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IDENTIFICATION OF KINETIC PARAMETERS IN A BIOCHEMICAL SYSTEM

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Introduction

The aim of this paper is to give an example of identification of kinetic parameters in a biochemical system (called the "model case"). For proofs, further details and other examples, we refer to Joly [4], [5], [6]. Other systems are described (a system with hysteresis and a system with oscillations). Many other enzyme artificial systems are described in Kernevez et al. [7], and numerical and experimental results are compared.

For artificial enzyme membranes, owing 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 a stage between classical enzymology in solution and the study of properties of enzymes in very complex distributed biological systems.

These problems were originally raised by a team of biochemists (ERA 338 CNRS, Laboratoire d'Enzymologie Médicale, Université de Technologie de Compiègne) lead by Dr. D. Thomas.

The plan is as follows:

§ 1. Description of the model case.
§ 2. Identification of parameters in the model case.
§ 3. An example of hysteresis.
§ 4. An example of oscillation.

Notations

$s(x, t)$ = concentration of substrate at point x and at time t $(0 < x < 1)$.
$s(x)$ = concentration of substrate at point x (in steady state).
$F(s) = \sigma s/(1+|s|+as^2)$.
h and k are the space and time steps: $Jh = 1$, $Nk = T$.
s_j^n = approximation of $s(jh, nk)$.

1. Description of the model case

An enzyme E is homogeneously distributed inside an artificial membrane (thickness e). The membrane is immersed in a solution of substrate S, the reference axis is chosen perpendicular to the membrane. Obviously the S concentration is a function of space and time (x and t).

For each x, $\partial[S]/\partial t$ is linked to enzyme reaction and metabolite diffusion

$$\frac{\partial[S]}{\partial t} = D_S \frac{\partial^2[S]}{\partial x^2} - V_M \frac{[S]}{K_M+[S]}, \tag{1.1}$$

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

Let us replace $\frac{x}{e}$ by x and $\frac{t}{e^2/D_S}$ by t. x and t are now dimensionless.

K_M is chosen as the concentration unit and equation (1.1) can be written as

$$\frac{\partial s}{\partial t} - \frac{\partial^2 s}{\partial x^2} + \sigma \frac{s}{s+1} = 0, \tag{1.2}$$

with

$$\sigma = \frac{V_M}{K_M} \cdot \frac{e^2}{D}; \tag{1.3}$$

obviously, σ is a perfect characterization of the membrane system.

The boundary conditions are:

The concentration at the membrane boundaries is constant (Dirichlet conditions)

$$s(0, t) = s(1, t) = \alpha \quad (\alpha \geqslant 0). \tag{1.4}$$

Initial conditions:

$$s(x, 0) = 0, \quad 0 < x < 1, \tag{1.5}$$

or

$$s(x, 0) = \alpha, \quad \forall x. \tag{1.6}$$

2. Identification of parameters in the model case

Examples of optimal control of such biochemical systems have already been given by Kernevez (1972), (1973), Brauner et al. [1], [2], Kernevez et al. [7], [8], and Yvon [15].

In this paper we give an example of identification of parameters which is dependent on the same technique, i.e., we have to minimize some cost function, to do that we use a gradient method, and to get the gradient we use an adjoint state.

2.1. *Description of the problem*

The (steady) state of the system is defined by

$$\begin{cases} -\frac{d^2 s}{dx^2} + v(x)\frac{s}{1+s} = 0, & 0 < x < 1, \\ s(0) = \alpha, \quad s(1) = \alpha, \end{cases} \tag{2.1}$$

where $v(x)$ is proportional to the concentration of the enzyme at point x.

v is an unknown function of x in

$$\mathcal{U}_{ad} = \{v \mid v \in L^2(0, 1) \text{ and } 0 \leqslant v \leqslant M\}, \tag{2.2}$$

M being some positive constant.

Let α_i ($i = 1, \ldots, N$) be N choices of the boundary concentrations of the substrate.

We shall call $s_i(x; v)$ the *solution of* (2.1) for the function v and for $\alpha = \alpha_i$.

We observe the fluxes z_i of the substrate entering the membrane at $x = 0$ and $x = 1$ for the different values of i ($i = 1, \ldots, N$):

$$\text{observation} = z_i \ (i = 1, 2, \ldots, N), \tag{2.3}$$

and we define the cost function

$$J(v) = \frac{1}{2} \sum_{i=1}^{N} \left| -\frac{ds_i(\cdot, v)}{dx}(0) - z_i \right|^2. \tag{2.4}$$

The problem is to find a u such that

$$J(u) \leqslant J(v) \quad \forall v \in \mathcal{U}_{ad}. \tag{2.5}$$

2.2. *Lagrangian, adjoint state and gradient*

G. Joly ([4], [5]) shows that this problem has at least one solution and gives a justification for the following formal instructions to find a solution.

First step: Define the Lagrangian

$$\mathcal{L}(v, s, p) = \frac{1}{2} \sum_{i=1}^{N} \left| -\frac{ds_i}{dx}(0) - z_i \right|^2 + \frac{1}{2} \sum_{i=1}^{N} \left| \frac{ds_i}{dx}(1) - z_i \right|^2 + \sum_{i=1}^{N} \int_0^1 p_i \left(-\frac{d^2 s_i}{dx^2} + v(x)\frac{s_i}{1+s_i} \right) dx \tag{2.6}$$

where v, $s = (s_1, \ldots, s_N)$ and $p = (p_1, \ldots, p_N)$ are *independent* and such that

$$v \in \mathcal{U}_{ad}, \tag{2.7}$$

$$s_i - \alpha \in H_0^1(\Omega) \cap H^2(\Omega) \quad (\Omega =]0, 1[), \tag{2.8}$$

$$p_i \in L^2(\Omega). \tag{2.9}$$

Second step: For every s define a p such that

$$\frac{\partial \mathcal{L}}{\partial s} = 0, \tag{2.10}$$

$$\left(-\frac{ds_i}{dx}(0) - z_i \right)\left(-\frac{d\psi}{dx}(0) \right) + \left(\frac{ds_i}{dx}(1) - z_i \right)\left(-\frac{d\psi}{dx}(1) \right) + \int_0^1 p_i \left(-\frac{d^2\psi}{dx^2} + v(x)\frac{1}{(1+s_i)^2}\psi \right) dx = 0 \quad \forall \psi \in H_0^1(\Omega) \cap H^2(\Omega), \tag{2.11}$$

which is equivalent to

$$(2.12)\qquad \begin{cases} -\dfrac{d^2p_i}{dx^2}+v(x)\dfrac{1}{(1+s_i)^2}\,p_i=0, \\ p_i(0)=-\dfrac{ds_i}{dx}(0)-z_i, \quad p_i(1)=\dfrac{ds_i}{dx}(1)-z_i \end{cases} \qquad (i=1,2,\ldots,N),$$

Third step: We know that

$$(2.13)\qquad J(v)=\mathscr{L}(v,s(v),p)\quad \forall p,$$

$$(2.14)\qquad (J'(v),\varphi)=\left(\frac{\partial\mathscr{L}}{\partial v},\varphi\right)+\left(\frac{\partial\mathscr{L}}{\partial s}\circ\frac{\partial s}{\partial v},\varphi\right)=\left(\frac{\partial\mathscr{L}}{\partial v},\varphi\right)$$

if we choose p as indicated in (2.12). (φ is an arbitrary function in $L^2(\Omega)$ and (f,g) denotes $\int_\Omega f(x)g(x)\,dx$.)

$$(2.15)\qquad (J'(v),\varphi)=\sum_{i=1}^{N}\int_0^1 p_i(x)\varphi(x)\frac{s_i}{1+s_i}\,dx.$$

2.3. *Numerical method*

We work with the discrete Lagrangian

$$(2.16)\qquad \mathscr{L}=\frac{1}{2}\sum_{i=1}^{N}\left|\frac{s_{i,0}-s_{i,1}}{h}-z_i\right|^2+\frac{1}{2}\sum_{i=1}^{N}\left|\frac{s_{i,J}-s_{i,J-1}}{h}-z_i\right|^2+$$
$$+h\sum_{i=1}^{N}\sum_{j=1}^{J-1}p_{i,j}\left(-\frac{s_{i,j+1}+s_{i,j-1}-2s_{i,j}}{h^2}+v_j\frac{s_{i,j}}{1+s_{i,j}}\right),$$

which corresponds to the discrete state

$$(2.17)\qquad \begin{cases} -\dfrac{s_{i,j+1}+s_{i,j-1}-2s_{i,j}}{h^2}+v_j\dfrac{s_{i,j}}{1+s_{i,j}}=0, \\ s_{i,0}=\alpha_i, \quad s_{i,J}=\alpha_i, \end{cases}$$

to the discrete adjoint state

$$(2.18)\qquad \begin{cases} -\dfrac{p_{i,j+1}+p_{i,j-1}-2p_{i,j}}{h^2}+v_j\dfrac{1}{(1+s_{i,j})^2}\,p_{i,j}=0, \\ p_{i,0}=\dfrac{s_{i,0}-s_{i,1}}{h}-z_i, \quad p_{i,J}=\dfrac{s_{i,J}-s_{i,J-1}}{h}-z_i, \end{cases}$$

and to the gradient

$$(2.19)\qquad \frac{\partial\tilde{J}}{\partial v_j}=h\sum_{i=1}^{N}p_{ij}\frac{s_{i,j}}{1+s_{i,j}}.$$

The algorithm is the steepest descent method.

Numerical results are given in Fig. 1.

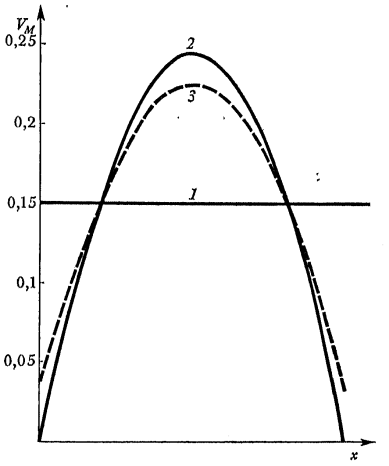

Fig. 1

1: initial guess for the function $V_M(x)$; *2*: estimate after 1 iteration; *3*: after 2 iterations there is almost superposition with the optimal curve.

3. An example of hysteresis

The only difference with respect to the "model case" (but of great importance for the behaviour of the system) is that in equation (1.1) the term denoting reaction must be changed into

$$(3.1)\qquad V_M\frac{[S]}{K_M+[S]+\dfrac{[S]^2}{K_{SS}}}.$$

This means that there is inhibition by excess of the substrate, and equations (1.2), (1.4), (1.5) and (1.6) must be replaced by

$$(3.2)\qquad \begin{cases} \dfrac{\partial s}{\partial t}-\Delta s+\sigma F(s)=0 \quad \left(\Delta=\dfrac{\partial^2}{\partial x^2}\right), \\ s|_\Gamma=\alpha, \quad s|_{t=0}=0 \text{ or } \alpha, \\ F(s)=s/(1+|s|+as^2), \end{cases}$$

where σ has the same meaning as in (1.3) and

$$(3.3)\qquad a=\frac{K_M}{K_{SS}}.$$

We are interested also in the steady states

$$(3.4)\qquad \begin{cases} -\Delta s+\sigma F(s)=0, \\ s|_\Gamma=\alpha_i, \end{cases}$$

and in the quasi steady states, i.e., an evolution of the steady states:

$$(3.5)\qquad \begin{cases} -\Delta s + \sigma F(s) = 0, \\ s|_\Gamma = \alpha(t). \end{cases}$$

Now, for systems (3.2) and (3.4) we have the following existence theorems, using monotone methods as indicated in Sattinger (1972):

$u_0 = \alpha$ (resp. $v_0 = 0$) is an upper (resp. lower) solution of (3.2):

$$(3.6)\qquad \begin{cases} -\Delta u_0 + \sigma F(u_0) \geqslant 0, & u_0|_\Gamma = \alpha, \\ -\Delta v_0 + \sigma F(v_0) = 0, & v_0|_\Gamma \leqslant \alpha. \end{cases}$$

Then we have the following results from Sattinger [13]:

(i) *There exists a regular solution s of* (3.4) *such that* $v_0 \leqslant s \leqslant u_0$.

(ii) *Moreover, for any continuous* $\varphi(x)$ *with* $v_0(x) \leqslant \varphi(x) \leqslant u_0(x)$ *we obtain a global regular solution of the initial value problem* (3.2) *with initial data* φ, *and the solution* $s(x, t)$ *satisfies* $0 = v_0(x) \leqslant s(x, t) \leqslant u_0(x) = \alpha$.

It is possible to prove (Kernevez et al. [9]) that (3.4) *admits more than one solution if* σ *and* α *are suitably chosen.*

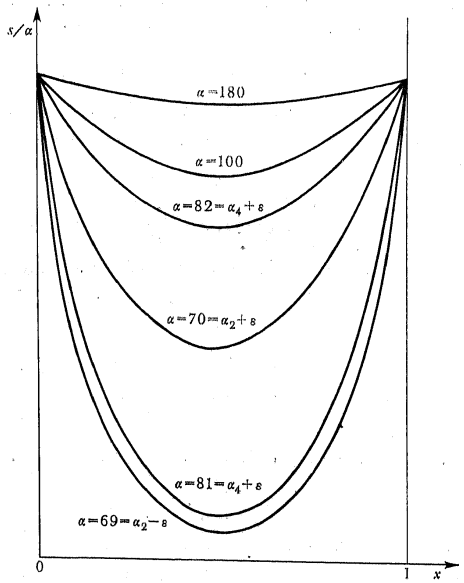

Fig. 2

The flux of the substrate entering the membrane under quasi-stationary state conditions has been calculated as a function of the external substrate concentration for increasing and decreasing values (Fig. 2).

In Fig. 2 there is evidence for hysteresis phenomenon: the activity substrate concentration relationship does not give a 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 the substrate inside the membrane (Fig. 3). Two families of curves are given in Fig. 3, showing the strong variation of the concentration level for each critical point. The numerical results are similar to the experimental results presented by Naparstek et al. [12].

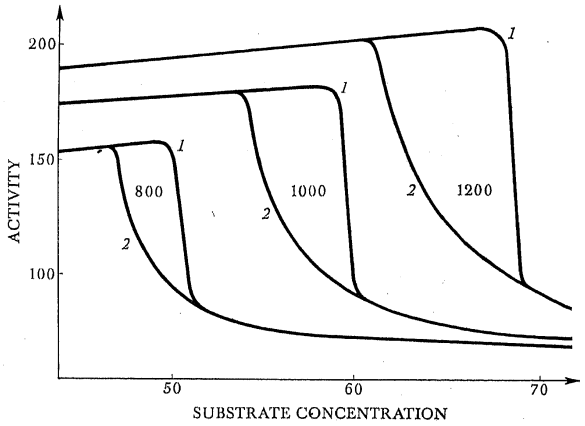

Fig. 3

4. An example of oscillation

The system was experimentally built and studied in Harvard by Naparstek et al. [12] and a numerical analysis was given by Naparstek et al. [3]. A coating (thickness L) bearing an enzyme activity is along a glass electrode. One side of the coating is impermeable to any molecule.

In this system, H^+ is a product with a concentration p ($[S] = s$) and due to the reaction between H^+ and OH^- a function $a(x, t)$ is introduced in the equation ruling the system.

$$\frac{\partial s}{\partial t} - D_S \frac{\partial^2 s}{\partial x^2} + F(s,p) = 0, \quad 0 < x < L, \tag{4.1}$$

$$\frac{\partial a}{\partial t} - D_H \frac{\partial^2 a}{\partial x^2} - F(s,p) = 0, \tag{4.2}$$

$$a = p - \frac{10^{-20}}{p}, \tag{4.3}$$

$$F(s,p) = Q_2 Q_3 \frac{E_0}{Q_2 + Q_3 + Q_3 \dfrac{5{,}45 \cdot 10^{-5}}{s}}, \tag{4.4}$$

$$Q_2 = \frac{64{,}5}{1 + 10^{7{,}29} p + \dfrac{10^{-11{,}49}}{p}}, \tag{4.5}$$

$$Q_3 = \frac{20.2}{1 + 10^{6{,}92} p}, \tag{4.6}$$

$$D_S = 0{,}13 \cdot 10^{-5}, \quad D_H = 0{,}64 \cdot 10^{-5}, \quad L = 10^{-2}. \tag{4.7}$$

Boundary conditions:

$$s(0,t) = 5{,}5 \cdot 10^{-7}; \quad p(0,t) = 10^{-13}, \tag{4.8}$$

$$\frac{\partial s}{\partial x}(L,t) = \frac{\partial p}{\partial x}(L,t) = 0. \tag{4.9}$$

Initial conditions:

$$s(x,0) = 5{,}5 \cdot 10^{-7}; \quad p(x,0) = 10^{-13}. \tag{4.10}$$

The existence and unicity of solution for equations (4.1)–(4.10) 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 fractionary derivatives, using the Fourier transform with respect to time, and extraction of a subsequence converging towards a solution of (4.1)–(4.10). The property used in demonstrating the existence is mainly the boundedness of the function F. The unicity proof uses the fact that F is Lipschitz continuous.

The system described by the equations (4.1) to (4.10) was solved numerically by Naparstek et al. [11]. A periodic behaviour for the system was shown and a sharp front of the 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 Naparstek et al. ([11], [12]) and the experimental results of Naparstek et al. [11].

Conclusion

An example of identification of a function appearing as a coefficient in the (non-linear) P.D.E. describing the "model case" has been given. References for optimal control problems related to such biochemical systems have been indicated. Two more examples of biochemical systems have been shown, as an indication of the wide area opened to the applied mathematician in the field of artificial enzyme membranes.

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