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Dynamic analysis of an enzymatic membrane reactor

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***Abstract*— This paper is focused on the analysis of nonlinear dynamics of an enzymatic process involving three chemical species. The work is based on the immobilization of Acetylcholinesterase enzyme into an artificial proteinic membrane. Our objective is to study oscillations observed in the membrane potential difference during experimental works. Stability analysis is provided for the system from a theoretical point view. Reported results allow the analysis of the profile of diffusion motion of molecules inside the membrane.**

I. INTRODUCTION

This study concerns the neuron-signaling Acetylcholinesterase ($AChE$) enzyme. Experimental works carried out in [1] showed that $AChE$ immobilized into a membrane can result, not only in a quantitative modification of the enzymatic kinetic properties but also in the existence of complex behaviors of its catalytic activity. This was verified latter in [2] through a developed model for investigating the enzymatic diffusion-reaction process. The authors analyzed the dynamic behavior of the three different chemical species and the existence of hysteresis loop resulting from the catalytic activity induced by immobilization of $AChE$ into the enzymatically inactive membrane. A second approach for describing such enzymatic process is called distributed system [3]. Two continuous independent variables, space and time, i.e., (x, t) in the Partial Differential Equations (PDEs) are used to characterize the states of the physical system. Their main contribution was to show the concentration profile for each chemical species involved in the reaction on space and time simultaneously. As part of this ongoing effort to understand the biological process, we study the system's behavior for different values of Thiele modulus, i.e., parameter σ in (1).

II. EXPERIMENTAL PROCEDURE

The artificial $AChE$ membranes were prepared with a co-cross-linked method as presented in [4] and illustrated in Figure 1. Notice that the $AChE$ membrane is put inside the diffusion cell. The buffer solution circulates continuously. Experiments were performed in a diffusion cell where the membrane produced with bovine serum albumin containing varying $[AChE]$ separates into two 25-ml compartments. The diffusion surface area was 0.5cm^2. Identical 10^{-3}M phosphate buffer solutions at pH 7.5 were stirred continuously in both compartments. ACh chloride was injected into one compartment and the potential difference between

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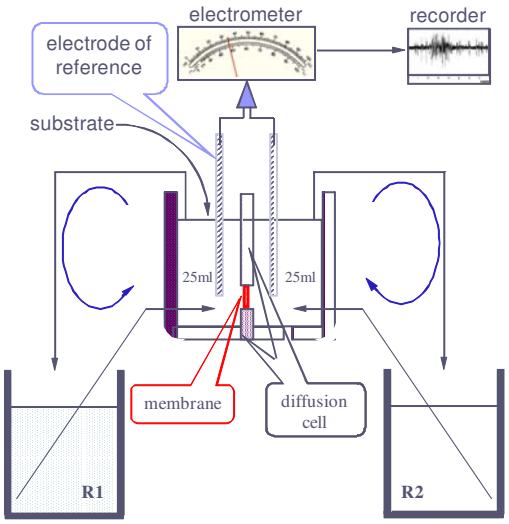

Fig. 1. Scheme used during experimental work.

the two compartments was measured using a vibrating-reed electrometer with calomel reference electrodes. The pH was regulated on each side of the cell with pH stats. The determination of the $AChE$ effect was obtained by comparing the resulting potential difference with membranes, with and without $AChE$. The steady state condition of $[ACh]$ is assured by small dimensions of the two compartments-reactor in relation to R1, R2. The potential difference was studied as a function of both $[ACh]$ and $[AChE]$. Experimental work indicates that the non-linearity of the $AChE$ reaction coupled with the diffusion constraints can, over a limited range of parametric values, cause some instabilities such as oscillations of the membrane potential. We verified these oscillations via numerical simulations and determined under which values they may be observed.

III. $AChE$ INSIDE THE MEMBRANE

The mathematical description of the kinetic reaction is presented in this section. The main feature of the distributed system is the fact that the $AChE$ is homogeneously distributed inside the membrane. In fact, enzyme molecules are mostly located inside cell membranes. In Figure 2 we have summarized the enzymatic process explained in the previous section. Note that physical constraints are imposed onto the system so that the phenomena of diffusion and reaction take place inside the same physical space, i.e., inside the membrane enzymatically active. These constraints are included because of the $[ACh]$ outside the membrane

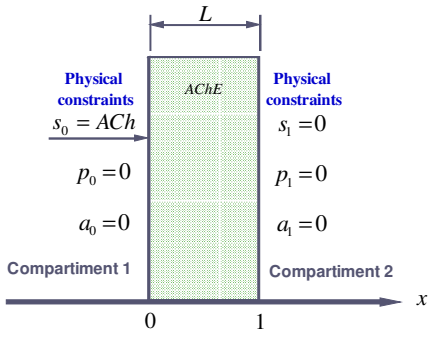

Fig. 2. Diffusion & reaction take place inside the active membrane.

is kept constant due to R1 and R2. The interpenetration of the three chemical species is the result of a natural movement of their particles throughout the membrane and it can be appropriately described by Fick's Second Law. In the hydrolysis regulation the $AChE$-catalyzed reaction can be inhibited by excess of ACh^+. This means that for a given $[AChE]$ the initial reaction velocity increases with the initial $[ACh]$ until it reaches the limiting value V_m. Similarly, the high concentration of Choline, Ch^+, has a negative effect on the reaction by inhibiting the enzymatic activity. The pH plays a key role since it can act either as an inhibitor or as an accelerator, directly on the $AChE$, as well as indirectly, by means of the ACh and Ch. This is explained by the fact that changes of pH charge affect the activity, the structural stability and the solubility of the enzyme [5]. These three factors that intervene in the enzymatic reaction rate are taken into account in the kinetic mechanism model for hydrolysis of ACh by $AChE$ (also called E) as showed in Figure 3. The ACh molecule and the free enzyme E are combined ($E + ACh$) and after a first reversible reaction the E binds to its substrate ACh to yield the substrate-enzyme intermediary complex ($E.ACh$) which breaks down into Ac and Ch. The red arrows correspond to inhibitors. High concentrations of Ch may appear during the reaction and exert a feedback control inhibiting the enzyme action as well as inhibiting the free enzyme E and the acetyl-enzyme intermediary ($E.Ac$). The inhibition of the reaction by excess of $[ACh]$ is described in [6] and it is explained by the binding of an additional ACh molecule to the intermediary complex to form a dead-end ternary complex ($E.Ac.ACh$). At the end of the catalytic process the active site is free to accept another ACh molecule and the E returns to its original state to start a new cycle. This enzymatic reaction combined with the diffusion of the chemical species is described in [7] by

$$\frac{\partial u(x,t)}{\partial t} = \begin{cases} \partial_t s &= D_s \partial_{xx} s - \sigma R(s,p,a) \\ \partial_t p &= \alpha \partial_{xx} p + \sigma R(s,p,a) \\ \partial_t a &= \beta \partial_{xx} a + \sigma R(s,p,a) \end{cases} \quad (1)$$

where $x \in (0,1)$, $t > 0$. The constants $D_s = 1$, $\alpha = (D_p/D_s) = 1$, $\beta = (D_a/D_s)$ correspond to the ratio of the diffusion coefficients for different components of the substrate, $s(x,t)$, the Choline, $p(x,t)$, and the Acetate, $a(x,t)$. The term $\sigma R(s,p,a)$ is the nonlinear reaction kinetics described by (4), $\partial_t u = \partial u/\partial t$; $\partial_{xx} u = \partial/\partial x\,(\partial u/\partial x)$, where $\sigma = \frac{V_M}{K_S}\frac{L^2}{D_S}$ is a non-dimensional constant called Thiele modulus corresponding to the ratio between the maximum rate of reaction and the maximum rate of diffusion of the three reactants. As will be discussed later on, σ allows us to determine the zones where instabilities may occur and subsequently the domains where oscillations may appear.

The diffusion term in (1) models the movement of the chemical particles of three different species denoted by $u(x,t)$, where u corresponds to $s(x,t)$ for the ACh, $p(x,t)$ for Ch and $a(x,t)$ for Ac, with $x \in \Omega \in \mathbb{R}^n$ (the $n-th$ dimensional space with Cartesian coordinate system). These chemical particles are transported from regions of high concentrations to regions of lower concentrations throughout $AChE$ membrane in fixed steps Δx that are taken in a fixed time Δt. Each particle will move a distance $\pm\,\Delta x$, moving right $(+\Delta x)$ or left $(-\Delta x)$ following a random motion. The number of particles to cross the membrane from left to right (positive mass flux) is $(u_0 \Delta x A)$, where A is the area of the membrane and $u_0 = [s_0\ p_0\ a_0]$ as illustrated in Figure 2. This movement of molecules across a membrane is defined by the rate of diffusion which is proportional to the concentration gradient [8]. To complete the physical description of the process we specify Initial Conditions (ICs) corresponding to initial state of each variable. Also, Boundary Conditions (BCs) are imposed because the physiological conditions in the reactor can vary with respect to x, t. ICs and BCs were taken from the physical constraints imposed to (1) during experiments. At $t = t_0$ and $0 \leq x \leq 1$ the ICs that specify the states of the system are $s(x,t_0) = s_0(x) = 853$, $p(x,t_0) = p_0(x) = 0$ and $a(x,t_0) = a_0(x) = 0$. Dirichlet BCs for the left side and right side of the membrane are given in section VI. These BCs are required so that the second order parabolic PDEs take the form of boundary value problem. They denote the concentration of the different chemical species in the one-dimensional membrane. The variable x is defined in the interval $[0, L]$, with $L = 1$. A discretization procedure is used for finding approximate solutions of the PDEs into an approximating system of Ordinary Differential equations (ODEs). The ODEs resulting from discretization in x space are integrated to obtain approximate solutions in t. The solver function in section VI returns values of the solution on a mesh provided in x.

IV. REACTION TERM FORMULATION

A. Inhibition by ACh

This section summarizes the reaction term developed in [9]. The $[ACh]$ can inhibit the reaction since the enzymatic reaction rate is proportional to $[ACh]$. However, beyond a certain threshold this rate will no longer increase [2], namely

$$R(S) = \frac{V_m + V_{SS}([S]/K_{SS})}{1 + K_m/[S] + [S]/K_{SS}} \quad (2)$$

where $[S]$ denotes the $[ACh]$. $V_m = [E]_t k_2 k_3/(k_2 + k_3) = k_{cat}[E]_t$, $V_{SS} = [E]_t k_2 k_5/(k_2 + k_5)$, $K_m = \frac{k_3(k_{-1}+k_2)}{k_1((k_2+k_3))}$

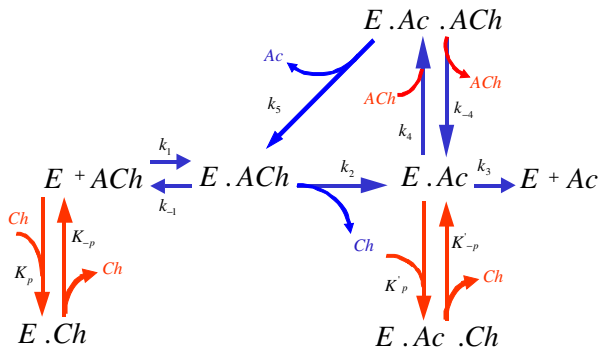

Fig. 3. Kinetic mechanism for hydrolysis of ACh by $AChE$.

and $K_{ss} = \frac{(k_2+k_3)(k_4+k_5)}{k_4(k_2+k_5)}$ where k_{-1}, $k_1, ..., k_5$ are kinetic constants, k_{cat} is the catalytic rate constant, $[E]_t$ is the total enzyme concentration, V_m is the maximum velocity reaction rate of the enzyme reaction, K_m is the Michaelis-Menten constant, V_{SS} is the maximum theoretical velocity in a given $[AChE]$, and K_{SS} is the substrate inhibition constant. k_{cat}, $[E]_t$, V_m, V_{SS}, K_{SS} and all kinetic constant were obtained from scheme for the enzyme-catalyzed reaction in Figure 3.

B. Inhibition by Ch

In immobilized enzymatic systems high concentrations of Ch may appear during reaction and alter the catalytic action of the enzyme by exerting a negative feedback control and inhibit the $AChE$ activity. This property implies that the rate for hydrolysis of ACh by $AChE$ is described by the following non dimensional form [10]

$$R(s,p) = s/\left(1 + p/kp + s(1 + p/k^{'}p + ks)\right) \quad (3)$$

$p = [P]/K_m$, $[P] = [Ch]$, $kp = K_P/K_m$, $k^{'}p = K_P/K_m$.

C. The effect of pH

In the hydrolysis of ACh by $AChE$ the reaction produces acetic acid which rapidly dissociates to give acetate and protons at pH values higher than 5. This local production of protons may induce an important modification of pH in the vicinity of the enzymatic sites. It is therefore reasonable to express the $[H^+]$ influence on the $AChE$ activity as follows

$$R(s,p,h) = \frac{sF(h)}{\left(1 + \frac{p}{k_P} + s(1 + \frac{p}{k_P^{'}}) + k_i s^2\right)} \quad (4)$$

where $s = [S]/K_m$, $p = [P]/K_m$, $h = [H^+]/H_0$,$k_P = K_P/K_m$, $k_P^{'} = K_P^{'}/K_m$ and $k_i = K_m/K_{ss}$.

K_{SS} describes H^+ influence on inhibition by ACh^+ while K_P, $K_P^{'}$ describe the effect of pH on the inhibition by $[P]$ corresponding to $[Ch]$. The $[H^+]$ influence is described by

$$F(h) = 1/\left(1 + h/c_a + c_b/h\right) \quad (5)$$

with catalytic constants $k_i = k_i^*/(1 + h/c_k)$, $k_p = k_p^*(1 + h/c_p)$, $k_p^{'} = k_p^{'*}(1 + h/c_p)$, where $c_a = C_{H1}/K_m$, $c_b = C_{H2}/K_m$, $c_k = C_{Hss}/K_m$, $c_p = C_{Hp}/K_m$.
The h value is calculated as described in the next subsection.

D. Determination of the local pH

The solution of (1) requires to calculate the local pH resulting from Ac dissociation and buffer solution effect. We assume that the different ionic equilibria related to the electroneutrality are fast when compared to the diffusion of hydronium ions. This local concentration of free hydrogen ion (H^+) is obtained from considerations $i)$, $ii)$ and $iii)$:

$i)-$ **Acid-base chemical equilibrium:** We represent an acid by HA and its conjugate base as A^-. The actual electric charges of the species depend on the particular nature of A, but the base always has one more negative charge than HA. This pair of species constitutes an acid-base system. When an HA dissolves in water some molecules of the acid dissociate to form hydronium ions and A^- as related by reaction

$$HA + \mathrm{H_2O} \rightleftharpoons \mathrm{H_3O^+} + A^- \quad (6)$$

Omitting $\mathrm{H_2O}$ on both sides of the Bronsted and Lowry equilibrium (6) one obtains

$$HA \rightleftharpoons H^+ + A^-. \quad (7)$$

The equilibrium expression is referred to as the acid-dissociation constant, K_A, of a weak acid, HA, which is given by the mathematical product of the equilibrium concentrations of the products of this reaction, divided by the equilibrium concentration of the original acid. For Ac dissociation, this constant at a particular temperature is expressed by the product of the concentrations of the products over the reactant concentration (not including water)

$$K_A = [A^-][H^+]/[AH] \quad (8)$$

K_A represents the ionization constant of an acid in an acid-base equilibrium. $[H^+]$ is the hydrogen ion concentration. $[A^-]$ is the concentration of the conjugate base of the acid and $[AH]$ is the concentration of undissociated acid. Similarly, the equilibrium expression for the base-dissociation constant K_B of a weak base, HB, is given by the generic base dissociation reaction with water

$$HB \leftrightharpoons H^+ + B^-. \quad (9)$$

The base-ionization equilibrium constant is the mathematical product of the equilibrium concentrations of the products of this reaction divided by the equilibrium concentration of the original base. In other words, K_B is the product of the concentrations of the products over the reactants concentration (not including water). The base constant corresponds to a measure of the relative strength of a base defined as

$$K_B = [B^-][H^+]/[BH] \quad (10)$$

K_B represents the ionization constant of a base in an acid-base equilibrium. $[B^-]$ is the concentration of conjugate acid, and $[BH]$ is the concentration of undissociated base. Water is not included in the base-ionization equilibrium expression because the $[\mathrm{H_2O}]$ has no effect on the equilibrium.

Relationship between K_A and K_B: For any conjugate acid-base pair, which are an acid and a base whose structures differ by only one ionizable proton, the values of K_A

for the acid and K_B for its conjugate base are related by $K_A \times K_B = K_W$, where K_W is an equilibrium (or ionization) constant for the autoionization reaction of water with itself. The inherent or intrinsic strength of an aqueous acid (or base) is its ability to remove a proton from (or donate a proton to) the solvent water or other ions and molecules in aqueous solutions. The ionization of pure water can be treated as the acid-base equilibrium because the water can act either as an acid or a base. The product of the activities of H^+ and OH^- ions in pure water is given by

$$K_W = \left[OH^-\right]\left[H^+\right] \tag{11}$$

where $[H^+]$ is the molar concentration of hydrogen and $[OH^-]$ is the molar concentration of hydroxide ion.

$ii)-$**Mass balance of the different species:** This is a relation that expresses the nominal concentration of the acid and buffer solution in terms of their two conjugate forms (12) and (13). All the weak acids in the system are represented collectively as HA. The anion for each acid will be different but because they all behave similarly, all the weak acids are represented as though they were a single HA which has a single apparent dissociation constant (6). The law of conservation of mass means that the total amount of A (symbol: $[A]_T$) must be constant, i.e., none of the reactions produce or consume A, whose conservation of mass is

$$[AH] + \left[A^-\right] = [A]_T . \tag{12}$$

The independent variable $[A]_T$ represents total concentration of non-volatile weak acids in the solution. Similarly, the total amount of concentration of conjugate acid $[B]_T$ in the solution is constant, i.e., conservation of mass for B is

$$[BH] + \left[B^-\right] = [B]_T \tag{13}$$

$iii)-$**The law of electroneutrality:** According to this law a solution must have an equal amount of positive and negative charges, i.e., it is neutral. If we add some sodium hydroxide to a solution of an acid, then an equivalent amount of the acid will be converted into its base form, resulting in a partly neutralized solution in which both the acid and its conjugate base are present in significant amounts. The equilibrium expressions for such a solution are given by equilibrium relations (8) for acetic acid, (10) for buffer and (11) for pure water, while the charge balance is given by

$$\left[H^+\right] + \left[Na^+\right] = \left[OH^-\right] + \left[B^-\right] + \left[A^-\right]. \tag{14}$$

The following equilibrium constant is used to replace the conjugate base concentration

$$x = \left[Na^+\right] / [B]_T . \tag{15}$$

From (8) we get the relation

$$K_A[AH] = [A^-][H^+]. \tag{16}$$

By multiplying both sides of (12) by K_A and substituting (16) we get the expression

$$\underbrace{[A^-][H^+]}_{K_A[AH]} + K_A[A^-] = K_A [A]_T . \tag{17}$$

The factoring $[A^-]$ in (17) implies

$$[A^-]\left([H^+] + K_A\right) = K_A [A]_T \tag{18}$$

leading to concentration of the conjugate base of the acid

$$[A^-] = K_A [A]_T / \left([H^+] + K_A\right). \tag{19}$$

Similarly, from (10) we get the relation

$$K_B[BH] = [B^-][H^+]. \tag{20}$$

Substituting the above into (13) and multiplying both sides by K_B leads to

$$\underbrace{[B^-][H^+]}_{K_B[BH]} + K_B \left[B^-\right] = K_B [B]_T . \tag{21}$$

Factoring $[B^-]$ in (17) we obtain

$$[B^-]\left([H^+] + K_B\right) = K_B [B]_T . \tag{22}$$

From (22) we get the concentration of conjugate acid

$$[B^-] = K_B [B]_T / \left([H^+] + K_B\right). \tag{23}$$

Adding (19) and (23) leads to

$$[A^-] + [B^-] = \frac{K_A [A]_T}{([H^+] + K_A)} + \frac{K_B [B]_T}{([H^+] + K_B)}. \tag{24}$$

From (11), (15) and (24) the charge balance (14) becomes

$$\left[H^+\right] + \underbrace{x [B]_T}_{Na^+} = \underbrace{\frac{K_W}{[H^+]}}_{[OH^-]} + \underbrace{\frac{K_B [B]_T}{([H^+] + K_B)}}_{[B^-]} + \underbrace{\frac{K_A [A]_T}{([H^+] + K_A)}}_{[A^-]} \tag{25}$$

which is rewritten as

$$\left[H^+\right] + x [B]_T - \frac{K_W}{[H^+]} - \frac{K_B [B]_T}{([H^+] + K_B)} = \frac{K_A}{([H^+] + K_A)} [A]_T \tag{26}$$

giving rise to total concentration of non-volatile weak acid

$$[A]_T = \left([H^+] + x [B]_T - \frac{K_W}{[H^+]} - \frac{K_B [B]_T}{(K_B + [H^+])}\right)\left(\frac{K_A + [H^+]}{K_A}\right). \tag{27}$$

Under experimental conditions $[H^+] << x [B]_T$ and $\frac{K_W}{[H^+]} << \frac{K_B [B]_T}{(K_B + [H^+])}$. Consequently, (27) is simplified to

$$[A]_T = \left(1 + \frac{[H^+]}{K_A}\right)\left(x [B]_T - \frac{K_B [B]_T}{(K_B + [H^+]}\right). \tag{28}$$

Equation (28) is rewritten in its dimensionless form as

$$a = (1 + h/k_a)\left(xb - bk_b/(h + k_b)\right), \tag{29}$$

where $a = [A]_T / K_M$, $b = [B]_T / K_M$, $k_w = K_W / K_{M^2}$, $k_a = K_A / H_0$ and $k_b = K_B / H_0$.
Equation (29) is rewritten as

$$a = xb - \frac{bk_b}{(h + k_b)} + \frac{h}{k_a} xb - \frac{h}{k_a}\left(\frac{bk_b}{(h + k_b)}\right) \tag{30}$$

which is expressed as

$$a = xb\left(\frac{k_a(h+k_b)}{k_a(h+k_b)}\right) - \left(\frac{k_a}{k_a}\right)\frac{bk_b}{(h+k_b)} + \frac{hxb}{k_a}\left(\frac{(h+k_b)}{(h+k_b)}\right) - \frac{hbk_b}{k_a(h+k_b)}. \tag{31}$$

Equation (31) is rewritten by reducing fractions to their lowest equivalent as follows

$$a = \frac{xbk_a(h+k_b) - k_abk_b + hxb(h+k_b) - hbk_b}{k_a(h+k_b)} \quad (32)$$

that multiplied in both sides by $k_a(h+k_b)$ leads to

$$ak_ah + ak_ak_b = xbk_a(h+k_b) - k_abk_b + hxb(h+k_b) - hbk_b. \quad (33)$$

From (33) we obtain a polynomial of degree 2 given by

$$\begin{aligned} h^2xb + h\left(xbk_a + xbk_b - bk_b - ak_a\right) + xbk_ak_b \\ -k_abk_b - ak_ak_b = 0. \end{aligned} \quad (34)$$

By dividing (34) by k_a we obtain a row vector with the coefficients of the polynomial

$$h^2(\frac{bx}{k_a}) + h(bx + b(x-1)\frac{k_b}{k_a} - a) + bk_b(x-1) - ak_b = 0. \quad (35)$$

Solving (35) results in a column vector whose elements are the roots, h. As the hydrogen ion cannot be negative, we take the positive roots corresponding to $[H^+]$.

V. STABILITY ANALYSIS

The stability analysis of (1) is done by a linearization process obtained by defining a neighborhood of a given equilibrium point $u^*(x,t)$ and introducing a small perturbation in the state variables around u^*

$$u = \delta u + u^* \quad (36)$$

where $\delta u = [\delta s, \delta p, \delta a]^T$ and δs, δp and δa are displacements from the equilibrium point $u^* = [s^*, p^*, a^*]^T$. Note that δu depends on both time and space as follows

$$\partial u/\partial t = \partial(\delta u)/\partial t, \quad \partial^2 u/\partial x^2 = \partial^2(\delta u)/\partial x^2.$$

The linearization of (1) around $u^*(x,t)$ is given by

$$\partial \delta u/\partial t = D\partial^2 \delta u/\partial x^2 + \sigma A \delta u \quad (37)$$

where $\sigma A\delta u$ represents the linearization of the reaction term $\sigma R(s,p,a)$. A is the Jacobian matrix $A = \partial R_i(u(x,t))/\partial u_j|_{u^*}$ evaluated at the equilibrium point, *i.e.*

$$A = \begin{bmatrix} -R_s & -R_p & -R_a \\ R_s & R_p & R_a \\ R_s & R_p & R_a \end{bmatrix}_{\{s^*,\ p^*,\ a^*\}} \quad (38)$$

and D is the diffusion coefficients matrix $D = diag[1, 1, \beta]$. Let us assume that the solution for (37) is given by

$$\delta u = e^{\lambda t} e^{ikx} \bar{u}. \quad (39)$$

$e^{\lambda t} = diag[e^{\lambda_1 t}, e^{\lambda_2 t}, e^{\lambda_3 t}]$ is the time-dependent part of the solution and $\bar{u}$ is the steady state solution of δu. The term e^{ikx} is the space-dependent part of the solution rewritten as

$$e^{ikx} = \cos(kx) + i\sin(kx) \quad (40)$$

where k is called wave number.

By substituting the proposed solution (39) into (37) and cancelling the common factor $e^{\lambda t}\ e^{ikx}$ we obtain $\lambda\bar{u} = -k^2 D\bar{u} + \sigma A\bar{u}$ which is rewritten as

$$\left\{\lambda I + k^2 D - \sigma A\right\}\bar{u} = 0 \quad (41)$$

The behavior of the linearized system (37) will depend on the roots λ_i and k of the characteristic polynomial

$$\det(\lambda I + k^2 D - \sigma \underbrace{A}_{\{a_{ij}\}}) = 0 \qquad \text{i.e.,} \quad (42)$$

$$\begin{vmatrix} \lambda + k^2 - a_{11} & a_{12} & a_{13} \\ -a_{21} & \lambda + k^2 - a_{22} & -a_{23} \\ -a_{31} & -a_{32} & \lambda + k^2\beta - a_{33} \end{vmatrix} = 0. \quad (43)$$

From (43) we obtain

$p\left[\lambda(k)\right] = \lambda^3 + (k^2\beta + 2k^2 - a_{22} + a_{11} - a_{33})\lambda^2 + (-a_{11}a_{22} - ka_{22} + a_{22}a_{33} - a_{11}a_{33} - a_{23}a_{32} - 2k^2a_{33} + 2k^4\beta + a_{21}a_{12} + a_{11}k^2 - a_{22}k^2\beta + a_{11}k^2\beta + k^4 + a_{13}a_{31})\lambda + a_{21}a_{13}a_{32} - a_{21}a_{12}a_{33} + a_{21}a_{12}k^2\beta - a_{11}a_{23}a_{32} + a_{11}a_{22}a_{33} - a_{31}a_{13}a_{22} - a_{11}k^2a_{33} + k^6\beta + a_{31}a_{13}k^2 - k^2a_{23}a_{32} + k^2a_{22}a_{33} - k^4a_{22}\beta - a_{11}a_{22}k^2\beta + a_{31}a_{12}a_{23} + a_{11}k^4\beta - k^4a_{33} = 0.$

We highlight that if λ_i is real then the corresponding solution diverges for $\lambda_i > 0$ and converges for $\lambda_i < 0$ as $t \to \infty$. The solution has oscillatory behavior if λ_i and λ_{i+1} $(i = 1, 2)$ are complex conjugate. The oscillations decrease if $\mathrm{Re}(\lambda_i) < 0$ and increase if $\mathrm{Re}(\lambda_i) > 0$. Sustained oscillations appear when $\mathrm{Re}(\lambda_i) = 0$. The term e^{ikx} represents oscillations of the solution with respect to spatial domain. For $k = 0$ the system behaves as in [2] since the space variable is not taken into account. For $k > 0$ the behavior depends on both λ_i and k. As $k\Delta x$ represents a displacement on the membrane, then for each k there is a spatial solution $u(k\Delta x, n\Delta t)$.

VI. NUMERICAL IMPLEMENTATION

The roots of $p\left[\lambda(k)\right]$ were computed via XPPAUT [11] and indicate as observed in experimental works, the existence of an unstable zone (see Figure 4) under the form of oscillations in the range of $38860 \leq \sigma \leq 41988$, with a mesh of 115 points and a grid size equal to 0.09. As the discretized model has 115 grid points for each state variable, then we have 345 roots i.e., $\lambda_n(k_1), \lambda_n(k_2), \cdots, \lambda_n(k_{115})$ with $n = 1, 2, 3$. For $\sigma \in [38860, 41988]$ we have found different equilibria. In $\sigma = 41000$ we have 10 complex conjugate roots: 2 with positive real part, 8 with negative real part and 335 negative real roots. The effect of the BCs is best observed by using the solver PDEPE [12] of Matlab® by arranging (1) in the form

$$\begin{aligned} C(x,t,u,\partial u/\partial x)\partial u/\partial t = \\ x^{-m}\partial/\partial x(x^m f(x,t,u,\partial u/\partial x)) + R(x,t,u,\partial u/\partial x) \end{aligned} \quad (44)$$

The PDEs hold for $t_0 \leq t \leq t_f$ and $0 \leq x \leq 1$, where x is the spatial variable, $m = 0$ corresponds to slab geometry of the $AChE$ membrane. $f(x,t,u,\partial u/\partial x)$ is the flux term of diffusion and $R(x,\ t,\ u,\ \partial u/\partial x)$ the reaction term. $C(x,t,u,\partial u/\partial x)$ is an identity matrix that restricts the coupling of the partial derivatives with respect to t. The model (1) is then rewritten in the form (44), i.e.,

$$\begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix} .* \frac{\partial}{\partial t}\begin{bmatrix} s \\ p \\ a \end{bmatrix} = \frac{\partial}{\partial x}\begin{bmatrix} \partial s/\partial x \\ \partial p/\partial x \\ \beta\partial a/\partial x \end{bmatrix} + \begin{bmatrix} -\sigma R(s,p,a) \\ \sigma R(s,p,a) \\ \sigma R(s,p,a) \end{bmatrix} \quad (45)$$

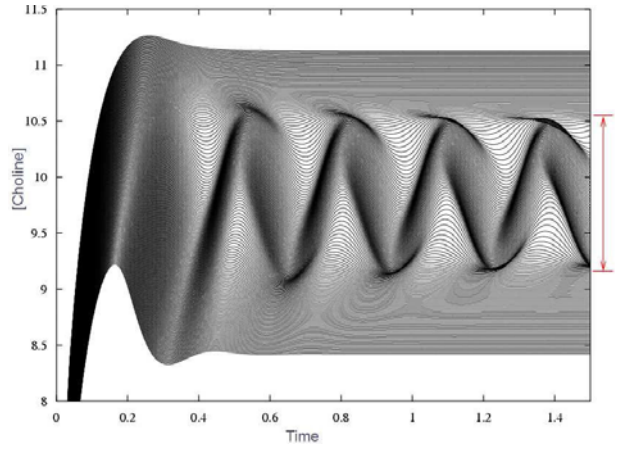

Fig. 4. Oscillations observed in computation of $[Ch]$. Mesh of 115 points.

where $D_s = 1$, $\alpha = 1$. The symbol ".$*$" represents element by element multiplication .

A. Diffusion Measurement and Reaction Rates

The estimation of the diffusion of molecules inside the membrane is determinant for understanding the dynamic properties behind an enzymatic process. Let us consider the relation between the flux versus spacial & temporal variables

$$u(x,t) = \partial/\partial x \underbrace{(D\partial u(x,\ t)/\partial x)}_{Flux(x,\ t,\ u,\ \partial u/\partial x)} \tag{46}$$

The number of particles crossing the space x per unit time t corresponds to the flux which is represented by the expression inside the parentheses in (46). This flux is calculated iteratively for each grid point representing the membrane surface. This may be useful for instance to compute the amount of ACh consumed during the formation of Ch and Ac. The molecular motion depends on the molecule size and polarity, temperature and more particularly as in our case study, the permeability and thickness of the membrane. As the numerical solution for both diffusion and reaction equations is not continuous in t and x, then the corresponding curves will not be continuous either. Consequently, due to the discretization process the solutions obtained iteratively are given by a set of points as for example, the diffusion-kinetic rate of ACh quantified by element $(1,1)$ of the vector from the expression inside the parentheses in (46). Such diffusibility corresponds to consumption of ACh in the formation of Ch and Ac. The quantification of diffusion rates can be computed also for products Ch and for Ac via elements $(2,1)$ and $(3,1)$ of that vector respectively. Numerical simulations were performed with parameter values from in vivo experimentation as presented in Table I.

TABLE I

$AChE$ CATALYZED REACTION - PARAMETER VALUES

$ca = 10$	$k^* = 0.01$	$ka = 631$	$cb = 0.01$
$k_p^* = 2.4$	$kb = 0.316$	$ck = 1$	$k_p^{'*} = 2.21$
$b = 225$	$cp = 0.5$	$kw = 6.32e-3$	$\beta = 5.76$

VII. CONCLUSIONS

The Results of this work represent a step forward on the hypothesis of the existence of "tunnels" for the transit of ACh through the membrane. By establishing a relation between the observed oscillations and the hysteresis phenomenon observed in [1], we concluded that under certain conditions, a wave of ACh could cross the membrane at higher velocity than in a simple diffusion. Indeed, in [13] it is showed that with a similar system (the immobilized papaine) a displacement front of pH could be obtained under particular conditions. This is in agreement with the results presented in [14] in which it was observed that a wave of substrate could propagate throughout a membrane.
Since diffusion and reaction exist in a metabolic system, we are extending the concept of compartimentation to the metabolism of lipid synthesis under study in our laboratory.

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