The pulsatory liposome works as a neuronal network (I)

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***Abstract**: In this paper we consider a unilamellar liposome (lipid vesicle) filled with aqueous solution of osmotic solute. This liposome is introduced in a hypotonic aqueous medium of large dimensions. Due to the osmosis process the liposome swells to a critical size, when a trans bilayer pore suddenly appears. Some of the internal solution leaks through this pore and the liposome relaxes and returns to its initial size. The swelling starts again and the liposome begins a new cycle and so on. The evolution of the liposome is a dynamic and cyclical process. The swelling of the liposome is described by a differential equation. The appearance of the pore changes the evolution of the liposome. The internal solution comes out through the pore and the liposome starts its deflation (relaxation) and reaches to its initial size. All the processes which contribute to the liposome relaxing and its coming back to the initial size (pore evolution and internal solution delivery) are described by three differential equations. This system of differential equations describes the evolution of the pulsatory liposome during a cycle and can be integrated using numerical methods. After performing a number of cycles, the pulsatory liposome stops. It can be assimilated to an intelligent biophysical engine and a neural network. A neural layer corresponds to a cycle. A neural layer corresponds to a cycle. Each neuronal layer learns to solve the equations that describe the evolution of the liposome during a cycle. So, a pulsed liposome works according to its own program that can be learned by a neural network.*

***Key Words:** Pulsatory liposome, intelligent biophysical engine, neuronal network*

1. INTRODUCTION

The liposome is an artificial model of cell membrane and are used in biophysical membrane researches.

The transport of molecules, especially of large ones, through cellular membrane is a very important process for biological processes and for some biotechnological applications [1, 2].

The transport through trans bilayer pores, across lipid bilayer, is a new strategy for biological material exchange between the two adjacent media [1, 3-5]. The liposome is a lipid vesicle formed by one or more bilayers of phospholipids that separates the external aqueous environment from the internal aqueous medium.

Some pores can appear due to structural and dynamic properties of lipid bilayer. These are named stochastic pores [4–9].

On the other hand, there are pores that appear due to some mechanical tensions induced in different ways [10, 11], electroporation [12], addition on porous surface [13], optical induced mechanical tension [14, 15]and osmotic stress [1, 10, 16, 17]. A sequence of 30-40 pores was recently observed in [11, 18,19].

There are two biotechnology applications which require an increase in membrane permeability: targeted delivery of special compounds and gene therapy. The first application uses special molecules encapsulated in vesicles, which have to be transported to a specified cellular or subcellular site [13, 20 – 24].

In gene therapy, it is necessary to transport DNA fragments through cellular and nuclear membranes, assuming that the liposome discharges its content to the external medium/ environment by its breakdown [25 - 27].

Lately, the formation of transmembrane pores in osmotically stressed vesicle, is the most studied mechanism. In this article we studied the dynamics of a pulsatory liposomes.

2. PHENOMENOLOGICAL BASES OF THE PULSATORY LIPOSOME WORKING

A liposome is a lipid vesical of spherical shape. Its membrane is most often composed of a bilayer of phospholipid molecules, oriented with the polar head towards the internal core and the external aqueous environment and the hydrophobic tail towards the inside of the lipid bilayer (fig. 1). Liposomes can have different sizes and several lipid bilayers.

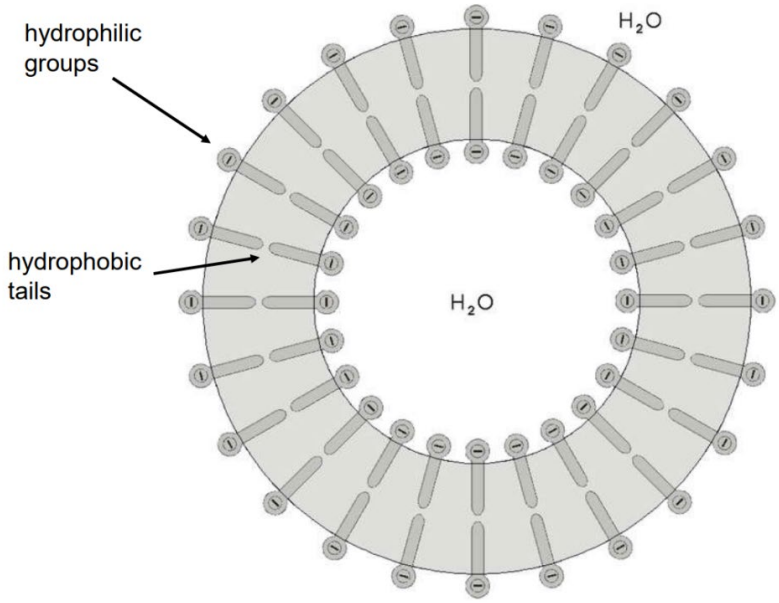

Fig. 1 The structure of a liposome

Let us consider a unilamellar liposome filled with aqueous solution of an osmotic solute. An osmotic solute is a solute for which the lipid bilayer is impermeable. This liposome is inserted into a large bath which containing water or a hypotonic aqueous solution.

The initial state of the liposome is an equilibrium one and is characterized by smooth and unstretched lipid bilayer. Due to the osmosis process, created by the transmembrane gradient of solute concentration, water molecules enter inside the liposome through liposome bilayer. The osmotic flow of solvent determines the swelling of the liposome and the dilution of the internal solution.

Swelling liposome is a rather slow process, so that when it reaches a critical size, suddenly appears a transient pore in the liposome bilayer [1, 28].

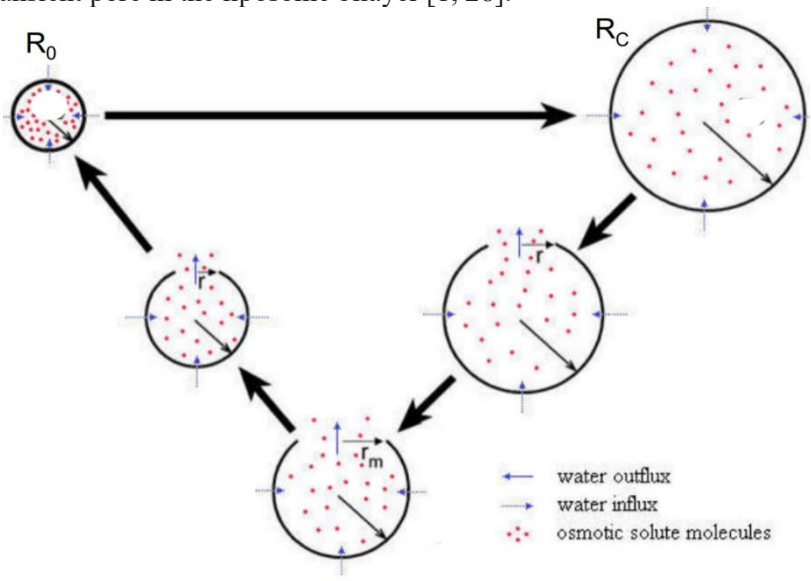

Fig. 2 A cycle of the pulsatory liposome.

In the first stage, named the swelling stage,the liposome swells from the initial state of radius R_0 to the critical state of radius R_c, when a trans bilayer pore appears (the upper part of the picture).
In the second stage, named the relaxing stage,the pore radius increases up to a maximum value, r_m (the bottom right part of the picture); after that, the pore radius decreases up to the pore disappearance (the bottom left part of the picture). Simultaneously with the pore evolution, the liposome relaxes until its radius becomes equal to R_0 and the bilayer becomes smooth and unstretched.

The formation of the transbilayer pore is an important event, because it changes the direction of the liposome evolution [1, 28-31]. The swelling of the liposome stops and its deflation begins. The transbilayer pore evolution consists from 2 phases: 1) the pore radius increases up to the maximum value, rm and 2) the pore radius decreases until the closure of the pore (Fig. 1). A quantity of the internal aqueous solution comes out through the pore and the liposome returns to its original size. Now, 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 other words, the liposome has a cyclic activity. This is why we named the liposome, as pulsatory [1, 23, 24].

Also, it is called “pulsatile liposome”, too [32, 33]. But liposome pulse can be considered like a two-stroke engine, for which the fuel is the osmotic solute and the energy is provided by the transmembrane concentration gradient [1, 31].

3. MATHEMATICAL DESCRIPTION OF THE PULSATORY LIPOSOME DYNAMICS

3.1The swelling stage

In the first stage, the liposome swells from the initial state of radius, R_0, to the critical state of radius, *Rc*, when o transbilayer pore appears (the upper part of the fig. 1) [25 - 30].

The equation that describes the evolution of the liposome in the swelling stage of the n-th cycle is:

$$\frac{dR}{dt} = P_w V_{\mu w}\left(\frac{C_{0,i}R_0^3}{R^3} - \frac{2\beta E}{R_0^2}\frac{R^2 - R_0^2}{R}\right) \tag{1}$$

where, *R(t)* is the liposome radius; R_0 is the pulsatory liposome radius in the initial unstreched state; $C_{0,i}$ is the initial solute concentration; P_w is the water permeability through the liposome membrane; $V_{\mu w}$ the water molar volume; E is the elastic modulus for surface stretching or compression; $\beta = 1/(N_A k_B T)$, N_A is the Avogadro constant, k_B is the Boltzman constant and $T =$ 300K is the absolute temperature.

3.2 The Relaxing Stage

Due to the appearance of the pore, the swelling of the liposome stops, its evolution direction changes and the liposome deflates [1, 28 - 31].

In this the second stage of a cycle, the pore radius increases to a maximum value, r_m, after which it decreases to the pore disappearance. It is observed that, simultaneously with the pore evolution, the liposome relaxes until its radius equals to R_0 (the bottom part of the fig. 1).

The dynamic evolution of the liposome in the deflation stage is described by three differential equations: one each for the liposome radius, the pore radius and the solute concentration.

3.2.1 The evolution of the radius *R* of the liposome

The decrease of the liposome radius, *R(t)*, is described by the following differential equation:

$$\frac{dR}{dt} = P_w V_{\mu w}\left(1 - \frac{r^2}{4R^2}\right)\left[\frac{3Q}{4\pi R^3} - \frac{2\beta E}{R}\left(\frac{R^2}{R_0^2} - 1 - \frac{r^2}{2R_0^2}\right)\right] - \frac{Er^3}{6\pi R^3 \eta_S}\left(\frac{R^2}{R_0^2} - 1 - \frac{r^2}{2R_0^2}\right) \tag{2}$$

3.2.2 Time evolution of the radius r of the transbilayer pore

The formation of a circular pore of radius, *r,* in lipid bilayer is driven by the difference between the membrane tension force and the edge tension force. Once it has appeared, the liposome free energy changes due to the driving forces responsible for the pore dynamics in a stretched bilayer [1, 30]:

$$\Delta E_m = \Delta E_\sigma + \Delta E_\gamma = 2\pi r(r\sigma - \gamma) \tag{3}$$

The free energy change is actually dissipated into lipid bilayer volume as a result of two internal viscosity forces, namely the intermolecular friction forces corresponding to the membrane viscosity, η_b, and the friction of the solvent with the pore wall. The solvent viscosity is η_s. The energy change related to these internal viscosity forces is:

$$\Delta E_\eta = 2\pi r(h\eta_b + C\eta_s r)\frac{dr}{dt} \tag{4}$$

Such that, on equating the two energy changes, one obtains a differential equation:

$$\frac{dr}{dt} = \frac{r\sigma - \gamma}{2h\eta_{b+}C\eta_s r} \tag{5}$$

3.2.3 The change of internal solute concentration

The amount of solute inside the liposome is modified by solute efflux due to the internal pressure and diffusion through the open pore according to equation [1, 28 - 30, 33, 34]:

$$\frac{dQ}{dt} = -\pi r^2\left(\mathrm{v}\Delta C + D\frac{\Delta C}{R}\right) \tag{6}$$

which is equivalent to:

$$\frac{d(lnQ)}{dt} = -\frac{r^3}{2R^4}\left(\frac{\sigma}{\eta_s} + \frac{3D}{2r}\right) \tag{7}$$

Equations (3), (5) and (7) form a system of three differential equation which can be numerically solved in order to obtain the time dependence of vesicle radius, pore radius, and internal solute amount ($R(t)$, $r(t)$ and $Q(t)$) during each cycle of the pulsatory liposome life.

4. ARTIFICIAL NEURAL NETWORKS

Artificial neural networks were inspired by biology and are composed of elements that work in a manner similar to natural neurons (fig. 3).

These elements are organized in successive layers and are strongly interconnected. Artificial neural networks perform functions that are specific to the human brain [36].

Neural networks can be learned so that they perform a certain function or solve a certain problem. The data to be processed are entered into neuronal network through the first layer of neurons (input layer). Then, they are processed by the hidden layers according to the learned algorithm. The final results are given by the output layer. Many algorithms for learning a neural network have been developed.

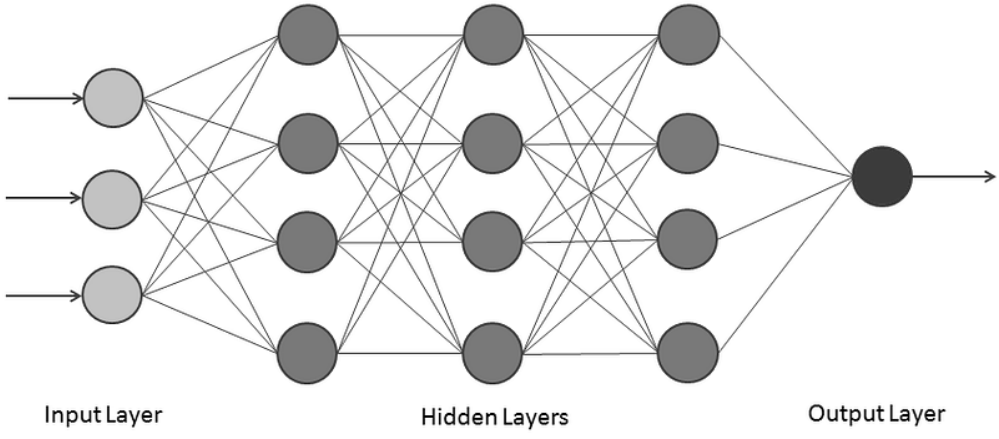

Fig. 3 An example of an artificial neural network

5. RESULTS

We have used the following physical parameters to solve the system of differential equations using the analytical method:

The water permeability through the liposome membrane $P_w = 3.10^{-5}$ m/s; the water molar volume $V_{\mu w} = 18.04\ 10^{-6}$ m^3/ mol; $P_w V_{\mu w} = 5.412\ 10^{-10}$ m^4 mol^{-1}s^{-1}[28 -30].

$\beta = 1/(N_A k_B T) = 4.00914\times10^4$ molJ^{-1}; Avogadro number, $N_A = 6.022\times10^{23}$; the Boltzman constant, k_B =1,380 649×10^{-23} J·K^{-1}; the absolute temperature T = 300 K.; the viscosity of aqueous solution $\eta_l = 3.2\times10^2$ N.s.m^{-2} [19]; the lipid bilayer viscosity, $\eta_m = 100\times10^2$ N.s.m^{-2} [11]; the two dimensional stretch modulus of the lipid bilayer, E = 0.2 N.m^{-1} [19]; the edge tension was $\gamma = 8{:}10^{12}$ N [19]; the thickness of the liposome membrane is $2h = 3.5\times10^{-9}$m.

Fig. 4 shows an artificial neural network that simulates the activity of a pulsatory liposome.

The input data for the neural network are: r_0 - the initial pore radius, R_0 - the initial liposome radius and C_0 - the initial solute concentration.

The network calculates the amounts of solvent released after each cycle in the external medium.

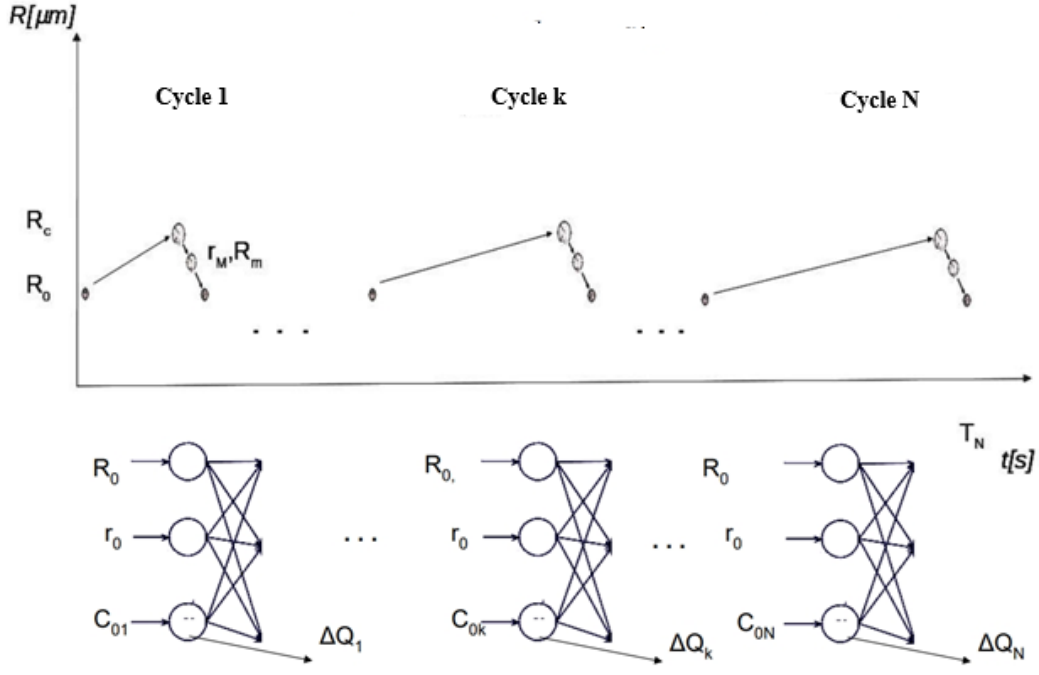

Fig. 4 Correspondence between pulsatory liposome cycles and neuronal layers of an artificial neural network

6. DISCUSSIONS AND CONCLUSIONS

Lately, interest in pulsatory liposomes has increased a lot. Many experimental and theoretical researches have been carried out because their application in biomedicine, nanomedicine, and nanoelectronics [1, 34]. Pulsatory liposome is a bionic example [35].

Pulsatory liposome can be a biomotor for the controlled release of solvent (which can be a drug) into the external environment.

This means that pulsating liposomes can be used to deliver drugs to diseased sites [28-34]. For this purpose, two parameters must be known: the time intervals between two successive cycles and the amount of drug released with the internal liquid leaked out through each pore. In this article we have presented a method for determining these 2 parameters by an artificial neural network.

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