Simulation of pulsatory liposome working using a linear approximation for transmembrane pore dynamics

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Abstract: *This paper presents an analytical solution of the differential equations describing the pulsatory liposome dynamics. We consider a unilamellar liposome filled with an aqueous solution of osmotic solute inserted in a hypotonic aqueous medium. Due to the osmosis process the liposome has a cyclic evolution. The lipid vesicle swells to a critical size, at which point a transbilayer pore suddenly appears. Part of the internal solution leaks through this pore. The liposome relaxes and returns to the initial size. The swelling starts again and the liposome goes through a periodical 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). The evolution of the pore has two phases: first, the radius of the pore increases to its maximum value, then the radius decreases until it disappears, and the liposome reaches its initial size. During each cycle, the liposome will release a quantity (a pulse) of the solution from its interior. All the processes which contribute to the liposome relaxing and its coming back to the initial size are described by three differential equations. This system of differential equations can be integrated using numerical methods. The functions – which model our biological engine in three stages, are as follows: R(t) - the liposome radius, r(t) - the pore radius, C(t) - solute concentration, Q(t) - the osmotic solute amount inside the liposome. The graphs representing these functions contain important linear portions, which suggested a solution using analytical methods. Based on some analytical methods, we solve these equations, and their explicit solutions are validated by comparing with numerical results of previous studies.*

Key Words: *Pulsatory liposome, analytical method, biophysical engine*

1. INTRODUCTION

We consider a unilamellar liposome filled with an aqueous solution of an osmotic solute for which the liposome membrane is impermeable. A liposome is a small artificial spherical vesicle. Its membrane is composed from a bilayer of phospholipids molecules. A phospholipid molecule has a hydrophobic tail nonpolar carbon chain and a hydrophilic polar head. The

liposome membrane (also named lamella) is composed from two phospholipids layers located in mutually opposite positions so that the hydrophobic zone is inside the lamella, and the polar interfaces are located at the interface with the external environment and internal medium, respectively (fig. 1). The internal medium may have a different composition in relation to the external environment. In our case, an aqueous solution of an osmotic solute is found inside the liposome. This liposome is introduced into an aqueous medium. Water molecules enter the interior of the liposome due to the osmosis process.

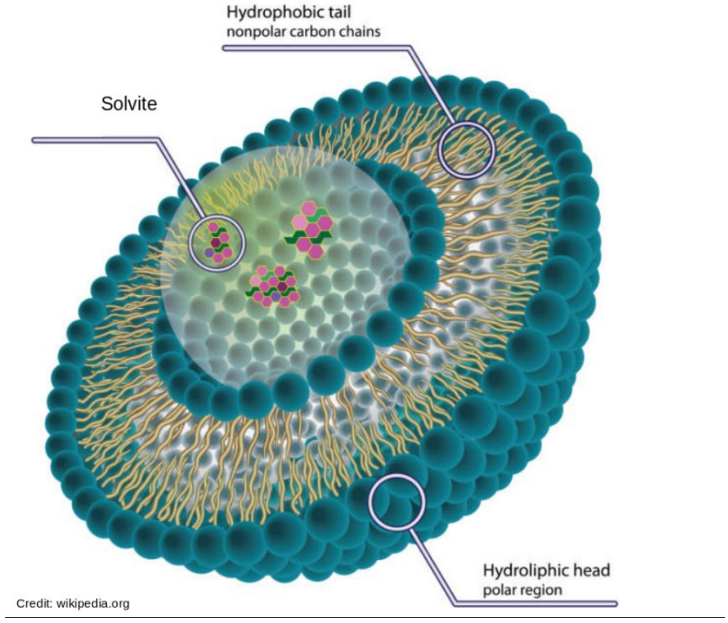

Fig. 1 A unilamellar liposome. Its membrane (named lamella) consists of two layers of phospholipid molecules

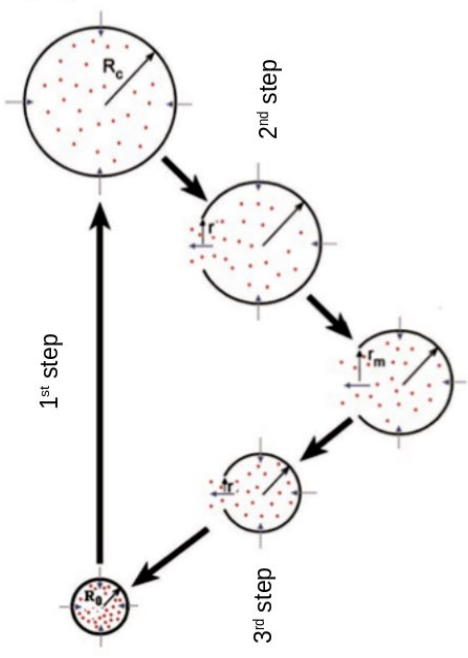

Fig. 2 The evolution of the liposome during a cycle

The liposome swells up to a critical size, at which point a transmembrane pore suddenly appears (STEP 1) (Fig. 2).

The appearance of the pore changes the evolution of the liposome. The pore can occur anywhere in the liposome membrane. It is the result of selective association and lateral diffusion of phospholipids in the lipid bilayer [1] – [10]. Obviously, the appearance of the pore is stimulated by the swelling of the liposome due to the process of osmosis.

The internal solution comes out through the pore and the liposome starts its deflation (relaxation).

Then the radius decreases until it disappears, and the liposome reaches its initial size.

A new cycle will begin. So, this liposome will have a cyclic evolution. Each cycle has two stages, swelling and relaxing [11] – [14]. On the other hand, in the relaxation stage, the evolution of the pore has two phases: first, the radius of the pore increases to its maximum value, r_M (STEP 2). Then the radius decreases until it disappears, and the liposome reaches its initial size (STEP 3). In this paper we will consider the pulsatory liposome as a ***three-stage biological engine model*** (BE3s model) [15].

2. THE CONSTITUTIVE EQUATIONS OF THE PULSATORY LIPOSOME FUNCTION

2.1 The three-stage biological engine model

Due to the appearance of the pore, the swelling of the liposome stops, its evolution changes and the liposome deflates [16] – [22].

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

$$\frac{dR}{dt} = \frac{Er^2}{6\eta_l R_0^2}\left(\frac{R^2}{R_0^2} - \frac{r^2}{4R_0^2} - 1\right) + P_w V_{\mu w}\left(1 - \frac{r^2}{4R_0^2}\right)\left[\Delta C_m - \frac{2\beta E}{R}\left(\frac{R^2}{R_0^2} - \frac{r^2}{4R_0^2} - 1\right)\right] \quad (1)$$

where, R_0 is the pulsatory liposome radius in the initial unstarched state and r(t) is the pore radius.

$\Delta C_m = C_{int} - C_{out}$ is the transmembrane gradient of the osmotic solute concentration. $C_{int} = C$ is the internal solute concentration; $C_{out} = 0$ is the external solute concentration.

η_l is the viscosity of aqueous solution.

$\beta = 1/(N_A k_B T)$; N_A – Avogadro constant; k_B – the Boltzman constant; T – the absolute temperature.

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.

The following differential equation describes the pore radius, r(t), evolution:

$$\frac{dr}{dt} = \frac{Er}{4\eta_l R_0^2}\left(\frac{R^2}{R_0^2} - \frac{r^2}{4R_0^2} - 1\right) - \frac{\gamma}{2\eta_m h} \quad (2)$$

where, 2h is the thickness of the lipid bilayer, η_m is the viscosity of the liposome membrane, γ is the line tension.

The change of the osmotic solute concentration, C(t), is given by the equation:

$$\frac{d\left[\ln\left(CV_{lip}\right)\right]}{dt} = \frac{Er^2}{2\eta_l R^4}\left(1 - \frac{R^2}{R_0^2} + \frac{r^2}{4R_0^2}\right) \quad (3)$$

$V_{lip} = 4\pi R^3(t)/3$ is the liposome volume. C V_{lip} is the quantity of osmotic solute from internal solution.

3. THE ANALITICAL METHODS

We are looking for the solution in a specific form for equation (1). Consider for $\Phi(t)=R^2(t)$ an ansatz form with the following expression [23]:

$$\Phi(t) = t.\rho + R_0^2 \tag{4}$$

In this approximation, eq. (1) becomes:

$$\frac{d^2\Phi(t)}{d^2t} = -a.\Phi^2(t) + b.\Phi(t) + c, \quad \Phi(0) = R_0^2 \tag{5}$$

With the following notations: $a = 8\mu\beta E/R_0^2$, $b = 8\mu\beta E$, $c = 4\mu C_0 R_0^3$, $\mu = P_w V_{\mu w}$

Most part of the graph representing the pore radius versus time is linear. Based on this observation, we propose a linear behavior for the pore evolution. We will refer to this as the liniar approximation of pore dynamics. As a result, we define an increasing linear law for pore radius, $r_a(t)$, in the 2nd step:

$$r_a(t) = d(t - t_u) + r_0, \ d > 0, \ r_0 > 0, \ t_u \leq t < t_M \tag{6}$$

and a decreasing one for pore radius, $r_b(t)$, in the 3rd step:

$$r_b(t) = q(T_f - t), \ q > 0, \ t_M < t \leq T_f \tag{7}$$

We also point to a linear approximation of the solute concentration function, which we term the linear solute concentration approximation, based on the earlier research [11], [14], [21]. In the second and third steps, we specifically take into account a decreasing law, as follows:

$$C_{ab}(t) = k(tu - t) + Cu \ k > 0 \text{ and } tu \leq t < T_f \tag{8}$$

In the BE3s-model, where d, q, k, and naturally t_u, t_M, and T_f still need to be calculated.

4. RESULTS

4.1 The physical parameters

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}.

$\beta = 1/(N_A k_B T) = 4.00914\times10^4$ mol.J^{-1}; Avogadro number, $N_A = 6.022\times10^{23}$; The Boltzman constant, $k_B = 1{,}380\ 649\times10^{-23}$ J·K^{-1}; The absolute temperature T = 300 K.

The viscosity of aqueous solution $\eta_l = 3.2\times10^2$ N.s.m^{-2} [23]. The lipid bilayer viscosity, $\eta_m = 100\times10^2$ N.s.m^{-2} [24].

The two dimensional stretch modulus of the lipid bilayer, E = 0.2 N.m^{-1} [25]. The edge tension was $\gamma = 8{:}10^{12}$ N [25] – [28].

The thickness of the liposome membrane is $2h = 3.5\times10^{-9}$ m.

4.2 The swelling stage

For the swelling stage equation (1) has an analytical solution t =t(R). It is difficult to obtain function R = R(t) (the invers function of the t = t(R)). In our model, to obtain function R = R(t) we have operate in the ansatz approximation. Thus, by solving equation (5), we may determine an analytical function for the liposome radius in the swelling stage:

$$R_{sw}(t) = \sqrt{\emptyset(t)} = \sqrt{\frac{2\emptyset_0 + bt + \sqrt{\emptyset_0^2 + 4b\emptyset_0 t + (b^2 + 4ac)t^2 + 8ct}}{2(at + 2)}} \tag{9}$$

where, $\Phi_0 = \Phi(0)$ and $0 \leq t \leq t_u$
For the first step, r(t) = 0 and dr(t)/dt=0.
We get the C(t) expression from equation (3):

$$C_{sw}(t) = \frac{k}{R_{sw}^3(t)}, \qquad k = C_0 R_0^3 \tag{10}$$

In the differential equation for swelling stage, the initial value for pulsatory liposome radius is $R(0) = R_0 = 19.7$ μm and the initial solute concentration is $C(0) = C_0 = 10$ mol/m^3.

4.3 The relaxing stage

The swelling time of the pulsatory liposome is t_u. At the end of the swelling stage (at the moment t_u), the pulsatory liposome radius is $R_u = 20.6$ μm. The pore radius at the moment t_u is r(0) = 1.57 μm. The maximum pore radius is 9,8 μm at the moment t_M. Also, we have C_u = 8.74575 mol/m^3 for solute concentration at the moment t_u. Such unilamellar vesicle were used in experimental studies [18], [25].

Further, depending on the chosen values of the parameters for BE3s-model which we specify in section 4.1, we compute the numerical values for the analytical expressions of the solutions and present these values below.

Taking into account the input data, we follow the next scenario as an illustration. Thus, we obtain the following numerical results:
d = 0.00412, q = G =0.000001157, k = 0.006228.

The swelling time of the liposome is t_u = 1114.9847 s. The moment when the pore reaches its maximum radius is t_M = 1114.9867 s. Also the moment when the pore disappears (the liposome reaches its initial size and the cycle ends) is T_f = 1123.559 s.

From these values we can calculate:

– The time required for the pore to reach its maximum radius is equal $t_M - t_u$ = 1114.9867s – 1114.9847s = 0.002 s

– The duration of the pore radius decrease stage, from the maximum value until the pore disappears $T_f - t_M$ = 1123.559 s. – 1114.9867 s = 8.5723 s

– The relaxation time of pulsatory liposome is $T_f - t_u$ = 1123.559 s – 1114.9847 s = 8.5743 s

– The length time of the cycle is $T_c = T_f$ = 1123.559 s

– When the pore radius is maximum, the liposome radius is equal to R_m =20.30 μm and the solute concentration is C_M = 8.74574 mol/m^3.

The solute concentration at the end of cycle is C_f = 8.6930 mol/m^3, This value is equal to the initial solute concentration for the second cycle.

5. CONCLUSIONS

We have proved that the functioning of the pulsatory liposome is determined by the transmembrane concentration gradient of the osmotic solute and by the appearance of the pore through the liposome membrane. The transmembrane osmotic gradient is the motrice force which causes swelling of the liposome.

We also showed that the pore changes the direction of the liposome evolution, bringing it back to its original geometric size.

Pulsatory liposomes can be considered as biotechnological tools and can be used in biological and medical applications [29[- [33]. The operating energy is ensured by the transmembrane concentration gradient of the osmotic solute. So, the solute is the fuel of the pulsatory liposome. The osmotic solute (the fuel) may be a pharmacological substance, or any other special substance [34] - [37].

The preparation of pulsatory liposomes with such properties and their delivery at a site of action remains a biotechnology challenge [38] – [41].

We note that for the characterization of the other cycles, the analytical description of the first operating cycle of the pulsating liposome is a good solution.

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