TRIGONOMETRIC ANALYTICAL SOLUTION OF THE PULSATORY LIPOSOME

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The model of the pulsatory liposome as a three-phase biophysical motor is solved analytically using the basic constitutive differential equations. Adopting a trigonometric approximation for the pore radius, analytical expressions for the model functions described by the system of three differential equations are derived. Using these solutions, the graphical representations as functions of time for the first cycle of the pulsatory liposome evolution are given and the durations of the 3 phases are calculated. In this context, the solute concentration functions inside the liposome at the transition moments between phases are deduced and we calculate the liposome radius when the pore radius becomes maximum.

Keywords: pulsatory liposome, pore mechanism, analytical solution, trigonometric representation
MSC2000: 34A34, 76D45, 92C05

1. Introduction

A liposome can be made of a lipid bilayer (unilamellar liposome, fig.1a) [1]. If the liposome is filled with an aqueous solution of an osmotic solute and is placed in a watery environment, it becomes a pulsatory liposome [2], denoted LP. Due to the process of osmosis, the lipid vesicle swells to a maximum size, at which point a transbilayer pore suddenly appears [3–5]. Part of the internal solution leaks through this pore [6], and the liposome deflates and returns to its initial size. The swelling starts again [7], and the liposome begins a cyclic

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process (fig.1b). After performing a number of cycles [8], the pulsatory liposome stops when the outer and inner concentration becomes equal and it can be assimilated to a biophysical engine [6, 9]. This biophysical engine may can have many applications in medicine [10, 11], genetics [12], astrobiology [13]. In previous modeling, using numerical [14, 8] and analytical methods [15–17], we determined the functions for this model (called in the present work LP-model). In this paper, we propose a method to find an analytical solution of the differential equation system that describe the evolution of the liposome using trigonometric functions for pore behaviour processes. This provides a broad and detailed description of the evolution of LP. In this context, the system of constitutive equations of the LP-model is solved and we explain the advantage of the analytical approach in modeling this three-phase biophysics engine, consisting mainly in a high-accuracy analysis of the process functions and equations, also highlighting qualitative aspects of the model such as calculating the durations of each phase.

The structure of the article, together with the definition of the expressions that appear, is as follows: a) in Section 2, we present the mathematical description of the model and the analysis method containing the working hypothesis of LP, which includes the investigation of key moments in LP evolution as well as the consequences of the working this hypothesis; b) in Section 3, we obtain the analytical expressions and graphical representations for the functions of the model as well as finding the relevant relations in LP-model evolution; c) in Section 4, we validate the results obtained in the trigonometric approximation, performing a simulation on a set of input data and comparing our results with previous work.

We conclude by emphasizing the importance of analytical solutions and their effects in describing the functioning of the pulsatory liposome.

2. **Mathematical model description and the method**

The lipid bilayer separates an aqueous environment into two parts: the internal compartment (which belongs to the liposome) and the external compartment in which the liposome coexists [18]. The interfaces of the lipid bilayer with the two aqueous compartments are formed by the polar heads of the constituent phospholipid molecules, which have two opposite types of interactions with water: attraction (hydrophilic, due to the polar head) and repulsion (hydrophobic, due to the methyl chains, fig. 1a) [17, 18]. The lipid bilayer of the liposome is also called the lamella. Evolution of a pulsatory liposome during a cycle consists in swelling (bottom side in fig. 1b) and deflation (upper side in also fig.1b) steps. So, a cycle of LP evolution consists of 3 phases (fig.1b):

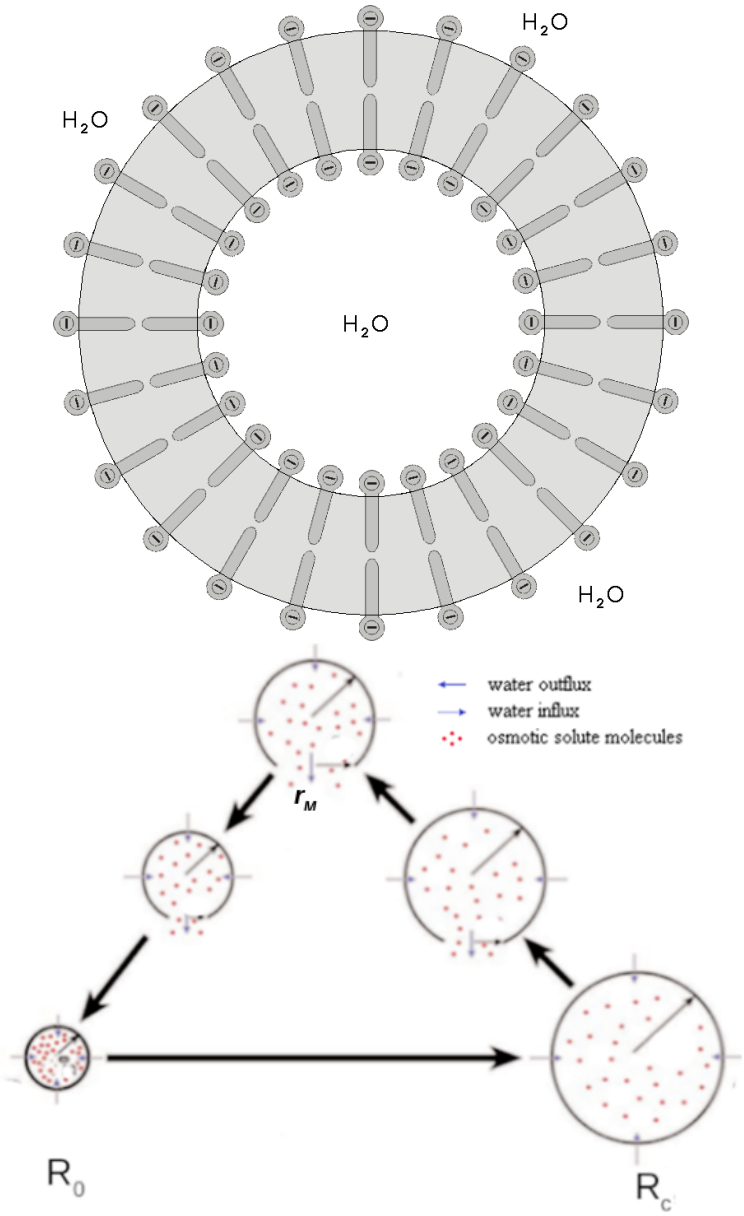

FIGURE 1. a) The liposome structure (a central section) in top panel, adapted by credit www.wikipedia.org; b) A cycle of the pulsatory liposome in sweling stage (phase f1) and relaxing stage (phases f2-f3) in bottom panel.

– phase 1st, denoted f1, corresponding to the swelling stage of the liposome;
– phase 2nd, denoted f2, for the stage of pore growth until it reaches the maximum radius;
– phase 3rd, denoted f3, for the stage of pore shrinkage until it disappears (r=0) [15].

2.1. **The basic equations and constitutive parameters**

The system of differential equations describing the evolution of a pulsatory liposome consists by the next three equations in three phases f1-f3 described by the model functions:

$$\frac{dr}{dt} = \frac{E}{4\eta_m h} \cdot r \cdot \sigma(R,r) - \frac{\gamma}{2\eta_m h} \tag{1}$$

$$\frac{dR}{dt} = \frac{-E \cdot r^3}{6\eta_\ell R^3} \cdot \sigma(R,r) + P_w V_{\mu w} \left(1 - \frac{r^2}{4R_0^2}\right) \left[C - \frac{2\beta E}{R} \cdot \sigma(R,r)\right] \tag{2}$$

$$\frac{d[ln(C \cdot V_{lip})]}{dt} = \frac{-E \cdot r^3}{2\eta_\ell \cdot R^4} \cdot \sigma(R,r) \tag{3}$$

where $\sigma(R,r) = \left(\frac{R^2}{R_0^2} - \frac{r^2}{4R_0^2} - 1\right)$ and $V_{lip} = \frac{4\pi}{3} \cdot R^3$ liposome volume.

The state vector is constituted by the liposome radius, R(t), the pore radius, r(t), and the solute concentration, C(t).

We use the following physical parameters for the liposome [2, 15]: the two dimensional stretch modulus of the lipid bilayer is $E = 0.2N \cdot m^{-1}$, $P_w \cdot V_{\mu w} = 5.412 \cdot 10^{-10} m^4 \cdot mol^{-1} \cdot s^{-1}$ with P_w, the membrane permeability coefficient for water and $V_{\mu w}$ water molar volume; $\beta = 4.00914 \cdot 10^4 \frac{mol}{J}$, the edge tension is $\gamma = 8.1 \cdot 10^{-12} N$; the lipid bilayer viscosity is $\eta_m = 100 \cdot 10^2 N \cdot s \cdot m^{-2}$; the aqueous solution viscosity is $\eta_\ell = 3.2 \cdot 10^2 N \cdot s \cdot m^{-2}$; liposome membrane thickness is $h = 1.75 \cdot 10^{-9} m$.

The swelling stage of a pulsatory liposome during a cycle is described by the following equation:

$$\frac{dR}{dt} = P_w V_{\mu w} \left(\frac{C_0 R_0^3}{R^3} - \frac{2\beta E}{R_0^2} - \frac{R^2 - R_0^2}{R}\right) \tag{4}$$

This equation has an analytical solution which is presented in the next section.

2.2. **Trigonometric representation for the analytical solutions**

In the trigonometric hypothesis, we define the function $r(t)$ as follows:
In phase f2, we consider:

$$r_a(t) = r_M \cdot sin\left(\frac{\pi}{2T_a} \cdot (t - t_c)\right), T_a = t_M - t_c \tag{5}$$

and, in phase f3, we consider:

$$r_b(t) = r_M \cdot sin\left(\frac{\pi}{2T_b} \cdot (t_f - t)\right), T_b = t_f - t_M \tag{6}$$

following that the durations of phases f2, f3 are determined in section 3.1. Below, we present several useful consequences of the trigonometric hypothesis.

Proposition 2.1. *The function $r_a(t)$ is increasing and concave.*

Proof. We calculate the derivatives of the function r(t) for phase f2:

$$\dot{r}_a(t) = r_M \cdot \frac{\pi}{2T_a} \cdot cos\left(\frac{\pi}{2T_a} \cdot (t - t_c)\right) > 0 \tag{7}$$

$$\ddot{r}_a(t) = -r_M \cdot \frac{\pi^2}{4T_a^2} \cdot sin\left(\frac{\pi}{2T_a} \cdot (t - t_c)\right) < 0 \tag{8}$$

□

Proposition 2.2. *The function $r_b(t)$ is decreasing and concave.*

Proof. We calculate the derivatives of the function r(t) for phase f3:

$$\dot{r}_b(t) = r_M \cdot \left(\frac{-\pi}{2T_b}\right) \cdot cos\left(\frac{\pi}{2T_b} \cdot (t_f - t)\right) < 0 \tag{9}$$

$$\ddot{r}_b(t) = -r_M \cdot \frac{\pi^2}{4T_b^2} \cdot sin\left(\frac{\pi}{2T_b} \cdot (t_f - t)\right) < 0 \tag{10}$$

□

Proposition 2.3. *The ratio of the second order derivative of r to r is a constant that depends on the duration of the phase considered, f2, respectively f3.*

Proof. We use formulas (5)–(9) and calculate the ratio:
$\frac{\ddot{r}}{r} = -\left(\frac{\pi}{2T}\right)^2$, where T is the duration of that phase.

□

Proposition 2.4. *The state variable R is expressed in terms of the state variable r and its first-order derivative.*

Proof. We calculate the formula of R using the constitutive equation for r (Eq.(1)) and obtain:

$$R(t) = R_0 \left(1 + \left(\frac{r(t)}{2R_0}\right)^2 + \frac{G + \dot{r}(t)}{\tilde{E}r(t)}\right)^{1/2} \tag{11}$$

□

Proposition 2.5. *The first-order derivative of the state variable R is a function of the state variable r and its derivatives.*

Proof. Derive the expression for R from Proposition 2.4. and also use the property from Proposition 2.3:

$$\dot{R}(t) = \frac{R_0^2}{2R(t)} \left(\frac{r(t) \cdot \dot{r}(t)}{2R_0^2} - \frac{1}{\tilde{E}} \cdot \left(\frac{\pi}{2T} \right)^2 - \frac{(G + \dot{r}(t)) \cdot \dot{r}(t)}{\tilde{E} \cdot r^2(t)} \right) \tag{12}$$

□

Proposition 2.6. *The formula for the state variable $C(t)$, named the solute concentration, is dependent on R, r and the first-order derivative of R.*

Proof. From the constitutive equation for R and Proposition 2.5. we successively deduce equation (2):

$$C(t) = \frac{\dot{R}(t)}{\mu \left(1 - \frac{r^2(t)}{4R^2(t)} \right)} + \left(\frac{\frac{E \cdot r^3(t)}{6\eta_\ell R^3(t)}}{\mu \left(1 - \frac{r^2(t)}{4R^2(t)} \right)} + \frac{2\beta E}{R(t)} \right) \cdot \sigma(R, r) \tag{13}$$

In addition to the above properties, we reiterate the analytical formula [15] for the explicit dependence of time values on the state variable R during phase f1 (swilling liposomes). □

$$t = \left(\frac{8\alpha\beta E P_w V_{\mu w}}{R_0^2} \right)^{-1} \left[(\alpha + 1) ln \left| \frac{\alpha - 1}{2z - \alpha - 1} \right| + (\alpha - 1) ln \left| \frac{\alpha + 1}{2z + \alpha - 1} \right| \right] \tag{14}$$

with $z(t) = \frac{R^2(t)}{R_0^2}$ and $\alpha = \sqrt{1 + \frac{2C_0 R_0}{\beta E}}$.

If formula (14) is considered an equation in the variable R then an expression of R as a function of t could be found. The difficulty of such an approach requires a distinct analysis. An approximate solution is possible by looking for a representation of the curve $R(t)$ from the graph $t(R)$ (see figure 2, panel a).

3. **Results**

In this section, we obtain the analytical expressions for the *r, R, C* functions in the phases f2-f3. By analytically solving the constitutive equations in the "trigonometric hypothesis" in section 2.2, we present the analytical expressions of the LP-model functions . The functions r(t), R(t), C(t), which describe the evolution of LP, must respect the continuity conditions at the moments of transition between phases. In the following, we analyze in turn the constitutive equations and the key moments of LP evolution for the first cycle, as follows:

-t_c is the moment of transition between phases f1 and f2, when the pore has radius $r = 0$,

-t_{0c} is the moment within phase f2 chosen so that R takes the value denoted R_c.

-t_M is the moment of transition between phases f2 and f3, when the pore has maximum radius, denoted $r = r_M$,

-t_f is the moment at the end of phase f3, when the pore disappears [15].

3.1. **The first cycle: durations of the stages and analytical solutions**

Working in the trigonometric hypothesis and taking into account Prop 2.4, we obtain the analytical expressions for the function R(t) in phases f2-f3. The function $R_a(t)$ is the state variable $R(t)$ in phase 2 and has the analytical expression:

$$R_a(t) = R_0 \left(1 + \left(\frac{r_M \cdot sin\left(\frac{\pi}{2}\frac{(t-t_c)}{T_a}\right)}{2R_0} \right)^2 + \frac{\left(G + \frac{\pi}{2} \cdot r_M \frac{cos\left(\frac{\pi}{2}\frac{(t-t_c)}{T_a}\right)}{T_a} \right)}{\tilde{E} \cdot r_M sin\left(\frac{\pi}{2}\frac{(t-t_c)}{T_a}\right)} \right)^{\frac{1}{2}} \tag{15}$$

The function *Rb(t)* is the state variable *R(t)* in phase 3 and has the analytical expression:

$$R_b(t) = R_0 \left(1 + \left(\frac{r_M \cdot sin\left(\frac{\pi}{2}\frac{(t_f-t)}{T_b}\right)}{2R_0} \right)^2 + \frac{\left(G - \frac{\pi}{2} \cdot r_M \frac{cos\left(\frac{\pi}{2}\frac{(t_f-t)}{T_b}\right)}{T_b} \right)}{\tilde{E} \cdot r_M sin\left(\frac{\pi}{2}\frac{(t_f-t)}{T_b}\right)} \right)^{\frac{1}{2}} \tag{16}$$

We note that to eliminate the singularity in relation (11), corresponding to the moment t_c, we consider the moment t_{0c}; also, to eliminate the singularity in relation (11), corresponding to the moment t_f, we use formula (16), namely, by considering the limit in t_f.

Furthermore, tacking into account the constitutive equation in the trigonometric hypothesis for the pore radius at the times t_{0c} and t_f, we calculate the durations T_a, T_b of the phases f2-f3: We specify that the solutions are given for the independent treatment of each phase, f2-f3.

$$T_a = \frac{\pi}{2} \cdot \frac{\sqrt{r_M^2 - r_0^2}}{(\dot{r})_{t_{0c}}} \tag{17}$$

$$T_b = -\frac{\pi}{2} \cdot \frac{r_M}{(\dot{r})_{t_f}} \tag{18}$$

where the moment t_{0c} is such as $R(t_{0c}) = R_c$, $(\dot{r})_{t_{0c}} = \frac{E}{4\eta_m h} \cdot r_0 \cdot \sigma(R_c, r_0) - \frac{\gamma}{2\eta_m h}$ and $(\dot{r})_{t_f} = -\frac{\gamma}{2\eta_m h}$.

Proposition 3.1. *For fixed R_0 and r_M, the radius liposome in t_M moment has a constant value independent of the method of solving the differential system, and has the formula:*

$R_a(t_M) = R_b(t_M) = R_m$ *with*

$$R_m = \frac{1}{2} R_0 \left(4 + \frac{r_M^2}{R_0^2} + \frac{4G}{\tilde{E} r_M} \right)^{1/2} \tag{19}$$

Proof. At time t_M, when the maximum radius for the pore is denoted by r_M, we have the derivative of r zero and we use this condition in its differential equation (1) (see also Eqs. (15)–(16)).

□

Proposition 3.2. *We have the following dependence between T_a, T_b and R_m:*

$$\dot{R}_a(t_M) \cdot T_a^2 \cdot R_m = \dot{R}_b(t_M) \cdot T_b^2 \cdot R_m = -\frac{1}{8} \frac{R_0^2 \pi^2}{\tilde{E}} \tag{20}$$

Proof. We start with Prop 2.5, equations (10)–(12), expressed for the moment t_M. □

On the other hand, from the process equations for the state variables R and C, the analytical expression for the function Q(t) is

$Q(t) = Q_0 \cdot exp(-I^*)$, $I^* = \frac{\tilde{E}}{2\eta_l} \cdot \int_0^T \left(\frac{r}{R^2}\right)^2 \cdot \sigma(R, r) dt$, where T is the duration of that phase (Eqs. (17)–(18)) and $Q = C \cdot V_{lip}$ as in Eq. (3) and Eq. (13).

3.2. **Analytical solutions behaviour in all three phases**

In the phase f1, namely for liposome radius growth, using formula (14), we obtain in figure 2 the following graphical representations of the time functions t (fig. 2 panel a), respectively concentration C (fig. 2 panel b), in which the free variable is considered R.

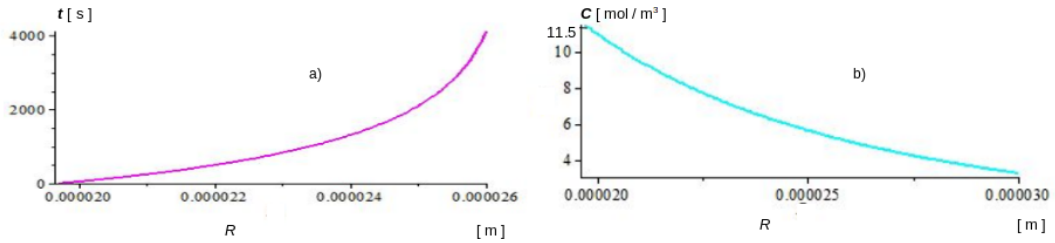

FIGURE 2. The time and solute concentration functions in variable R in the phase f1 in panel a), respectively panel b).

We remark that, from process equation (3) applied to phase f1, we have $Q = C \cdot V = const$ and, therefore, $C = C_0 \left(\frac{R_0}{R}\right)^3$, from which we obtain as consequences $C(t_{0c}) = C_c$ and $\tau = t(R_c)$.

Further, the graphical representations of the state variables r, R, C, dependent on the free time variable t, are given in Figure 3, for which we use the analytical formulas in the trigonometric representation obtained in section 3.1. We recall that the two phases f2 and f3 are connected at the moment t_M.

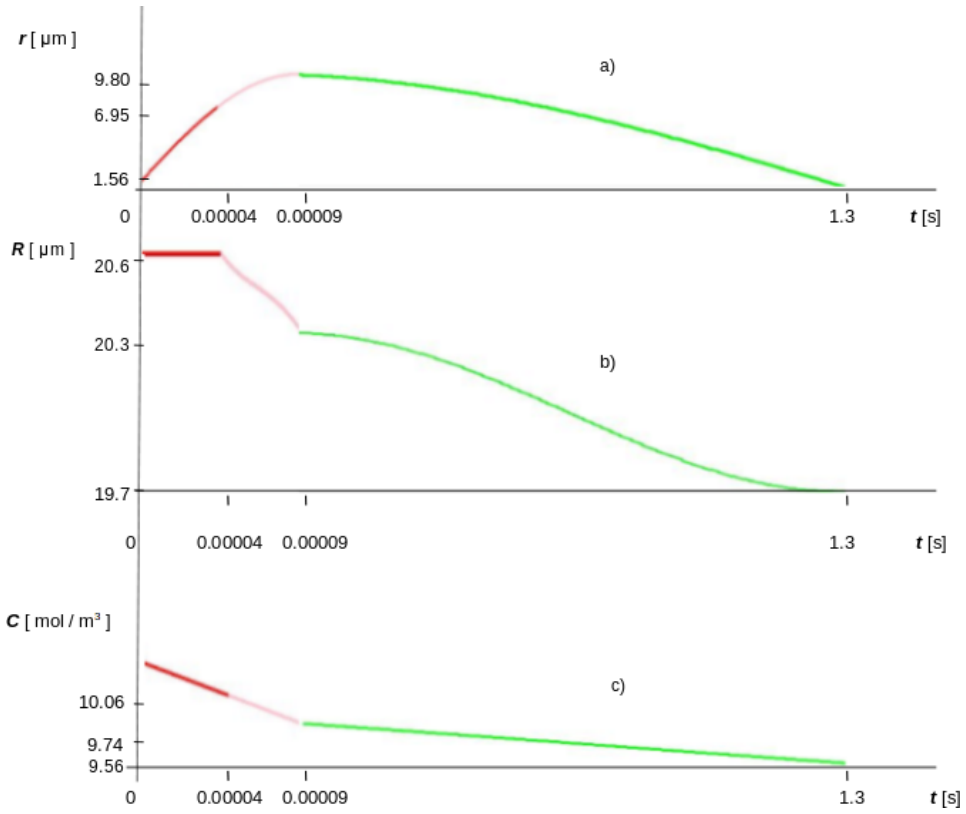

FIGURE 3. The pore and liposome radii and solute concentration functions in the trigonometric representation for phases f2 and f3, in panels a) to c).

The color code for the graphical representation is red/pink for phase f2, corresponding to the growth of the pore, with separation at time t_{0c}, and is green for phase f3, corresponding to the decrease and disappearance of the pore. In figure 3, panels a)-c), the graphical representations highlight the analytical properties of the state variables in trigonometric representation, such as: monotonicity, extreme points, rate of variation.

We can conclude that the model functions behaviour in figures 2 and 3 confirm the analytical properties of the state variables r, R and C in the trigonometric representation and also with the previous studies [16, 6, 7].

4. **Validation of the analytical solutions**

The validation of the trigonometric representation, which leads to analytical formulas for the state variables, is done by comparing the results of this representation with those obtained experimentally and numerically from the references [1, 8, 14]. The liposome functioning scenario is the one that has as input values for the state variables the values existing in the experimental works[11,16]:$R_0 = 19.7, R_c = 20.6, r_0 = 6.95, r_M = 9.8$.

For the first cycle of LP evolution, we obtain durations, key moments and the solute concentrations functions in the all three stages:

- $\tau = 160.624305, t_{0c} = 0.00004, T_a = 0.00009, T_b = 1.3$ in seconds;

- $R_m = 20.3[\mu m]$, see Prop. 3.1, Eqs. (19)–(20);

-concentrations at key moments:$C_0 = 11.5, C_c = 10.06, C_M = 9.74, C_f = 9.56$ in $mol \cdot m^{-3}$.

From the validation results and the properties of the analytical formulas for the state variables R, r, C, the following qualitative aspects are noted:

- the durations T_a, T_b do not depend on the initial value of the C_0 solute concentration and, in addition, $T_a << T_b < \tau(C_0)$;

- for concentrations, we note that $(C_c - C_M) \sim (C_M - C_f) < (C_0 - C_c)$.

In the scenario presented in section 4, we validated the solutions obtained for the trigonometric representation and conclude that the pulsatory liposome modeling results obtained by us in this paper are in harmony with previous theoretical studies as well as with experimental works [1, 11, 16].

5. **Conclusions**

In this paper, we obtained the analytical solutions of differential equations system Eqs.(2)–(4) in the trigonometric approximation, as well as the explicit analytical expressions for the functions r(t), R(t), and C(t). From the graphical representation of the functions r(t), R(t), and C(t), we deduce that the results obtained by the trigonometric method describe the dynamics of pulsating liposomes better than the solutions obtained by integrating the system of differential equations through numerical methods [1]. The trigonometric hypothesis describes more precisely the behaviour of the pore radius, highlighted in a previous numerical study [7, 8]. Although it is an approximation method and this could be considered a limitation, the method provides

the analytical expressions of the solutions and a sufficiently precise description of the evolution of the pulsatory liposome (section 4), our results being in very good agreement with those obtained in laboratory works [1, 17]. We have also tried other analytical methods [6, 16] to solve the system of differential equations, and the trigonometric method becomes one of these that is more accurate and complete. Futher, the pulsatory liposome is an example of a bionic object [19]. It can be likened to a three strokes bioengine. The potential operating energy is given by the transbilayer gradient of the osmotic solute concentration, which represents the fuel for the bioengine [20, 9]. If the osmotic solute is a pharmaceutically active substance, the pulsatory liposome can be used in the medical field.

A more rigorous description of liposome evolution is compatible with the requirements of considering pulsating liposomes as an elementary mechanism [8] for explaining forms of biological existence that are at the center of attention in frontier fields such as astrobiology [13].

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