NUMERICAL ANALYSIS OF ARTIFICIAL ENZYME MEMBRANE -
HYSTERESIS, OSCILLATIONS AND SPONTANEOUS STRUCTURATION

J.P. KERNEVEZ

Département de Mathématiques Appliquées
Université de Technologie de Compiègne
60206 - COMPIEGNE - FRANCE

D. THOMAS

E.R.A. n° 338 du C.N.R.S.
Département de Génie Biologique
Université de Technologie de Compiègne
60206 - COMPIEGNE - FRANCE

1 - INTRODUCTION

Over the past few years there has been an upsurge of interest in partial differential equations with multiple solutions, prompted largely by their probable biological importance, and reflected by a growing number of papers (1). The systems resulting from the coupling between enzyme reactions and diffusion constraints can be ruled by this kind of partial differential equations (2). The binding of enzymes into artificial membranes makes possible the study of the interaction between diffusion and enzyme reaction within a well-defined context (3). This paper deals with the numerical analysis of three experimental systems very recently produced: an hysteresis phenomenon observed by Naparstek et al (4) ; an oscillation phenomenon studied by Naparstek et al (5) and Caplan et al (6) ; an asymmetrical structuration shown by Thomas et al (7).

Among the various mechanisms describing information storage in the phase of "short term memory", the most favoured one is based on an all or none process, due to phase transition in an excitable membrane, accompanied with metastable states (8,9). These phase transitions require structural changes at the molecular level, in membrane permeability, or in high molecular weight molecules involved in the process (10).

On the other hand chemical engineers studied intensively cooperative effects, due to non-linear temperature dependance of rate coefficients, and their relation to the stability of chemical reactors. In these works, hysteresis and oscillations resulting, are discussed (11). At the biological point of view, sustained oscillations have been

experimentally observed and established beyond doubt for glycolysis by Hess (12) and Betz and Chance (13). Sel'Kov (14) and Higgins (15) have worked out models to represent the observed oscillations. Recently Goldbeter and Lefever (16) have constructed a model explicitly taking into account the allosteric effects which is free from phenomenological factors. According to Prigogine and Nicolis (17) these systems are also likely to occur, under different experimental conditions, in spatially ordered states (18). When associated with diffusion constraints the non-linear enzyme reaction can produce a pattern or structure due to an instability of an homogeneous state.

The experimental systems will be described briefly in order to introduce the equations ruling them. It is possible to get more details from the original papers cited above (4,5,6,7). The existence of one or several solutions will be demonstrated for both transient and steady states. The numerical methods used to solve the described equations will be given together with the numerical results.

2 - STATEMENT OF THE PROBLEMS AND EQUATIONS RULING THE SYSTEMS

2.1. - Hysteresis phenomenon

An enzyme giving an inhibition by excess of substrate is homogeneously distributed inside an artificial membrane (thickness L). The membrane is immersed in a solution of substrate S . The concentration of S is changing slowly enough to enable the quasi steady state hypothesis about the enzyme reaction and the metabolite diffusion which occur inside the membrane. The reference axis is chosen perpendicular to the membrane. S concentration is a function of space and time (x and t).

$$(2.1) \quad -D_S \frac{\partial^2 [S]}{\partial x^2} + V_M \frac{[S]}{K_M + [S] + \frac{[S]^2}{K_{SS}}} = 0$$

with D_S substrate diffusion coefficient, K_M Michaelis constant and V_M maximum activity by volume unit of membrane.

Let us replace $\frac{x}{L}$ by x and $\frac{[S]}{K_M}$ by s . x and s are now dimensionless.

Equation (2.1) can be written like

$$(2.2) \quad -\frac{\partial^2 s}{\partial x^2} + \sigma \frac{s}{1+s+as^2} = 0$$

with

$$\sigma = \frac{V_M}{K_M} \frac{L^2}{D} \quad \text{and} \quad a = \frac{K_M}{K_{SS}}.$$

Obviously, σ and a are a perfect characterization of the membrane system.

The boundary conditions are:

$$(2.3) \quad s(0,t) = s(l,t) = \alpha(t) \quad (\alpha(t) \geq 0)$$

2.2. - Oscillation phenomenon

The system was experimentally built and studied in Harvard by Naparstek et al (4) and a numerical analysis was given by Caplan et al (5). A coating (thickness L) bearing an enzyme activity is along of glass electrode. One side of the coating is impermeable to any molecule. In this system a substrate S (of concentration $[S] = s$) is transformed into the product H^+ (of concentration $[H^+] = p$), and due to the reaction between H^+ and OH^- a function $a(x,t)$ is introduced in the equations ruling the system:

$$(2.4) \quad \frac{\partial s}{\partial t} - D_S \frac{\partial^2 s}{\partial x^2} + F(s,p) = 0 \quad , \quad \frac{\partial a}{\partial t} - D_H \frac{\partial^2 a}{\partial x^2} - F(s,p) = 0$$

With

$$a = p - \frac{10^{-20}}{p} \quad , \quad F(s,p) = Q_2 Q_3 \frac{E_0}{Q_2 + Q_3 + Q_3 \frac{5.45 \cdot 10^{-5}}{s}}$$

$$Q_2 = \frac{64.5}{1 + 10^{7.29} p + \frac{10^{-11.49}}{p}} \quad , \quad Q_3 = \frac{20.2}{1 + 10^{6.92} p}$$

$$D_S = 0.13 \cdot 10^{-5} \quad , \quad D_H = 0.64 \cdot 10^{-5} \quad , \quad L = 10^{-2}$$

The boundary and initial conditions are:

$$(2.5) \quad s(0,t) = 5.5 \cdot 10^{-7} \quad ; \quad p(0,t) = 10^{-13} \quad ; \quad \frac{\partial s}{\partial x}(L,t) = \frac{\partial p}{\partial x}(L,t) = 0.$$

$$(2.6) \quad s(x,0) = 5.5 \cdot 10^{-7} \quad ; \quad p(x,0) = 10^{-13}.$$

2.3. - Asymmetrical structuration

When pH is taken into account, it is necessary to replace V_M by αV_M , where α is a function of pH ($0 \leq \alpha \leq 1$).

Two enzymes E_1 and E_2 are homogeneously immobilized. Reaction E_i is

With an excess of substrates reaction rates are:

$$(2.7) \quad v_i = \alpha_i \sigma_i \quad i = 1, 2$$

σ_i being a constant and α_i being a function of pH as indicated in fig. 1.

Figure 1. Graphs of α_1 (...), α_2 (---) and $\alpha_2 - \alpha_1$ (—).

Enzyme E_1 (resp. E_2) has a range of activity at low pH (resp. high pH). It is a catalyst of the transformation of the substrate S_1 (resp. S_2) into the product P_1 (resp. P_2). This product is an acid (resp. a base) so that when it appears the pH is decreasing (resp. increasing). E_i activity is maximum at $\text{pH} = a_i$ ($i = 1, 2$).

When the two enzymes are homogeneously immobilized inside a membrane, there is no variation of pH for a pH value a_0 .

With a pH value of $a_0 + \varepsilon$ in the first compartment and $a_0 - \varepsilon$ in the second one there will be amplification of the concentration difference because on both sides there

will be an autocatalytic reaction.

Product concentrations are ruled by

$$(2.8) \quad \frac{\partial p_i}{\partial t} - \frac{\partial^2 p_i}{\partial x^2} - \alpha_i \sigma_i = 0 \quad i = 1, 2$$

with $\sigma_1 = \sigma_2 = \sigma$. pH is linked to p_1 and p_2 by

$$(2.9) \quad \text{pH} = (\text{pH})_0 + \beta(p_2 - p_1).$$

It is possible to write

$$(2.10) \quad \frac{\partial \text{pH}}{\partial t} - \frac{\partial^2 \text{pH}}{\partial x^2} = \beta \sigma (\alpha_2 - \alpha_1).$$

The function $(\alpha_2 - \alpha_1)$ is described by curve in Fig. 1.

The boundary and initial conditions are:

$$(2.11) \quad \text{pH} = a_0 - \varepsilon \text{ at } x = 0 \quad \text{and} \quad \text{pH} = a_0 + \varepsilon \text{ at } x = 1$$

$$(2.12) \quad \text{For } t = 0 \text{ the pH through all the system is equal to } a_0.$$

3 - MATHEMATICAL SECTION

3.1. - Hysteresis phenomenon

Using monotone methods as indicated in Sattinger (19) it is easy to check that

$$(3.1) \quad \begin{cases} -\Delta s + \sigma F(s) = 0 \\ s|_{\Gamma} = 0 \end{cases}$$

has at least one solution.

(Here the equation is written in a domain Ω , open and bounded in $\mathbb{R}^n$ ($n=1,2$ or 3) of boundary Γ , $-\Delta = -\sum_{i=1}^n \frac{\partial^2}{\partial x_i^2}$ and $F(s) = s / (1+s+a|s|^2)$).

In fact let $u_0 =$ (resp. $v_0 = 0$) is an upper (resp. lower) solution of (3.1):

$$(3.2) \quad \begin{cases} -\Delta u_0 + \sigma F(u_0) \geq 0 & , & u_0|_{\Gamma} = \alpha \\ -\Delta v_0 + \sigma F(v_0) = 0 & , & v_0|_{\Gamma} \leq \alpha \end{cases}$$

Then we have the following results from Sattinger (19):

There exists a regular solution s of (3.1) such that $v_0 \leq s \leq u_0$.

Now we can prove that there exists values of σ and α for which (3.1) has at least 3 solutions.

Multiple solutions (one dimensional case)

The system (3.1) is now

$$(3.3) \quad \begin{cases} -s'' + \sigma F(s) = 0 \\ s(0) = s(1) = \alpha \end{cases}$$

and we have a first integral

$$(3.4) \quad -s'(x)^2 + \sigma G(s(x)) = \sigma G(\mu)$$

Where G is some primitive of F and $\mu = s(\frac{1}{2})$ the minimum of $s(x)$ for $x \in [0, 1]$.

$$(3.5) \quad s'(x) = +\sigma^{1/2} (G(s(x)) - G(\mu))^{1/2} \quad 1/2 \leq x \leq 1$$

$$(3.6) \quad \sigma^{1/2}(x - 1/2) = \int_{\mu}^{s(x)} \frac{d\xi}{(G(\xi) - G(\mu))^{1/2}} \quad 1/2 \leq x \leq 1$$

and we have the constraint

$$(3.7) \quad \frac{1}{2} \sigma^{1/2} = \int_{\mu}^{\alpha} \frac{d\xi}{(G(\xi) - G(\mu))^{1/2}}$$

(3.6) will define a solution of (3.3) every time

(3.7) will have a solution μ .

We define f by

$$(3.8) \quad f(\mu) = \int_{\mu}^{\alpha} \frac{d\xi}{(G(\xi) - G(\mu))^{1/2}}$$

Then

$$(3.9) \quad f'(\mu) = -\frac{1}{(G(\xi) - G(\mu))^{1/2}} + \left(-\frac{1}{2}\right) \int_{\mu}^{\alpha} \frac{F(\xi) - F(\mu)}{(G(\xi) - G(\mu))^{3/2}} d\xi$$

our purpose is to show that we can find α and σ such that (3.7) admits 3 different solutions at least.

Let us choose μ^* such that $F'(\mu^*) < 0$ ($\mu^* > 0$).

It is easy to see that when $\alpha \rightarrow +\infty$ the first term in $f'(\mu^*)$ tends towards 0 and the second one towards a limit $\ell > 0$ ($\ell < +\infty$).

Taking α sufficiently large we have $f'(\mu^*) > 0$. With this value of α we have necessarily the graph of f like (c) in Fig. 2.

Figure 2. Graphs of f for different values of α .

(In particular because for every σ equation (3.7) has at least one solution).

Now we see on Fig. 2 that according to the value of σ equation (3.7) has 1, 2 or 3 solutions.

Let us take such a value of σ for which (3.7) can have 3 solutions, and assume that α is varying continuously, first increasing from α_1 to α_6 , then decreasing to α_1 . If we follow the corresponding values of μ , we pass successively by the points 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and there are 2 jumps: first, when we pass through α_4 , the point jumps from 4 to 5. Then, when we pass through α_2 , the point jumps from 9 to 10.

This corresponds to 2 kinds of profiles of concentration:

- The "low" profiles, for $\alpha_1 < \alpha < \alpha_4$ in the increasing phase and $\alpha_1 < \alpha < \alpha_2$ in the decreasing phase, and
- The "high" profiles, for $\alpha_4 < \alpha$ in the increasing phase and $\alpha > \alpha_2$ in the decreasing phase. (Fig. 3).

Figure 3. Hysteresis phenomenon: calculated substrate concentration profiles inside an artificial enzyme membrane. These profiles are given for several concentration values outside. The concentration unit in each case is the concentration in the external medium.

3.2. - Oscillation phenomenon

Existence of a solution for equations (2.4) - (2.6) can be proved by standard methods (LIONS (10)): construction of a sequence of Faedo Galerkin approximations, a priori estimates on these approximations, a priori estimates on the time fractionnary derivatives using Fourier transform with respect to time, extraction of a subsequence converging towards a solution of (2.4) - (2.6). The property used in the proof is mainly the boundedness of the function F . In the proof of unicity is used the fact that F is Lipschitz continuous.

3.3. - Spontaneous asymmetrical structuration

$$(3.10) \quad \begin{cases} \frac{\partial u}{\partial t} - \Delta u + F(u) = 0 \\ u|_{\Gamma} = g(x) \\ u|_{t=0} = u_0(x) \end{cases}$$

F being the full line curve of figure 1. $u_0(x) = a_4$ (resp. $v_0(x) = a_3$) is an upper (resp. lower) solution of

$$(3.11) \quad \begin{cases} -\Delta u + F(u) = 0 \\ u|_{\Gamma} = g(x) \end{cases}$$

provided $a_3 \leq g(x) \leq a_4$.

The same arguments than in § 3.1 insure that (3.11) has at least one solution. Moreover for any continuous $u_0(x)$ with $a_3 \leq u_0(x) \leq a_4$, we obtain a global regular solution of the initial value problem (3.10), this solution is unique and satisfies $a_3 \leq u(x,t) \leq a_4$.

4 - NUMERICAL METHODS AND RESULTS

4.1. - Hysteresis phenomenon

For a given boundary value α (2.2) was solved by the following scheme (s_j^k is the approximation of $s(j \Delta x)$ at iteration number k and $J \Delta x = 1$):

- i/ Define $s_j^0 = 0$ or according to the solution expected ("low" or "high" profile).
- ii/ Define s^{k+1} from s^k by

$$(4.1) \quad \begin{cases} -\frac{1}{h^2} (s_{j+1}^{k+1} + s_{j-1}^{k+1} - 2s_j^{k+1}) + \sigma s_j^{k+1} / (1 + s_j^k + a(s_j^k)^2) = 0 \\ s_o^{k+1} = s_J^{k+1} = \alpha \end{cases}$$

$$\text{iii/ Stop when } \frac{\sum_{j=0}^J |s_j^{k+1} - s_j^k|}{\sum_{j=0}^J |s_j^{j+1}|} < \varepsilon$$

To solve (3.3), where the boundary value $\alpha(t)$ varies continuously with time the initial approximation (s_j^0) for the solution when $\alpha = \alpha(t)$ was the last approximation s_j^k for the preceding value of α .

The flux of substrate entering the membrane under quasi-stationary state conditions was calculated as a function of the external substrate concentration for increasing and decreasing values (Figure 4).

Figure 4. Hysteresis phenomenon: boundary flux as a function of substrate concentration at the boundaries for 3 σ values = 800, 1000 and 1200.

The system parameters were:

$$\begin{aligned} V_M &= 4 \cdot 10^{-8} \text{ mole cm}^{-3} \text{ sec}^{-1} & D &= 2.8 \cdot 10^{-8} \text{ cm}^2 \text{ sec}^{-1} \\ K_M &= 4 \cdot 10^{-8} \text{ mole cm}^{-3} & e &= 4.5 \cdot 10^{-3} \text{ cm} \\ K_{SS} &= 4 \cdot 10^{-7} . \end{aligned}$$

On figure 4 there is an evidence for an hysteresis phenomenon, the activity-substrate concentration relationship does not give an unique curve. For increasing substrate concentration values after a critical point there is a strong drop of the enzyme activity. For decreasing values a similar effect is observed. The low and high activity values correspond respectively to high and low concentration profiles of substrate inside the membrane (Fig. 4). Two families of curves are given on Fig. 3, showing the strong variation of concentration level for each critical point. The numerical results are similar to the experimental results presented by Naparstek et al (4).

4.2. - Oscillation phenomenon

The system described by the equations (2.4) to (2.6) was solved numerically by Caplan et al (6). A periodic behavior for the system was shown and a sharp front of substrate concentration in the active layer oscillates between the boundaries ruled respectively by Dirichlet and Neumann conditions.

There is a qualitative agreement between the simulation of Caplan et al (6) and the experimental results of Naparstek et al (7).

4.3. - Spontaneous asymmetrical structuration

The system previously described by equations (2.10) to (2.12), was solved numerically by using the explicit method. At time zero the pH is equal to a_0 through all the membrane. Asymmetrical perturbations were introduced on both boundary values and pH profile in the membrane evolved as shown on Fig. 5. There is an evidence for a spontaneous structuration of the concentration from an homogeneous repartition. This phenomenon is due to the instability of the homogeneous state through the non-linearity and the autocatalysis of the enzyme reactions.

Figure 5. pH profile inside an artificial bienzyme membrane. These profiles are given for different time values.

CONCLUSION

It is interesting at both physical and biological points of view to study instabilities and possibilities of multiple solutions in systems ruled by partial differential equations. In this way, artificial enzyme membranes or at least artificial immobilization of the enzymes could be a mean to study this kind of phenomena, because enzyme kinetics are frequently non-linear or autocatalytic and the systems are ruled by diffusion-reaction coupling, that is to say by partial differential equations.

Moreover it is easily possible to change the boundary conditions, so critical for these kinds of systems.

For artificial enzyme membranes, due to the well-defined context it is possible to write in a simple way equations ruling the systems and to compare calculated and experimental results. This work is also of stage between the classical enzymology in solution and the study of properties of enzymes in very complex distributed biological systems.

REFERENCES

- (1) Lecture Notes in Mathematics n° 332, Non Linear Problems in the Physical Sciences and Biology, Springer Verlag 1973.
- (2) PRIGOGINE, I (1969). "Structure, Dissipation and Life". In Theoretical Physics and Biology: proceedings, et M. Marois (Amsterdam - North-Holland Co.) p.p. 23-52.
- (3) THOMAS, D. and CAPLAN, S.R., in "Membrane Separation Processes", Ed. Meares P. Elsevier Publishing Co. Amsterdam (1974).
- (4) NAPARSTEK, A., ROMETTE, J.L., KERNEVEZ, J.P. and THOMAS, D. Nature (1974), 249 , 490
- (5) NAPARSTEK, A., THOMAS, D. and CAPLAN, S.R., Biophys. Biochem. acta. (1973), 323 , 643.
- (6) CAPLAN, S.R., NAPARSTEK, A. and ZABUSKY, N., (1973), Nature, 245 , 364.
- (7) THOMAS, D., GOLDBETER, A. and LEFEVER, R., in press
- (8) CHANGEUX, J.P., and THIERY, J., in: "Regulatory Function of Biological Membranes" Vol. 11, Ed. Jänefelt, J., Elsevier Publishing Co. (1968).
- (9) KATCHALSKY, A. and OPLATKA, A. (1966), Neurosciences Res. Symp. 1 352 (1966)
- (10) KATCHALSKY, A. and SPANGLER, R., Quartely Revs. Biophys., 1 , 127 (1968).
- (11) DENBIGH, K.G. and TURNER, J.C.R. in "Chemical Reactor Theory", University Press Cambridge (1971).
- (12) HESS, B. (1962) in "Funktionelle und morphologische Organisation der Zelle". Springer Verlag, Berlin.
- (13) BETZ, A. and CHANCE, B. (1965). Phase relationship of glycolytic intermediates in yeast cells with oscillatory metabolic control. Archs. Biochem. Biophys., 109 , 585.
- (14) SEL'KOV, E.E. (1968), Self-oscillations in glycolysis. Euro. J. Biochem. 4 , 79.
- (15) HIGGINS, J. (1964), A chemical mechanism for oscillation of glycolysis intermediates in yeast cells. Proc. Nat. Acad. Sci. U.S.A. 51 , 989.
- (16) GOLDBETER, A. and LEFEVER, R. (1972). Dissipative structures for an allosteric model. Application to glycolytic oscillations. Biophys. J. 12 , 302.
- (17) PRIGOGINE, I. and NICOLIS, G. (1971). Biological order, structure and instabilities. Quarterly Reviews of Biophysics, 4 , 107.
- (18) ZHABOTINSKI, A.M. (1964), Biofizika, 2 , 306.
- (19) SATTINGER, D.H. Monotone Methods in Non-linear Elliptic and Parabolic Boundary Value Problems. Indiana Univ. Math. Journal, 21 , 979 - 1000 (1972).
- (20) LIONS, J.L. Quelques Méthodes de Résolution des Problèmes aux limites non linéaires, Dumod, Gauthier Villars, Paris, 1969. (English translation by Le Van, Holt, Rinehart and Winston, New-York (1971)).