Proceedings of the 28th IEEE
EMBS Annual International Conference
New York City, USA, Aug 30-Sept 3, 2006

Modelling of Acetylcholinesterase immobilized into artificial membrane

J. Santos[1] , A. Friboulet[2], R. Lozano[1]

***Abstract*— A model is developed for the investigation of an enzymatic diffusion-reaction system. The aim is to analyze the dynamic behavior of three different species, the modification of their enzymatic kinetic properties and the existence of complex behaviors resulting of the catalytic activity induced by immobilization of Acetylcholinesterase into artificial membrane enzymatically inactive. We report results that make possible the characterization and prediction of complex behaviors as well as a qualitative/quantitative analysis of the system stability via bifurcation diagram which allows to study: i) the effect of the initial substrate concentration in the reservoir and ii) the effect of reaction-permeation modulus of the membrane as bifurcation parameters.**

I. Introduction

The necessity of understanding the behavior of biological systems requires more than ever considerable efforts of the scientific community. Aware of that, researchers of different specialities have formed workgroups in order to develop strategies to quantify changes at the molecular level. A complete understanding of the enzymes' nature requires the use of an interdisciplinary approach based on fundamental physical and chemical principles. In our research we take advantage of concepts of nonlinear dynamics and control for modelling and predicting the biochemical behavior of the Acetylcholinesterase ($AChE$) enzyme immobilized into an artificial membrane.

When the enzymes are in solution with the reactants and/or products it is difficult and expensive to recover them from reactor effluents at the end of the catalytic process. Therefore, if they can be physically attached to the reactor by artificial membranes, they can be re-used for many reaction cycles, lowering the total production cost. This process is called enzyme immobilization [1] [2] and it provides many other advantages, for instance the ability to stop the reaction rapidly by removing the enzyme from the reaction solution (or vice versa), and the fact that the product is not contaminated with the enzyme (especially useful in the food and pharmaceutical industries).

A. The Neurotransmitters

Every time we move a muscle or even when we are thinking, our nerve cells are in activity. They are processing information: receiving signals, deciding what to do with them and dispatching new messages off to their neighbors. Some nerve cells communicate directly with muscle cells, sending them the signal to contract. Other nerve cells are involved solely in the central administration of information, communicating only with other nerve cells.

The nerves communicate with one another and with muscle cells by using neurotransmitters. These are small molecules that are released from the nerve cell and rapidly diffuse to neighboring cells, stimulating a response once they arrive. In spite of many different neurotransmitters are used for different functions, in our research work we are particularly interested in Acetylcholine (ACh), a neurotransmitter considered important in the transmission of nerve impulses in the body. After stimulation, acetylcholine, $CH_3COOCH_2CH_2N^+(CH_3)_3$ degrades to acetate (Ac) and choline (Ch) which are absorbed back into the first neuron to form another ACh molecule [3]. The main function of ACh is to carry the signal from nerve cells to muscle cells. When a motor nerve cell gets the proper signal from the nervous system, it releases ACh into its synapses with muscle cells. There, the ACh opens receptors on the muscle cells, triggering the process of contraction. The decomposition of old ACh is carried out by $AChE$ enzyme.

B. The cholinesterase

The cholinesterase enzyme, also called acetylcholinesterase has drawn the attention of scientists as it could play a striking role in Alzheimer's disease, cardiac diseases and other diseases [4]. The $AChE$ is found in the synapse between nerve cells and muscle cells. It starts its action soon after a signal is passed, breaking down the ACh molecule into its two component parts, Ac and Ch.

Our study is focussed on the results of experimental works on immobilized $AChE$ membranes in [5] in which it was observed the evidence of memory and oscillations when the ACh was injected on one side of an artificial proteinic membrane bearing $AChE$. Also, a potential difference was recorded as a function of time. The authors observed that the steady-state potential due to the enzyme activity for increasing and decreasing substrate concentration exhibited a hysteresis loop, and that the nonlinearity of the enzyme reaction coupled with diffusion constraints caused some instabilities (oscillations of the potential membrane). These phenomena are the main motivation of our work in which the following questions are addressed: Why does immobilization of $AChE$ leads to a complex behavior ? How can we determine their causes ? Is it possible to predict these phenomena ? We can answer these questions if we show that the results of [5]

Supported by Programme Alban, the European Union Programme of High Level Scholarships for Latin America (E04D047796MX)

[1] Jorge dos Santos and Rogelio Lozano UMR-CNRS 6599, Université de Technologie (UTC) de Compiègne, France,

[2] Alain Friboulet UMR-CNRS 6022, UTC-France,

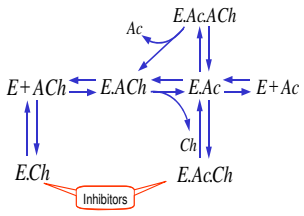

Fig. 1. kinetic mechanism for hydrolysis of ACh by $AChE$.

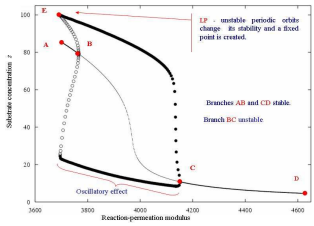

Fig. 2. HBs in points B and C

can be interpreted in terms of a coupling between the enzyme reaction and diffusion process. See figure 1]. In order to achieve this objective we develop in section II a model for the hydrolysis of ACh to obtain the reaction velocity of the enzymatic reaction. Then, in section III the reaction is coupled with the diffusion giving thus rise to a set of Ordinary Differential Equations (ODEs) that govern the enzymatic process. The main results are discussed in sections IV and V. Concluding remarks are given in section VI.

II. The rate equation for hydrolysis of ACh

In this section we present the model based on the scheme of hydrolysis regulation for $AChE$ depicted in Figure 1. The model takes into account the inhibition of the enzyme-catalyzed reaction by excess of substrate concentration because for a given enzyme concentration the initial reaction velocity increases with the initial substrate concentration to a limiting value V_m. The model also takes into account the inhibition by Ch (subsection $II-.1$), because the Ch in high concentrations becomes a competitive inhibitor of the $AChE$. Finally the model considers the effect of the pH (subsection II-.2), i.e., the changes in ionic charges of pH affect the activity, the structural stability and solubility of the enzyme [2]. The kinetic mechanism for hydrolysis of ACh by $AChE$ with the formation of an intermediary acetyl-enzyme complex and the reaction being inhibited by excess of substrate [6] is illustrated in Figure 1. The ACh molecule and the free E are combined in $(E + ACh)$ to form substrate-enzyme intermediary complex $(E.ACh)$ and the formation of an intermediary acetyl-enzyme complex $(E.Ac)$ with concomitant elimination of Ch. The inhibition by excess of substrate (ACh^{+}) is explained by the binding of an additional substrate molecule to the intermediary complex to form the dead-end ternary complex $(E.Ac.ACh)$. Also, high concentrations of Ch may appear during the reaction and exert a feedback control by inhibiting the free enzyme E and the acetyl-enzyme intermediary (EAc).

The compartment contains enzyme molecules that catalyze the transformation of the substrate whose rate expression is given by

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

where $V_m = \frac{[E]_t k_2 k_3}{(K_2+k_3)} = k_{cat}[E]_t$, $V_{SS} = \frac{[E]_t k_2 k_5}{(K_2+k_5)}$, $K_m = \frac{k_3(k_{-1}+k_2)}{k_1((k_2+k_3))}$ and $K_{ss} = \frac{(k_2+k_3)(k_4+k_5)}{k_4(k_2+k_5)}$

In (1), the $[S]$ denotes the substrate ACh. The k_{cat} is the catalytic rate constant, $[E]_t$ corresponds to the total enzyme concentration. V_m denotes the maximum velocity of the enzyme reaction, K_m is the Michaelis-Menten constant, V_{SS} is the maximum theoretical velocity in a given enzyme concentration, K_{SS} is the inhibition constant by substrate. k_1, k_{-1}, k_2, k_3, k_4 and k_5 are the kinetic constants that determine the reaction rates. $[E]_t$, V_m, V_{SS} and K_{SS} were obtained from the kinetic scheme for the reversible enzyme-catalyzed reaction in Figure 2. See [1] [7] and [8] for details.

*1) AChE **inhibition** by high concentration of Ch:* The fact that $AChE$ is inhibited by high concentrations of Ch is a property generally not considered when the hydrolytic reaction is studied in solution. However, in an immobilized enzymatic system high concentrations of product may appear during the reaction and exert a negative feedback control by inhibiting the enzyme. In this sense the equation (1) for hydrolysis of ACh becomes a function of the substrate and the product, and it may thus be rewritten as

$$R(S,P) = \frac{1 + a.k.s^2}{(1 + \frac{[P]}{K'_P} + \frac{K_m}{[S]})(1 + \frac{[P]}{K_P}) + \frac{[S]}{K_{ss}})} \qquad (2)$$

$[P]$ and $[S]$ indicate the concentration for product a (acetate) and substrate s respectively. By substituting $p = \frac{[P]}{K_m}$, $kp = \frac{K_P}{K_m}$ and $k'p = \frac{K_P}{K_m}$ into (2) we get the following non dimensional rate expression

$$R(s,p) = \frac{s + aks^2}{1 + \frac{p}{kp} + s(1 + \frac{p}{k'p} + ks)} \qquad (3)$$

After verify experimentally that the term corresponding to ternary complex $E.Ac.ACh$ does not affect the system's behavior we have decided to omit it. As a consequence the equation (3) can be rewritten as follows

$$R(s,p) = \frac{s}{1 + \frac{p}{kp} + s(1 + \frac{p}{k'p} + ks)} \qquad (4)$$

*2) Effect of pH **on the reaction**:* As mentioned above, the hydrolysis of ACh by $AChE$ depends on pH. This is an important feature which can not be neglected when dealing with the immobilized $AChE$. In fact the reaction produces Ac, which rapidly dissociates to give Ac plus

protons at pH values higher than 5. This local production of protons may thus be able to induce an important modification of pH in the vicinity of the enzymatic sites. The effect of pH on the hydrolytic reaction leads to rewrite (2) as follows

$$R(S,P,H) = \frac{[S]F(H)}{([S](1+\frac{[P]}{K_P^{'}}) + K_m(1+\frac{[P]}{K_P}) + \frac{[S]^2}{K_{ss}})} \tag{5}$$

where

$$F(H) = \frac{1}{1+\frac{[H^+]}{C_{H1}} + \frac{C_{H2}}{[H^+]}} \tag{6}$$

The H^+ depends on the pH value of the buffer solution, therefore $F(H)$ describes the pH-dependence of the catalytic constant. The expression $K_{ss} = K_{ss}^*(1+\frac{[H^+]}{C_{Hss}})$ describes the H^+ influence on the excess substrate inhibition. The equations $K_P = K_P^*(1+\frac{[H^+]}{C_{Hp}})$ and $K_P^{'} = K_P^{'*}(1+\frac{[H^+]}{C_{Hp}})$ describe the effect of pH on Ch.
In order to rewrite equation (5) in a dimensionless form we take K_m as the natural unit of concentration

$s = \frac{[S]}{K_m}$; $p = \frac{[P]}{K_m}$; $h = \frac{[H^+]}{K_m}$;
$k_P = \frac{K_P}{K_m}$; $k_P^{'} = \frac{K_P^{'}}{K_m}$; $k_i = \frac{K_m}{K_{ss}}$

which leads to the following expression

$$R(s,p,h) = \frac{sF(h)}{1+\frac{p}{K_P} + s(1+\frac{p}{K_P^{'}}) + k_i s^2)} \tag{7}$$

where $F(h)$ is similarly described in nondimensional form

$$F(h) = 1/(1+\frac{h}{C_{H1}} + \frac{C_{H2}}{h}), \tag{8}$$

with $k_i = \frac{k_i^*}{(1+\frac{h}{C_{Hss}})}$ which denotes the inhibition by ACh^+, $k_P = k_P^*(1+\frac{h}{C_{Hp}})$ and $k_P^{'} = k_P^{'*}(1+\frac{h}{C_{Hp}})$ correspond to the effect of pH on Ch, where $C_{H1} = \frac{C_{H1}}{K_m}$, $C_{H2} = \frac{C_{H2}}{K_m}$, $C_{Hss} = \frac{C_{Hss}}{K_m}$, $C_{Hp} = \frac{C_{Hp}}{K_m}$

In order to solve (8) we must calculate the local pH resulting from Ac dissociation and buffer solution effect. We assume that the different ionic equilibria, related to the electroneutrality are rapid when compared to the diffusion of hydronium ions. The local concentration of free H^+ results from the dissociation of Ac and buffer solution $\left(\frac{[B^-][Na^+]}{[BH]}\right)$ by taking the considerations **a)** and **b)** in the sequel.

*a) **Acid-base chemical equilibria**:* According to Bronsted-Lowry (BL) theory the equilibrium expression for the dissociation constant K_A of a weak acid HA is written as $HA \leftrightharpoons H^+ + A^-$ where H^+ is an acid and A^- is a base. For Ac dissociation K_A at a particular temperature is given by $K_A = \frac{[A^-][H^+]}{[AH^+]}$ where $[H^+]$ 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 $HB \leftrightharpoons H^+ + B^-$ where H^+ is an acid and B^- is the hydroxide concentration. For the buffer solution dissociation K_B is given by $K_B = \frac{[B^-][H^+]}{[BH^+]}$ where, $[B^-]$ is the concentration of conjugate acid, and $[BH^+]$ is the concentration of undissociated base. In pure water a special unitless equilibrium constant K_W can be defined as $K_W = [OH^-][H^+]$ where $[H^+]$ is the molar concentration of hydrogen and $[OH^-]$ is the molar concentration of hydroxide. By taking the principle of "Mass Conservation" the acid-base reaction can be written as $[AH]+[A] = [A]_T$, $[BH]+[B] = [B]_T$ with $x = \frac{[Na^+]}{[B]_T}$

*b) **Electroneutrality condition**:* We assume that the electroneutrality principle is verified, i.e., there exists a balance between cations (negative charge) and anions (positive charge). This implies the equality of the total positive ionic charges to the total negative ionic charges
$[H^+] + [Na^+] = [OH^-] + [B^-] + [A^-]$
From conditions **a)** and **b)** we get the following expression

$$[A]_T = \left([H^+] + x\,[B]_T - \frac{K_B[B]_T}{K_B+[H^+]} - \frac{K_W}{[H^+]}\right)\left(\frac{K_A+[H^+]}{K_A}\right) \tag{9}$$

By considering that in experimental conditions $[H^+] << x\,[B]_T$ and $\frac{K_W}{[H^+]} << \frac{K_B[B]_T}{(K_B+[H^+])}$, (9) may be simplified to

$$[A]_T = \left(1+\frac{[H^+]}{K_A}\right)\left(x\,[B]_T - \frac{K_B\,[B]_T}{(K_B+[H^+]}\right). \tag{10}$$

(10) can be rewritten in its equivalent dimensionless form as

$$a = \left(1+\frac{h}{k_a}\right)\left(xb + \frac{bk_b}{(h+k_b)}\right) \tag{11}$$

with $a = \frac{[A]_T}{K_M}$, $b = \frac{[B]_T}{K_M}$, $k_w = \frac{K_W}{K_{M^2}}$, $k_a = \frac{K_A}{K_M}$, $k_b = \frac{K_B}{K_M}$
From (11) the h value is computed as the positive root of the following quadratic equation

$$h^2(\frac{bx}{k_a}) + h(bx + b(x-1)\frac{k_b}{k_a} - a) + bk_b(x-1) - ak_b = 0 \tag{12}$$

III. Modelling the enzymatic diffusion-reaction

It is generally possible to explain qualitatively the experimental results of diffusion-reaction (RD) systems as two phenomena separately. We assume that the enzyme is perfectly agitated and homogeneous into the reactor separated of the reservoir by a passive membrane enzymatically inactive, through which the ACh diffusion occurs. This characterizes a zero dimensional system whose main particularity is that RD occur into the membrane and compartment respectively.

The reservoir contains the substrate at a fixed concentration S_0. The product concentrations are kept constants and equal to zero into the reservoir during all the time. The buffer solution is homogeneously distributed in the reservoir, in the membrane and the compartment reactor. S is the substrate concentration as a function of time in the compartment reactor. The membrane transports ACh from the reservoir to the compartment reactor with rate $D_s\frac{A}{L}(S_0 - S)$, where D_s is the diffusion coefficient for

the substrate S, A is the contact surface area, and L is the thickness of a passive membrane that separates the two compartments. The mass balance for S in the reactor is therefore expressed as

$$V_r \frac{dS}{dt} = (D_s \frac{A}{L})S_0 - S - VrR(S,P,H) \qquad (13)$$

where V_r is the reactor volume and $R(S,P,H)$ is the reaction term given by (5).
Let P be the mass transfer coefficient given by

$$P = D_s \frac{A}{(LV_r)} = \left(\frac{V_m}{V_r}\right)\frac{D_s}{L^2} \qquad (14)$$

where V_m is the membrane volume.
Substituting (14) into (13) leads to

$$\frac{dS}{dt} = P\,(S_0 - S) - R(S,P,H) \qquad (15)$$

Let $\theta = \frac{1}{P}$ be the characteristic time of the system (the inverse of the mass transfer coefficient). By taking θ and K_S as natural units of time concentration we obtain (15) in the dimensionless form

$$\frac{ds}{d\tau} = s_0 - s - \rho R(s,p,h) \qquad (16)$$

where $s = \frac{S_0}{K_S}$; $\tau = \frac{t}{\theta}$; $s_0 = \frac{S_0}{K_S}$.
ρ in (16) is given by $\rho = (\frac{V_m}{K_{ss}})\theta$ and represents the reaction-permeation modulus with $\theta = \left(\frac{V_r}{V_m}\right)\frac{L^2}{D_s}$. The dimensionless rate expression is denoted by $R(s,p,h)$ as described (7). By applying the same procedure used to determine (16) we can obtain the equations for the products Ch and Ac and construct the set of ODEs that governs the dynamic system as

$$\begin{array}{rcl} \frac{ds}{d\tau} & = & s_0 - s - \rho R(s,p,h) \\ \frac{dp}{d\tau} & = & \alpha(p_0 - p) + \rho R(s,p,h) \\ \frac{da}{d\tau} & = & \beta(a_0 - a) + \rho R(s,p,h) \end{array} \qquad (17)$$

with $\alpha = \frac{D_p}{D_s}$, $\beta = \frac{D_a}{D_s}$, where D_p and D_a are the diffusion coefficients to the Ch and Ac respectively.
As the product concentrations are kept equal to zero into the reservoir during all the time, it implies that $p_0 = 0$, $a_0 = 0$. By taking the diffusion coefficient $\alpha = 1$ to the Ch the system (17) can be rewritten as

$$\begin{array}{rcl} \frac{ds}{dt} & = & s_0 - s - \rho R(s,p,h) \\ \frac{dp}{dt} & = & -p + \rho R(s,p,h) \\ \frac{da}{dt} & = & -\beta a + \rho R(s,p,h) \end{array} \qquad (18)$$

where $s(t), p(t)$ and $a(t)$ correspond to the state variables: substrate, Choline and Acetate respectively.

IV. The role of the reaction-permeation modulus in the formation of choline

The model (18) represents the mathematical description of a coupling between the enzyme diffusion and reaction and it is the first step to help answering the questions addressed in section I. Our objective in this section and the next is therefore to verify if with (18) we can approximate the qualitative results experimentally observed in [5]. By paying close attention to (18) we can conclude that only two parameters could eventually cause the system to present behaviors not comprehensible at a glance. As s, p and a are the state variables, and β is a constant parameter, there is no doubt that the parameters s_0 and ρ, the ones that can be externally manipulated, correspond therefore to the most probable sources of the complex behavior. This conclusion leads us to analyze how the system's dynamics responds to external variation on the parameters s_0 and ρ. We proceed then to obtaining bifurcation diagrams [9] to get an overview of the qualitative behavior as well as quantitative results for assessing the sensitivity and robustness of the model to variation in the parameters s_0 and ρ. To plot the bifurcation diagrams was used XPPAUT [10]. The results are presented first with s_0 held fixed while ρ is allowed to change, see Figure 2. Then we change s_0 while ρ is held fixed, see section V. The parameter values were obtained from *in vivo* experimentation and are given in Table I [11]. The integration time was equal to 20 with $\Delta t = 0.0002$, besides, the error tolerance and absolute tolerance were both chosen equal to $1e-8$. The initial conditions for $s(t)$, $p(t)$ and $a(t)$ were defined equal to zero.

TABLE I
$AChE$ catalyzed reaction - parameter values

$ca = 10$	$k^* = 0.01$	$ka = 631$
$cb = 0.01$	$k_p^* = 2.4$	$kb = 0.316$
$ck = 1$	$k_p^{'*} = 2.21$	$b = 225$
$cp = 0.5$	$kw = 6.32e-3$	$x = 0.24$
$\beta = 5.76$	$\rho = 4000$	$s_0 = 300$

Figure 3 shows three regions (AB, BC and CD) in the bifurcation diagram, each one corresponding to a different form of qualitative behavior. Two Hopf bifurcations (HBs) are represented, one in B, ($\rho = 3766$) and the other in C, ($\rho = 4153$). In B the equilibrium point loses stability and a stable fixed point becomes unstable to form a stable limit cycle. A pair of complex conjugate eigenvalues touch the imaginary axis of the complex plane from the left half-plane to the right half-plane, causing the system to undergo a bifurcation, as consequence, oscillations appear between A and B. Note in the bifurcation how the trajectories leave a small neighborhood of the unstable $HB1$ and tend to a small stable limit cycle (filled circles) that emerges from B. The periodic solutions exist between points B and C for $3766 < \rho < 4153$. In this range of ρ values the equilibrium points are not attractors anymore but repellents, which implies that the limit cycle is the only attractor. Notice that after the branch BC the periodic orbits cease to exist and the system recovers its stability in branch CD. The biochemical interpretation is that these phenomena occur as consequence of the competition among the molecules in the coupled diffusion-reaction throughout the artificial enzyme membrane. In fact, in [1] the author highlights that just a single reaction coupled with diffusion is sufficient for the existence of oscillations.

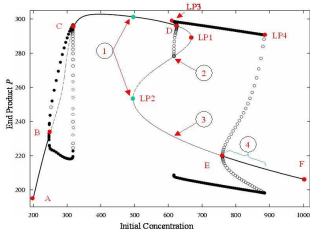

Fig. 3. HBs in B, C, D and E. The bistability associated to hysteresis is indicated by number 1.

V. Impact of variation in substrate initial concentration

The initial substrate concentration s_0 in the reservoir is the second parameter which affects the model's qualitative behavior. In Figure 3 the system starts up with $s_0 = 200$ in stable steady state (branch AB). As s_0 is increased, a HB arises at point B with $s_0 = 625.5$ followed by the birth of periodic orbits around it. As a consequence there is a subsequent loss of stability and generation of oscillations for all solutions in BC (thin dashed lines). A second HB represented by C with $s_0 = 316.8$ leads the final product to a new stable zone (CD) into which all eigenvalues have negative real parts. As s_0 becomes bigger and reaches $s_0 = 625.5$ in D, a third HB appears and changes the system's behavior on branch DE. Note a small unstable periodic orbit around D causing loss of stability for all solutions between D and the virtual point indicated by number 2. By paying close attention to the equilibrium points in branch CE we recognize a bistability phenomenon indicated by number 1, which is important in regulation of biological networks. Such a phenomenon is due to the non-linearities in the reaction rate. Note also that for $s_0 \approx 495.6$ there are two stable stationary states (green points) indicated by number 1. In this case s_0 is a stable fixed point in both branches CD and DE. It is easy to verify in CE the presence of three stationary states, two are stable (those indicated by 1) and the third one is unstable (indicated by $LP1$). This verification confirms the hysteresis phenomenon experimental observed in [5] in which the $AChE$ membrane exhibits an auto-catalytic effect with the enzymatic production of H^+. Depending on the number of steps taken along the branch CE, other points of bistability as well as multistability may be observed. It means that for a given set of s_0 values the system is neither stable nor totally unstable, but alternates between two or more states in response to a single s_0 value. The consequences of several attractors in this enzymatic reaction implies a system extremely sensitive to perturbations due to its complex interactions and the presence of several saddle-node bifurcation for fixed points (indicated by $LP1$, $LP1$, $LP1$and $LP1$). From the imaginary point represented by number 3 up to E the system reenters to the limit cycle and there is no possibility of bistability anymore. Finally, a fourth HB was found in E with $s_0 = 760.5$. Note that a periodic orbit emanates from E. The system is stable in branch EF. The small branch in green color by 4 indicates that the stable steady state presents yet oscillatory effect, but disappears gradually from $s_0 \approx 885.3$ on.

VI. Conclusions

We have addressed the problem of modelling three different species and their in kinetic properties induced by immobilization of the $AChE$ membrane. The mathematical description of the system allowed the prediction of its behavior for a particular operation range and specific initial conditions. Based on the results we conclude that the proposed model was shown to be a satisfactory representation of the $AChE$ immobilized into the artificial membrane since it allowed to characterize the phenomena experimentally observed [5] and showed that those phenomena can be interpreted in terms of a coupling enzyme diffusion-reaction.

The Hopf-bifurcations in both figures 2 and 3 allow to determine and predict the zone of sustained oscillations during the enzymatic reaction under particular values of initial conditions. This could be useful for instance as auxiliary information for determining the initial concentration in reservoir so that the products of the reaction can be formed inside or outside the instable zone.

This work may stimulate new research directions on cholinergic agents, since it is known that after the ACh neurotransmitter interacts with the cholinergic receptor, it is hydrolyzed very rapidly by the $AChE$ enzyme. On the other hand, the proposed approach could be useful to describe small subsystems that together perform a particular task in larger structured biochemical networks.

References

[1] J. P Kernevez, *Enzyme Mathematics*, *North-Holland, New York*, 1980.

[2] P. Trevor, Enzymes: Biochemistry, Biotechnology and Clinical Chemistry. Horwood Series in Chemical Science, 2004.

[3] B. Alberts, A. J. Johnson, M. Lewis, K. Raff Roberts and P. Walter. Molecular Biology of the Cell. New York: Garland Publishers, 2002

[4] M. Parveen, S. Kumar, *Recent Trends in the Acetylcholinesterase System*, IOS Press,US, 2005.

[5] Friboulet A. and Thomas D., Electrical excitability of artificial enzyme membranes. *Biophysical Cheministry*, vol 16, pp 153-157, 1982.

[6] Krupka R.M. & Laidler K.J. Molecular mechanisms for hydrolytic enzyme action. I. Apparent non-competitive inhibition, with special reference to acetylcholinesterase, J. Am. Chem. Soc., pp.1448–1454, 1983.

[7] R. S. Arthur, Enzyme.Kinetics - From Diastase to Multi-enzyme Systems, Cambridge University Press 1995.

[8] C. Gilles, A. Friboulet and D. Thomas, Horse serum butyryl-cholinesterase kinetics: a molecular mechanism based on inhibition studies with dansylaminothyltrimethylamminium, Biochm. Cell Biol., vol. 65, pp. 529-535, 1987.

[9] J.S. Ferreira, R. Lozano, M. Sabine and A. Friboulet, Bifurcation analysis of a biochemical network. Proceedings of the Second Multidisciplinary Int. Symposium on Positive Systems: Theory and Applications, august 30-September 1, Grenoble - France, 2006.

[10] B. Ermentrout, *Dynamics Simulating, Analyzing, and Animating Dynamical Systems: A Guide to Xppaut for Researchers and Students - Software, Environments, Tools*. Paperback, 2002.

[11] Friboulet A, *Propriétés structurales et dynamiques d'une enzyme: l'acétylcholinestérase, PHD thesis, Université de Technologie de Compiègne. Compiègne-France*, 1989.