ANALYTICAL MODELING OF THE FUNCTIONING OF THE PULSATORY LIPOSOLE

DIANA R. CONSTANTIN¹,a, DUMITRU POPESCU²,b

1 Astronomical Institute of the Romanian Academy,
   Cuitul de Argint street, nr. 5, Bucharest, Romania
Corresponding authora: ghe12constantin@yahoo.com

2 Institute of Mathematical Statistics and Applied Mathematics, “Gheorghe Mihoc-Caius Iacob”,
   Department of Mathematical Modelling in Life Sciences,
   Calea 13 Septembrie street, nr. 13, Bucharest 5, Romania
E-mail b: dghpopescu@gmail.com

Received September 24, 2023

Abstract. In this paper the analytical solutions of the differential equations are presented. These equations describe 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, when 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. All the processes which contribute to the liposome relaxing and its coming back to the initial size are described by three differential equations. Based on some analytical methods, we solve these equations and their explicit solutions are validated by comparing with previous study numerical results.

Key words: Pulsatory liposome, analytical method, biophysical engine.

DOI: https://doi.org/10.59277/RomJPhys.2024.69.701

1. INTRODUCTION

The transport of ions and small or large molecules through cellular membrane is a very important problem for many biological processes and for many biotechnological applications [1, 2]. Liposomes were initially used as artificial model of cell membrane in biophysical membrane studies. The pore appearance in lipid bilayer of liposomes following some controlled mechanisms may be an adequate and interesting way of transmembrane transport [3]. The great interest in exploring pores through experiments, computational and theoretical approaches increased dramatically not only for a better understanding of molecular traffic through biomembranes, but especially for biomedical applications [3]. Some pores, named stochastic pores, can appear due to structural and dynamic properties of lipid bilayer [4–8], but other ones may be favored by a mechanical tension induced in different ways [9–13]. The stochastic pores can appear due to thickness fluctuations determined by thermal motion of lipid molecules.

Romanian Journal of Physics 69, 701 (2024)
that superposes over local variations of the bilayer thickness which can appears due to selective association of phospholipids [4–6]. The stretching tension of liposome bilayer can be induced using physical and chemical methods [9–13]. For example, in a giant vesicle stretched by an optical induced mechanical tension was observed a sequence of 30-40 pores [12]. Only a single pore appears into the vesicle membrane at a time. There are two very interesting biotechnological applications, which require the increase of membrane permeability: gene therapy and targeted special substances delivery. In the first application, the transport of DNA fragments through cellular and nuclear membranes is requested [14]. The second application uses special substance molecules encapsulated in vesicles, which have to be transported and released to a target place [15–19]. Some liposomes release their contents by breaking them. It is possible for a lipid vesicle to release the drug molecules, in a fashion well-controlled [15–17]. Such a vesicle is called pulsatory liposome and has cyclic activity. The pulsatory liposome has a special importance because the osmotic solute can be a pharmaceutical substance that can be delivered to diseased sites of the body [20].

In this paper we realize a mathematical approach of the pulsatory liposome by modeling of its dynamics as a three-stages biological engine-model called by us BE3s model. So, we tried to find an analytical solution for the system of differential equations that describe these three stages of the model. In the second section, we give a description of the BE3s model along with the analytical methods. We proposes the modeling of pulsatory liposome considering the dependence of its physical parameters. In the third section, the explicit expressions of the analytical solutions of the model are obtained. The numerical values of these solutions are validated by comparing with the numerical results of the previous studies.

We summarize our results in some conclusions that close our paper.

2. DESCRIPTION OF THE DYNAMIC ACTIVITY OF PULSATORY LIPOSOME

The three-stage biological engine model (BE3s model) is a Pulsatory Liposome, more precisely an unilamellar liposome filled with an aqueous solution of an osmotic solution. This liposome will be placed in a bath of hypotonic solution. Due to osmosis process, the liposome swell up to suddenly a transmembrane pore appears. The appearance of the pore changes the evolution of the liposome (see Fig. 1). The internal solution comes out through the pore and the liposome starts its deflation (relaxation).

We consider an unilamellar liposome filled with an aqueous solution of an osmotic solution. This liposome will be placed in a bath of hypotonic aqueous solution. Due to osmosis process, the liposome swell up to suddenly a transmembrane pore appears. The appearance of the pore changes the evolution of the liposome, see Fig. 1. 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, \( r_M \), 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 three stages. For this reason it will be called pulsatory liposome in three steps [21–24]. In other words, the pulsatory liposome is a three-stage biological engine, shortly BE3s. During each cycle, the liposome will release a quantity (a pulse) of the solution from its interior. The functions which are modeling 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.

We define the stages of the model as follows:

The swelling step of the pulsatory liposome is described by a differential equation. The relaxation step is described by three differential equations: one for liposome evolution, one for pore dynamics and one for the solute concentration change. The relaxation stage of the pulsatory liposome is determined by the evolution of the pore. The pore dynamics has two phases: pore growth to a maximum radius, followed by its decrease to extinction (his disappearance). For this reason, we believe that the pulsatory liposome can be referred to as a three-stroke biomotor: the liposome swelling, the pore increasing and the pore decreasing during the pore relaxing.
2.1. PHYSICAL PARAMETERS OF THE MODEL

In order to solve the differential equations that describe the first cycle of a pulsatory liposome, considering an analytical approach, we used the following physical parameters for our model: The membrane permeability coefficient for water $P_w = 3 \cdot 10^{-5}$ m·s$^{-1}$ and water molar volume $V_{\mu w} = 18.04 \cdot 10^{-6}$ m$^3$·mol$^{-1}$ and $P_w \cdot V_{\mu w} = 5.412 \cdot 10^{-10}$ m$^4$·mol$^{-1}$·s$^{-1}$. The two dimensional stretch modulus of the lipid bilayer is $E = 0.2$ N·m$^{-1}$ [12], $\beta = 1/(R \cdot T) = 4.00914 \cdot 10^{-4}$ mol·J$^{-1}$, the universal gas constant $R = 8.314$ J·mol$^{-1}$·K$^{-1}$ and $T = 300$K is the absolute temperature. The edge tension was $\gamma = 8.1 \cdot 10^{-12}$ N [13]. The lipid bilayer viscosity was $\eta_{lm} = 100$ N·s·m$^{-2}$ [11]. The aqueous solution viscosity was $\eta_t = 3.2 \cdot 10^{-2}$ N·s·m$^{-2}$ [12]. Liposome membrane thickness is $2h = 3.5 \cdot 10^{-9}$ m.

In addition, 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$^{-3}$; then, we consider $R_u = 20.6$ μm for the liposome at the moment $t_u$, $r(0) = 1.57$ μm for the pore at the moment $t_u$ and the maximum pore radius is $9.8$ μm at the moment $t_M$. Also, we have $C_u = 8.7457$ mol·m$^{-3}$ for solute concentration at the moment $t_u$. Such unilamellar vesicles were used in experimental studies [12, 13].

2.2. BASIC EQUATIONS

The equations that describe the dynamics of the pulsatory liposome during stages 1-3 of BE3s model are as follows [21–24]:

For all three stages of the model, the liposome radius equation is:

$$\frac{\partial R}{\partial t} = \frac{-r^3}{6 \cdot \eta_t \cdot R^3} \cdot E \left( \frac{R^2}{R_0^2} - 1 - \frac{r^2}{4R_0^2} \right) +$$

$$+ P_w V_{\mu w} \cdot \left( 1 - \frac{r^2}{4R^2} \right) \cdot \left[ C - \frac{2\beta E}{R} \left( \frac{R^2}{R_0^2} - 1 - \frac{r^2}{4R_0^2} \right) \right]$$

(1)

where $P_w$ is the water permeability through liposome membrane, $V_{\mu w}$ is the water molar volume, $E$ is the elastic modulus for surface stretching, $\beta = 1/(R \cdot T)$, $R$ is the universal gas constant and $T$ is the absolute temperature. The $R_0$ is the initial liposome radius when the liposome is unstretched and $C_0$ is the initial solute concentration. Also, for all three stages the solute quantity equation is:

$$\frac{d[\ln(C \cdot V)]}{dt} = \frac{-r^3}{2 \cdot \eta_t \cdot R^4} \cdot E \left( \frac{R^2}{R_0^2} - 1 - \frac{r^2}{4R_0^2} \right),$$

(2)

where $\eta_t$ is the viscosity of aqueous solution, $C(t) \cdot V(t) = C_0 \cdot V_0 = Q(t)$ is the osmotic solute amount which remains constant during of the whole 1st stage.
For steps 2 - 3 of the model, the pore radius equation is:

\[
\frac{dr}{dt} = r \cdot \tilde{E} \left( \frac{R^2}{R_0^2} - \left( 1 + \frac{r^2}{4R_0^2} \right) \right) - G,
\]

where, \( \gamma \) is the line tension acting for pore closure, \( \eta_m \) is the membrane viscosity, and \( \tilde{E} = \frac{E}{F}, G = 2\gamma F, F = 4\gamma \eta_m, 0 < r \leq 2\sqrt{R^2 - R_0^2} \).

2.3. ANALYTICAL APPROXIMATION METHODS OF THE MODEL

Taking into account the behavior of the function \( R(t) \), in the equation (1) we propose to consider for \( \Phi(t) = R(t)^2 \) an ansatz form (the solution is sought in a certain form) \([27]\), with the following expression:

\[
\Phi(t) = t \cdot \rho(t) + R_0^2.
\]

Thus, in this approximation the eq. (1) becomes:

\[
\frac{\partial^2 \Phi(t)}{\partial t^2} = -a \cdot \Phi(t)^2 + b \cdot \Phi(t) + c, \quad \Phi(0) = R_0^2,
\]

where \( a = \frac{8\mu \beta E}{R_0^2}, b = 8\mu \beta E, c = 4\mu C_0 R_0^3, \) and \( \mu = P_w V_{\mu w} \).

For the pore evolution, we propose a linear behavior called by us the linear pore approximation. Thus, we consider an increasing law in the 2nd step and respectively a decreasing one in the 3rd step, as follows:

\[
r_a(t) = d(t - t_u) + r_0, d > 0, r_0 > 0, t_u \leq t < t_M.
\]

\[
r_b(t) = q(T_f - t), q > 0, t_M < t \leq T_f.
\]

Based on the previous studies \([1, 2, 7, 17, 21]\) for the solute concentration function, we also propose a linear behavior of it which we call linear solute concentration approximation. Specifically, we consider a decreasing law in the 2nd and 3rd stages, as follows:

\[
C_{ab}(t) = \kappa(t_u - t) + C_u, \kappa > 0, t_u \leq t \leq T_f.
\]

where \( d, q, \kappa \) and of course \( t_u, t_M, T_f \) remain to be determined into BE3s-model.

3. THE MODEL RESULTS: ANALYTICAL SOLUTIONS

In this section, in accordance to the parameters of our biophysical model and taking into account the section 2.1 with some physical restrictions, we calculate below the moments \( t_u, t_M, T_f \) and also the quantities \( d, q, \kappa \). Thus, we obtain the explicit analytical expressions for the model functions relative to the coefficients \( a, b, c, R_0, r_0, R_u, r_M, C_0 \) (see section 2.1).
For the first step of the model, we work in the ansatz approximation. Hence, solving the eq. (5), we find the analytical formula of liposome radius swelling function:

\[
R_{sw}(t) = \sqrt{\Phi(t)} = \sqrt{\frac{2\Phi_0 + bt + \sqrt{4\Phi_0^2 + 4b\Phi_0t + (b^2 + 4ac)t^2 + 8ct}}{2(at + 2)}},
\]

where \(\Phi_0 = \Phi(0), 0 \leq t \leq t_u\).

We recall for the 1st step that \(r_{sw}(t) = 0 = \frac{dr_{sw}(t)}{dt}\).

Using the eq. (2), we obtain the \(C(t)\) expression as:

\[
C_{sw}(t) = \frac{k}{R_{sw}(t)^3}, k = C_0 \cdot R_0^3.
\]

Solving eq. (9), we find the explicit time function expression in the first stage as:

\[
t_{sw}(\Phi) = -2\frac{\Phi(\Phi - \Phi_0)}{a\Phi^2 - b\Phi - c}, \Phi \in \left[\Phi_0, \frac{b + \sqrt{b^2 + 4ac}}{2a}\right]
\]

and obtain the \(t_u\) time value:

\[
t_{sw}(\Phi_u) = -2\frac{\Phi_u(\Phi_u - \Phi_0)}{a\Phi_u^2 - b\Phi_u - c} = t_u.
\]

Further, we work in the linear approximations for pore and solute concentration and also give the following explicit formulas for the model functions: \(R(t), r(t), C(t), Q(t)\). Also, the coefficients \(d\) and \(q\) are calculated below and relative to the model parameters \(R_u, r_M, r_0\) specified in section 2.1.

So, according to the eq. (3) at time \(t_M\) which corresponds to \(r = r_M\), we calculate the corresponding value \(R_m\) thus:

\[
R_m = R_0 \cdot \sqrt{\frac{G}{E} \cdot \frac{1 + \frac{r_M^2}{4R^2_0}}{t_M}}.
\]

According to the eqs. (2) and (8) for both at the moment \(T_f\), we calculate the expression of \(\kappa\) as follows:

\[
\kappa = \frac{3 \cdot P_{w}V_{\mu w} \cdot C_f^2}{R_0}.
\]

Then, using \(k\) expression in eqs. (1–2) and solving it, we obtain the concentration \(C_f\) and also the \(k\) numerical value.

After that, from eqs. (1) and (2) at the moment \(t_M\), we calculate the concentration \(C_M\):

\[
C_M = \frac{\left(\frac{r_M^2 \cdot F}{\eta_{\mu w}R_{sw}} + \frac{23F \cdot P_{w}V_{sw}}{r_M R_{sw}}\right) \cdot G}{P_{w}V_{\mu w} \cdot \left(1 - \frac{r_M^2 \cdot F}{3R_{sw}^2}\right)},
\]

(15)
On the other hand, using eqs. (8) and (14), we obtain time value $t_M$ thus:

$$t_M = t_u + \frac{C_u - C_M}{\kappa}.$$  

(16)

Then, taking into account eq. (1) and eq. (8) both in $t_u$ and $t_M$, we calculate the moment $T_f$, the quantities $d$ and $q$.

$$d = \frac{r_M - r_0}{t_M - t_u},$$  

(17)

$$T_f = t_M + \frac{C_M - \sqrt{\frac{\kappa R_0}{3P_w V_{\mu w}}}}{\kappa},$$  

(18)

$$q = \frac{r_M}{T_f - t_M}.$$  

(19)

Solving the eq. (3), we obtain $R(t)$-function expressions for steps 2 and 3 as follows:

$$R_a(t) = R_0 \sqrt{\frac{d + G}{E_{R_a}(t)}} + 1 + \frac{r_a(t)^2}{4R_0^2}, t_u \leq t \leq t_M,$$  

(20)

$$R_b(t) = R_0 \sqrt{\frac{G - q}{E_{R_b}(t)}} + 1 + \frac{r_b(t)^2}{4R_0^2}, t_M \leq t < T_f.$$  

(21)

Moreover, we give the expression of $Q_a(t) = \frac{4\pi}{3} \cdot C_{ab} \cdot R_a(t)^3$ for 2nd stage, $Q_b(t) = \frac{4\pi}{3} \cdot C_{ab} \cdot R_b(t)^3$ for 3rd stage.

Therefore, as a synthesis for all model functions in steps 1 to 3, we obtain the complete expressions of the eqs. (9–21).

### 4. VALIDATING OF THE MODEL

Further, depending on the chosen values of the parameters for BE3s-model which we specify in section 2.1, 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 exemplification. Thus, we obtain the following numerical results:

- $R_m = 20.30 \, \mu m$,
- $t_u = 1114.9847 \, s$, $t_M = 1114.9867 \, s$, $T_f = 1123.4559 \, s$,
- $C_M = 8.74574 \, mol \cdot m^{-3}$, $C_f = 8.6930 \, mol \cdot m^{-3}$,
- and
- $d = 0.00412$, $q = G = 0.000001157$, $\kappa = 0.006228$.

These numerical values are in good agreement with the values of prevision studies.

In adding, for more clarity, we make the following specifications:
We use the "u" index for the parameters that characterize the state in which the liposome reaches its maximum size (the end of swelling stage). Thus, we have \( R_u, r_u, C_u, t_u \). Similarly, the index "m/M" was associated for the state in which the pore has the maximum radius: \( R_m, r_M, C_M, t_M \). Finally, the index "f" characterizes the final state of the cycle, when the pulsatory liposome returns to its initial size: \( C_f, T_f \).

Moreover, in Fig. 2 we finish our analysis by performing of the whole representations of the liposome radius, the pore radius and the solute concentration over all three stages of our model.

5. CONCLUSIONS

In this paper we have obtained the explicit analytical formulas in the eqs. (9)-(21) for the model functions in the approximations of the liposome radius \( R(t) \), the pore radius \( r(t) \), and the solute concentration \( C(t) \) during of the 1st to 3rd steps of BE3s model. Giving the graphical representation (Fig. 2), we have provided the analytical solutions behavior during of these three stages.
In the last time, experimental and theoretical researches on the pulsating liposomes has increased very much, because their application in biomedicine, nanomedicine, sensing and nanoelectronics [28]. Pulsatory liposome is a bionic example [29]. In adding, we validated the BE3s model by obtaining of a good agreement between the theoretical values provided by our model (via analytical solutions from section 3) and also the expected values estimate in previous studies about pulsatory liposome by chosen values of the parameters for BE3s model.

REFERENCES

4. D. Popescu, Association probabilities between the single chain amphiphiles into a binary mixture in plan monolayers (II), Biochimica et Biophysica Acta 1152, 35–43 (1993).


