The pulsatory liposome works as a neuronal network (II)

Dumitru POPESCU*,[1], Diana Rodica RADNEF-CONSTANTIN[2],
Valentin Ioan Remus NICULESCU[3]

*Corresponding author
*,[1]"Gheorghe Mihoc-Caius Iacob" Institute of Mathematical Statistics and Applied Mathematics of Romanian Academy,
Department of Mathematical Modelling in Life Sciences,
Calea 13 Septembrie, 13, Bucharest, Romania,
dghpopescu@gmail.com
[2]Astronomical Institute of Romanian Academy,
Cutitul de Argint 5, Bucharest, Romania
ghe12constantin@yahoo.com
[3]National Institute for Lasers, Plasma and Radiation Physics,
Atomistilor 409, Magurele, Ilfov, Romania

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***Abstract**: In this paper we consider a unilamellar liposome (lipid vesicle) filled with aqueous solution of an 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. 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

Phospholipids represent the main components of cellular membrane. In relation to water, phospholipids have an amphiphilic character: hydrophilic (attraction) due to the polar group and hydrophobic (repulsion) due to the tail. [1 – 3]. Due to the amphiphilic property, when phospholipid molecules are introduced into an aqueous environment, they self-assemble into

supramolecular structures: micelles or liposomes. 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. The liposome is an artificial model of cell membrane and are used in biophysical membrane researches biomedical applications [4 – 6]. There are two biotechnology applications which require an increase in membrane permeability:

1.Targeted delivery of special compounds. Due to the biocompatibility and amphiphilicity of phospholipids, liposomes can be used for drug transport and delivery systems. In this application on uses special molecules encapsulated in vesicles, which have to be transported to a specified cellular or subcellular site [6 – 13].

2. Gene therapy. In gene therapy application, it is necessary to transport DNA fragments through cellular and nuclear membranes, assuming that the liposome discharges its content to the external environment by its breakdown [14 – 16].

The transport through trans bilayer pores, across lipid bilayer, is a new strategy for biological material exchange between the two adjacent media [3, 18 – 20].

The formation of transmembrane pores in osmotically stressed liposomes is a very interesting mechanism that determines a cyclic dynamic of pulsatory liposomes [21 –24].

A pulsatory liposome 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 [3, 25, 26]. Very interestingly, a pulsatory liposome works as an artificial neural network [27].

2. DYNAMIC EVOLUTION OF A PULSATORY LIPOSOME

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 external aqueous environment and to the internal core and the hydrophobic tail towards the inside to its lipid bilayer (fig. 1). Liposomes can have different sizes (20 nm-10μm) and several lipid bilayers. Taking into account by the size and structure of liposomes, there are three types: small unilamellar vesicles (SUVs, size 20–100 nm), large unilamellar vesicles (LUV, size > 100 nm) and multilamellar vesicles (MLV, size > 1 μm).

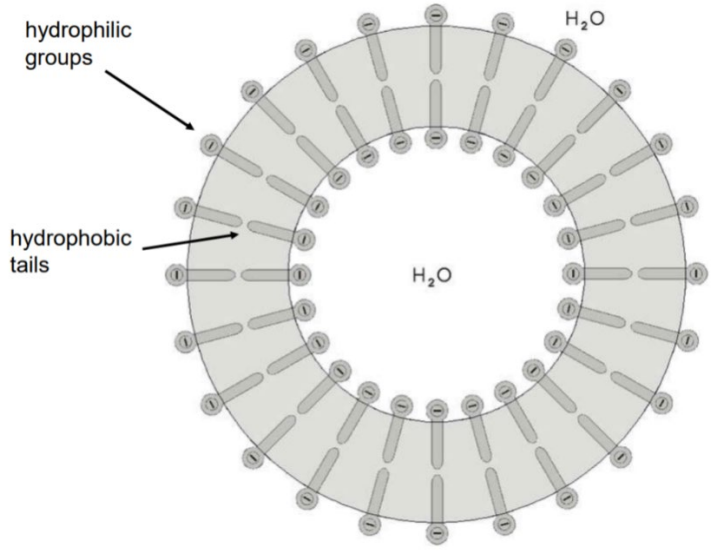

Fig. 1 The structure of a unilamellarliposome

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 [3, 28].

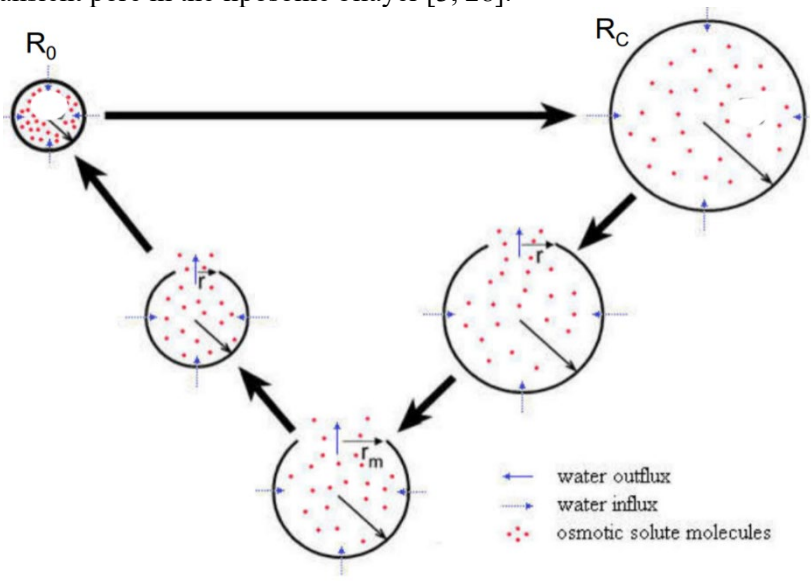

Fig. 2 A cycle of the pulsatory liposome.

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 [3, 29–31]. 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 [3, 19, 28 –30].

3. 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 [27].

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.

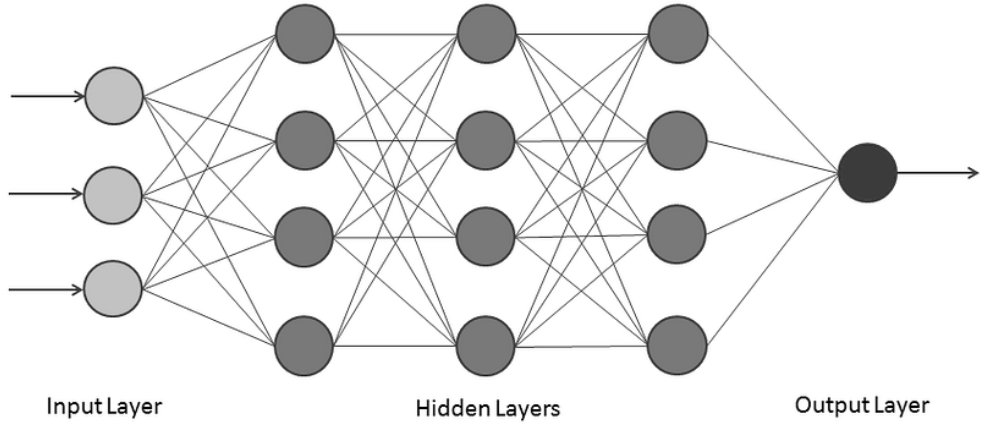

Fig. 3 An example of an artificial neural network

4. MATHEMATICAL DESCRIPTION OF THE PULSATORY LIPOSOME DYNAMICS

4.1 The swelling stage

In the first stage, the liposome swells from the initial state of radius, R_0, until a transbilayer pore appears (the upper part of the fig.1) [25–30]. This is named "critical state" of radius, Rc.

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

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

where, R_0 is the pulsatory liposome radius in the initial unstarched state; $R(t)$ is the liposome radius; $C_{0,n}$ 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 number, k_B is the Boltzman constant and $T = 300$K is the absolute temperature.

The concentration of the solute changes due to the increase of the amount of water that enters in the interior of the liposome through osmosis. The amount of solute does not change.

$$C_n = \frac{R^3}{R_0^3} C_{c,(n-1)} \tag{2}$$

where, $C_{c,(n-1)}$ is the solute concentration at the end of the previous cycle. For the first cycle $C_{c,0}$ is initial solute concentration C_0.

4.2 The relaxing stage

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

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

In this the deflation stage of a cycle, the pore radius increases to a maximum value, r_m, after which it decreases to the pore disappearance:

$$\frac{dr}{dt}=\frac{r\sigma-\gamma}{2h\eta_{b+}C\eta_s r} \tag{4}$$

The amount of solute inside the liposome is modified by solute efflux due to the internal pressure [3, 17, 21, 25, 30]:

$$\frac{\mathrm{d(lnQ)}}{\mathrm{dt}} = -\frac{\mathrm{r}^3}{2\mathrm{R}^4}\left(\frac{\sigma}{\eta_\mathrm{s}}+\frac{3\mathrm{D}}{2\mathrm{r}}\right) \tag{5}$$

Equations (3), (4) and (5) 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.

5. RESULTS

The artificial neural network was taught to solve the differential equation (1) for the inflation stage and the system of differential equations (3-5) for the deflation stage by numerical methods.

We have used the following parameters to solve the system of differential equations:

1. Physical parameters regarding water.
   - $P_w = 3.10^{-5}$ m/s for the water permeability through the liposome membrane;
   - $V_{\mu w} = 18.04\ 10^{-6}$ m^3/ mol for the water molar volume;
   - $P_w V_{\mu w} = 5.412\ 10^{-10}$ m^4 mol^{-1}s^{-1}[29 – 30].
   - $\eta_l = 3.2\times10^2$ N.s.m^{-2} for the viscosity of aqueous solution the lipid bilayer viscosity [25];
2. Physical parameters regarding the lipid bilayer:
   - $2h = 3.5\times10^{-9}$m is the thickness of the liposome membrane;
   - $\eta_m = 100\times10^2$ N.s.m^{-2} the viscosity of the lipid membrane [21];
   - $E = 0.2$ N.m^{-1} the two dimensional stretch modulus of the lipid bilayer [19];
   - $\gamma = 8{:}10^{12}$ N is the edge tension for the lipid bilayer pore [25].
3. Physical constants:
   - $N_A = 6.022\times10^{23}$, the Avogadro number;
   - $k_B = 1{,}380\ 649\times10^{-23}$ J/K the Boltzman constant;

T = 300 K the absolute temperature;
$\beta = 1/(N_A k_B T) = 4.00914 \times 10^4$ mol/J

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.

Using the above physical constants and numerical methods for solving differential equations the artificial neural network calculates the amounts of solvent released in the external medium after each cycle.

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

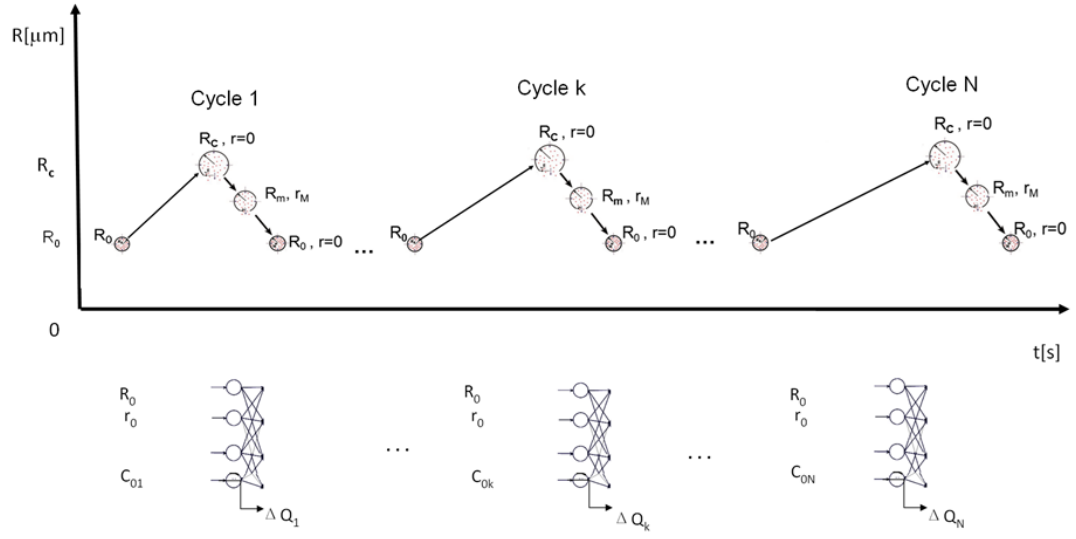

Fig. 4 Correspondence between pulsatory liposome cycles and neuronal layers of an artificial neural network

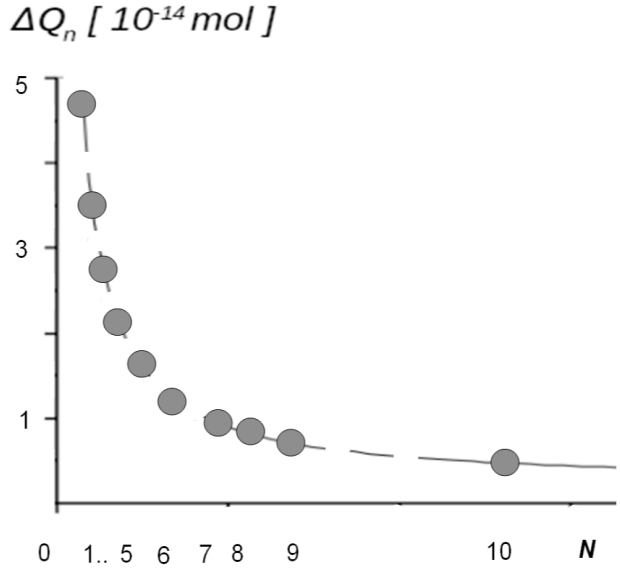

Fig. 5 The amounts of solute released after each cycle. On the 0N axis, the cycle order is plotted instead of the time at which each cycle ends.

6. DISCUSSIONS AND CONCLUSIONS

Because of their application in biomedicine, nanomedicine and nanoelectronics in the last time, the interest for pulsatory liposomes has increased a lot. Many experimental and theoretical researches have been carried out [3, 26]. A pulsatory liposome can be likened to an intelligent two-stroke biophysical engine. Very interestingly, the pulsatory liposome is considered a bionic example [33].

Pulsatory liposome can be a bioengine for the controlled release of the osmotic solution (which can be a drug) into the external environment.

This property suggests that pulsatory liposomes can be used as drug delivery systems to diseased sites [26, 29 –-31]. For this purpose, three parameters must be known: the number of cycles, the lifetime of each cycle and the amount of drug released with the internal liquid leaked out through transbilayer pore during each cycle. In this paper, we have presented a method for determining of these 3 parameters by an artificial neural network.

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