially, in a complete relaxation state the vesicle contains: $N_{x0} = C_{x0}V_0$ mols solute and $N_{w0} = C_{w0}V_0$ mols of water. At the end of the swelling stage, just before the pore opening, the same number of solute molecules is present in the vesicle, but this contains a larger number of water molecules. This water quantity (measured in mols), noted with N'_{w0}, is equal to:

$$N'_{w0} = N_{w0} + N^+ \qquad (1)$$

where N^+ is the amount of water which enters the liposome during the swelling process due to osmotic influx. The liposome internal radius increases from R_0 (vesicle bilayer is smooth and $\sigma = 0$) to the critical size R_c, when $\sigma = \sigma_c$, just before pore formation. So, N^+ is:

$$N^+ = \frac{V_c - V_0}{V_{\mu w}} = \frac{4\pi\left(R_c^3 - R_0^3\right)}{3V_{\mu w}} = \frac{4\pi R_c^3}{3V_{\mu w}}\left(1 - \frac{R_0^3}{R_c^3}\right) = N(1 - f) \qquad (2)$$

In the above formula the following notations have been introduced:

$N = V_c / V_{\mu w} = 4\pi R_c^3 / (3V_{\mu w})$ is the number of moles of water which would fill the stretched vesicle just before the pore appearance if only water would be present. The molar volum of water is noted with $V_{\mu w}$.

$f = V_0 / V_c = R_0^3 / R_c^3$ is the ratio between the vesicle volumes in the complete relaxed state (V_0) and in the stretched state just before pore formation (V_c).

The internal liquid concentration changes during each cycle. Let us analyse the first cycle in its both stages:

- swelling stage, is represented by the upper part of figure 1. At the beginning of the first cycle (vesicle is in a complete relaxation state), the vesicle contains:

$$N_{s0} = C_{s0}V_0 \text{ mols of solute, and} \qquad (3)$$

$$N_{w0} = C_{w0}V_0 \text{ mols of water.} \qquad (4)$$

At the end of the swelling stage, just before the pore opening, the same amount of solute is present in the vesicle, but this contains a larger amount of water:

$$N'_{w0} = N_{w0} + N^+ = C_{w0}V_0 + N(1 - f) \qquad (5)$$

The new concentrations at the end of the first cycle are:

$$C_{s1} = N_{s0}/V_c = C_{s0}V_0/V_c = fC_{s0} \qquad (6)$$

$$C_{w1} = N'_{w0}/V_c = \left[C_{w0}V_0 + N(1 - f)\right]/V_c = fC_{w0} + (1 - f)/V_{\mu w} \qquad (7)$$

- relaxation stage, represented by the lower part of figure 1. After the pore opening, the pore radius increases up to a maximal value, r_m, then it decreases and finally the pore is sealed (fig.1). During the pore evolution an amount of internal liquid leaks out.

At the beginning of the second cycle, the vesicle is in a complete relaxation state and contains:

$$N_{s1} = V_0 C_{s1} = fV_0 C_{s0} \text{ mols of solute and} \qquad (8)$$

$$N_{w1} = V_0 C_{w1} = fV_0 C_{w0} + V_0(1 - f)/V_{\mu w} \text{ mols of water.} \qquad (9)$$

By making the same reasoning as for the first cycle, one finds out the following recurrent formula for the characterization of the inside concentration of the vesicle at the end of the kth cycle:

$$C_{sk} = f^k C_{s0} \; ; \; C_{wk} = f^k C_{w0} + \left(1 - f^k\right)/V_{\mu w} \qquad (10)$$

$$N_{sk} = f^k V_0 C_{s0} \; ; \; N_{wk} = f^k V_0 C_{w0} + V_0\left(1 - f^k\right)/V_{\mu w} \qquad (11)$$

The number of pulses. As it was mentioned before, the liposome will work a cycle if at the end of the cycle is available the condition:

$$\pi_{osm} \geq \pi_L \qquad (12)$$

where,

π_{osm} is the osmotic pressure which appears due to transbilayer gradient of the solute, ΔC_s. According to Pfeffer law [18, 19], it is given by the following formula:

$$\pi_{osm} = \Re T\left(C_s^{in} - C_s^{out}\right) \qquad (13)$$

Here, C_s^{in} and C_s^{out} are the inside solute concentration and outside solute concentration, respectively, $\Re$ is the universal gas constant, and T is the absolute temperature.

The formula (13) is valid if the membrane is impermeable to the solute and for dilute solution.

π_L is the Laplace pressure due to separating surface curvature, and for a spherical surface is given by the formula:

$$\pi_L = \frac{2\sigma}{R} \qquad (14)$$

A liposome introduced into a hipoosmotic medium will swell if the osmotic pressure is higher than Laplace pressure:

$$\Re T\left(C_s^{in} - C_s^{out}\right) > \frac{2\sigma}{R} \qquad (15)$$

Take into account that in the external medium the solute concentration is zero, the inequality (15) becomes:

$$\Re T C_s^{in} > \frac{2\sigma}{R} \qquad (16)$$

According to condition (16) it can be stated that a liposome will work n cycles if the following equation is true at the end of the nth cycle:

$$\Re T C_{sn} = \frac{2\sigma_c}{R_c} \qquad (17)$$

The activity of pulsatory liposome ceases when the osmotic pressure becomes smaller than Laplace pressure. Therefore, the following condition is accomplished:

$$\Re T f^n C_{s0} \leq \frac{2\sigma_c}{R_c} \qquad (18)$$

where, n is the number of cycles realized by pulsatory liposome.

Also, the value of n obtained from this formula, represents the number of pulses of solute delivered by the

pulsatory vesicle, or the succesive number of pores formed in the vesicle submitted to a process of swelling, regardless of the method used for this.

From the relation (10) and (17) one finds that the number of cycles accomplished by the pulsatory liposome is given by the following relation:

$$n = \frac{\log \dfrac{2\sigma_c}{\Re T C_{s0} R_c}}{\log f} \qquad (19)$$

The timelength of each cycle [9] For the liposome considered here the swelling time, τ_k, in the k-th cycle is given by formula:

$$\tau_k = \frac{2(R_c - R_0)}{rv(2 - C_{s0}V_{\mu s}f^k)} \qquad (20)$$

where, r is the partition coefficient of water in the hydrophobic medium at the water/hydrophobic core interface and v is the mean transport velocity of water molecules through lipid bilayers.

Solute content of pulse. By making the difference between the solute mols number inside the vesicle after two successive cycles, we obtain the quantity of solute contained in the pulse of internal solution delivered between the two cycles:

$$\Delta N_{sk} = N_{s(k-1)} - N_{sk} = f^{k-1}(1-f)V_0 C_{s0} \qquad (21)$$

Applications

Let us consider a vesicle in a closed and large chamber filled with water. The vesicle contains also other solute molecules for which the bilayer is impermeable. The radius of the relaxed liposome is R_0 = 19.7 μm. The value of the critical radius is R_c = 20.6 μm. The giant vesicles obtained experimentally have such value of critical radius [16]. The critical membrane tension is σ_c = 10^{-5}N/m [20].

We will consider that a quarter of the internal volume of the vesicle is occupied by solute, at the beginning of the experiment. So, $C_{s0}V_{\mu s}$ = 0,25.

The transport velocity of water through the lipid bilayer is v = 10^{-5} m/s [21].The partition coefficient of water in the lipid bilayer is r = 64 10^{-6} [22].

For this liposome, the volumes ratio at the end of swelling stage is f = 0,8746.

Introducing the above data in the formula (20) we obtain the swelling time, τ_k, measured in minutes, as a function of cicle order, k:

$$\tau_k = \frac{3000}{16(8 - f^k)} \qquad (22)$$

The swelling time as a function of the number of cycles k is presented in figure 2. It can be seen that the duration of a cycle decreases and finally reaches a constant value, equal to 23.44 min.

Solute content. We will calculate the solute content of a pulse for the same liposome conditions as we calculated the swelling time for each cycle. We have considered that the solute concentration is C_{s0}=0.5 M. In order to see more intuitively the delivered amount of solute in each cycle, we have calculated it as a number of solute molecules.

Some value of ΔN_k, measured as number of molecules and calculated using the formula (21) for f = 0.8746 is represented in figure 3.
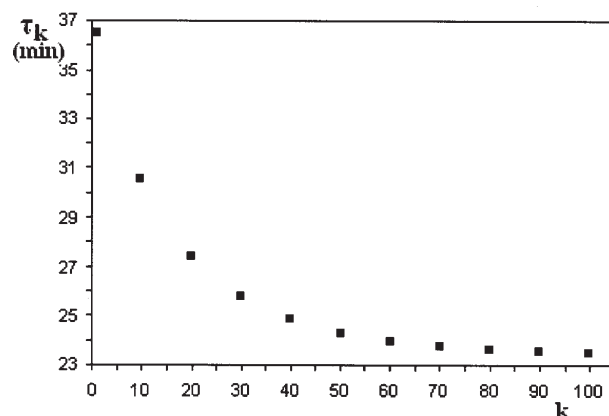

Fig. 2. Swelling time, τ_k versus k, the rank in sequence of pores for the pulsatory liposomes characterized in text
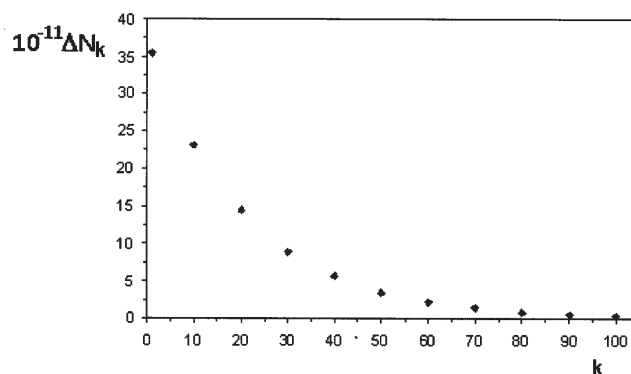

Fig.3. Quantity of solute delivered by each pore in its cycle as a function of the number of cycles, k

For the liposome characterized by the above properties we can calculate the number of cycles of its activity. Using the formula (19) we have obtained n=105 cycles.

Discussion

We have studied a lipid vesicle inside a box filled with water. This box is large enough, so that the solute quantity delivered by pulsatory liposome, does not change significantly the external medium composition. In other words, the solute concentration outside of liposome remains very small and does not influence the osmotic pressure.

In these condition, the pulsatory liposome works 105 cycles.

From thermodynamic point of view, the pulsatory liposome into a large bath filled with water is an open system at constant pressure and temperature.

If the pulsatory liposome would be introduced inside o closed box, for example a cell, the solute concentration between liposome and closed box increases after each cycle. In these conditions, the transbilayer gradient of solute concentration decreases mare rapidly and the cycle number is smaller than in the case of open (or large) box.

A cascade of 30 – 40 succesive pores were observed in a giant unilamellar vesicle stretched by visible light, but it is possible that the amount of lipids in a vesicle cannot be constant during entire process [15, 16].

The transbilayer gradient decreases due to both the decrease of solute concentration inside of the liposome and the increase of solute concentration outside the liposome.

This problem is relatively difficult and will be published in a following paper.

It is obvious, that the pulsatory liposomes use the energy of transbilayer gradient of concentration and the transient pores constitute its propelling force. After a number of cycles, both the periodicity and the amount of solute delivered during each cycle are constant. This phenomenon could be extremely beneficial in some practical applications in biology and medicine.

C_{s0} – initial solute concentration. Also, it could be named as the solute concentration at the beginning of the first cycle, or at the beginning of the pulsatory process of the liposome. It is the most important parameter for active life of the pulsatory liposome.

C_{w0} – initial water concentration, at the beginning of the first cycle.

C_{s1} – solute concentration at the end of the first cycle.

C_{w1} – water concentration at the end of the first cycle. We are considering that during the relaxing process both concentration does not change. So, at the beginning of the second cycle the solute and water concentrations are C_{s1} and C_{w1}, respectively.

C_{sk} – solute concentration at the end of the kth cycle.

C_{wk} – water concentration at the end of the kth cycle. We are considering that during the relaxing process during the kth cycle, both concentrations do not change. So, at the beginning of the $(k+1)^{th}$ cycle the solute and water concentrations are C_{sk} and C_{wk}, respectively. The concentrations are measured in mol/m³.

N – number of moles of water which would fill the liposome in the critical stretched state, just before the pore appearance.

N_{s0} – number of moles of solute existing initially inside the liposome.

N_{w0} – number of moles of water existing initially inside the liposome.

N_{w}^{+} – number of moles of water that enter inside the liposome during the swelling stage due to inward osmotic flux of water. The water amount that enters inside the liposome during swelling stage of each cycle is the same and is equal to N_{w}^{+}.

N'_{w0} – number of moles of water existing initially inside the liposome.

N_{s1} – number of moles of solute existing inside the liposome at the beginning of the second cycle.

N_{w1} – number of moles of water existing inside the liposome at the beginning of the second cycle.

N_{sk} – number of moles of solute existing inside the liposome at the beginning of the $(k+1)^{th}$ cycle.

N_{wk} – number of moles of water existing inside the liposome at the beginning of the $(k+1)^{th}$ cycle.

R_{0} – internal liposome radius in the complete relaxed state ($\sigma = 0$).

R_{c} – internal liposome radius in the critical stretched state, just before the pore appearance ($\sigma = \sigma_{c}$).

R – mean radius of the spherical liposome. In other words it is the radius of the spherical surface which separates the two lipid monolayers.

$\Re$ – universal gas constant

V_{0} - initial volume of the liposome in the complete relaxed state ($\sigma = 0$).

V_{c} – critical volume of the liposome in the critical stretched state, just before the pore appearance ($\sigma = \sigma_{c}$).

$V\mu_{w}$ – molar volume of water.

T – absolute temperature

f - ratio between the liposome volumes in the complete relaxed state (V_{0}) and in the critical stretched state (V_{c}).

r – partition coefficient of water in the hydrophobic medium at the water/hydrophobic core interface.

v – mean transport velocity of water molecules across the lipid bilayer

π_{L} – Laplace pressure due to the curvature of the closed surface

π_{osm} – osmotic pressure, due to the solute concentration gradient between the adjacent medium of a selective membrane.

σ – tension of the stretched membrane.

σ_{c} – tension of the stretched membrane in the critical state.

τ_{k} – swelling time of pulsatory liposome for the kth cycle.

References

1.POPESCU, D., RUCAREANU, C., VICTOR, G., Bioelectrochem. Bioenerg., **25**, 1991, p. 91
2.MOVILEANU, L., POPESCU, D., Recent Research Developments in Biophysics, **3**, Part 1 Transworld Research Network, Kerala, 2004, p. 61
3.VERMA, I. M., SOMIA, M., Nature (London), 389, 1997, p. 239
4.ZASADZINSKI, J. A., Curr. Opin. Solid State Mat. Sci. 2, 1997, p. 345
5.DIETRICH, A., BAHR, G., WINTERHALTER, M., Langmuir, 14, 1998, p. 4597
6.SAITOH, A., TAKIGUCHI, K., TANAKA, Y., HOTANI, H., Proc. Natl. Acad. Sci. USA 95, 1998, p. 1026
7.WEAWER, J. C., CHIZMADZHEV, Y. A., Bioelectrochem. Bioenerg. 41, 1996, 135
8.POPESCU, D., ZAHARIA, C. N., STELIAN, I., FLONTA, M. L., Romanian J. Biophys. 16, 2006, p. 189
9.POPESCU, D., MOVILEANU L., POPESCU A.G., Mathematical Biology Research Trends. Nova Science Publishers (ed. L. B. Wilson), 2008, p.275
10.de GENNES, P. G., TAUPIN, C., J. Phys. Chem. 86, 1982, p. 2294
11.GUEDEAU-BOUDEVILLE, M.-A., JULLIEN, L., DI MEGLIO, J.-M., Proc. Natl. Acad. Sci. USA, 92, 1995, p. 9590
12.BERNARD, A.-L., GUEDEAU-BOUDEVILLE, M.-A., SANDRE, O., PALACIN, S., Di MEGLIO, J.-M., JULLIEN, L., Langmuir, 16, 2000, p. 6801
13.BAR-ZIV, R., NELSON, P., MOSES, E., Biophys. J., 75, 1998, p. 294
14.BROCHARD-WYART, F., DE GENNES, P. G., SANDRE, O., Physica A, 278, 2000, p. 32
15.SANDRE, O., MOREAUX, L., BROCHARD-WYART, F., Proc. Natl. Acad. Sci. USA, 96, 1999, p. 10591
16.KARATEKIN, E., SANDRE, O., GUITOUNI H., BORGHI, N., PUECH P.-H., BROCHARD-WYART, F., Biophys. J., 84, 2003, p. 1734
17.LITSTER, J. D., Phys. Letters A, 53, 1975, p. 193
18.MARIA, G., Rev. Chim.(bucuresti), **59**, 2008, p. 318
19.MARIA, G., Chemical and Biochemical Engineering Quartely, 19, 2005, p. 213
20.NARDI, J., BRUISMA, R., SACKMANN, E., Phys. Rev. E Stat. Phys., 58, 1998, p. 6340
21.LAWACZECK, R., J. Membrane Biol., 51, 1979, p. 229
22.SACKMANN, E., ALBRECHT, O., GRULER, H., J. Physique, 39, 1978, p. 301