



Tihonov theory and center manifolds for inhibitory mechanisms in enzyme kinetics.

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Abstract

In this paper we study the chemical reaction of inhibition, determine the appropriate parameter ϵ for the application of Tihonov’s Theorem, compute explicitly the equations of the center manifold of the system and find sufficient conditions to guarantee that in the phase space the curves which relate the behavior of the complexes to the substrates by means of the tQSSA are asymptotically equivalent to the center manifold of the system. Some numerical results are discussed.

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1. Introduction

Let us consider a reaction where a substrate S binds reversibly to an enzyme E to form a complex C. The complex can decay irreversibly to a product P and the enzyme, which is then free to bind another substrate molecule. This is summarized in the scheme



where a , d , k are kinetic parameters (supposed constant) associated with the reaction rates. For notational convenience we will use the same variable

names to denote both a chemical species and its concentration. For example, E denotes both an enzyme and its concentration.

Assuming that the complex concentration is approximately constant after a short transient phase leads to the usual *standard quasi-steady-state approximation* (sQSSA) [1], which is valid when the enzyme concentration is much lower than either the substrate concentration or the Michaelis constant $K_M = \frac{d+k}{a}$. The *total QSSA* (tQSSA) [2,3] is another approximation, and is valid for a broader range of parameter values covering both high and low enzyme concentrations.

The sQSSA and tQSSA are related to the *asymptotic expansion* of the solutions of the ODEs governing the process with respect to an appropriate parameter [1,4–6]; the topic can also be read in terms of center manifold, normal forms and bifurcation theory [7–9]. They are both developed setting the derivative of the intermediate complexes equal to zero. The theoretical justification for this assumption is given by Tihonov’s Theorem 2.1 [10–13]. In Tihonov’s framework, the assumption $\dot{C}_i = 0$ is equivalent to impose the aforementioned “appropriate parameter” (*the perturbation parameter*, usually denoted by ϵ) equal to zero (see, for example, [14,15]).

In particular, Kumar and Josić [15] derive the mathematical expression of the *center manifold* for the tQSSA system just putting $\epsilon = 0$. Although not seemingly strictly theoretically founded, this result remains as true for a wide class of systems (including the tQSSA model) and has a mathematical explanation, as we proved in a different paper [16]. It should be remarked that we still lack the theoretical investigation of the validity of the tQSSA in the case of successive reactions — where more parameters appear — but nevertheless it can be generalized to them. In particular, Theorem 2.1, as stated in [11], represents the generalization of Tihonov’s Theorem [10,13] to the case of more parameters. The question is: what is the most appropriate parameter to develop an *asymptotic expansion* of the solutions of the ODEs modelling the reactions?

In a series of papers written by Palsson et al. [17] [18] [19] [20], the possibility to choose the required parameter has been related to the decoupling of the eigenvalues of the Jacobian matrix of the differential system. In [19] the authors focused on chains of reactions, obtaining a block form for the resulting Jacobian matrix.

In this paper we use the techniques shown in [19] in order to determine the appropriate parameter ϵ for the asymptotic expansions and give sufficient conditions to guarantee that in the phase space the curves which relate the behavior of the complexes to the substrates by means of the tQSSA are asymptotically equivalent to the center manifold of the system.

In Section 2 we recall the most important mathematical background

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concerning Tihonov's Theorem and Center Manifold Theory. In Section 3 we describe the mathematical model of the enzymatic inhibition reaction and, by means of an appropriate adimensionalization, we determine a suitable perturbation parameter ϵ which will be used to apply the tQSSA, in the framework of Tihonov's Theorem. In Section 4 we determine the Center Manifold of the system and discuss its asymptotic equivalence with the tQSSA. In Section 5 some perspectives of our research are discussed.

2. Preliminary results and notations on nonlinear dynamical systems.

2.1. Singular Perturbations

For this section we will refer to the widespread book by W. Wasow [13], and - in particular - to its relevant section on Singular Perturbations. A systematic study of the qualitative aspects of such singular perturbation problems can be found in a series of papers by Tihonov [10–12], written in Russian.

According to [11], we consider $c + s$ -dimensional differential systems of the form

$$(2) \quad \begin{aligned} \frac{dx}{dt} &= f(x, y) \\ \epsilon \frac{dy}{dt} &= g(x, y), \end{aligned}$$

where x is c -dimensional and y is s -dimensional. All variables are real, and ϵ is positive.

We assume that:

- (A) The functions f and g in (2) are continuous in an open region Ω of the (x, y) -space.
- (B) There is a m -dimensional vector function $\phi(x)$ continuous in $\xi_1 \leq x \leq \xi_2$ such that the points $(x, \phi(x))$, for all $\xi_1 \leq x \leq \xi_2$, are in Ω and

$$g(x, \phi(x)) \equiv 0.$$

- (C) There exists a number $\eta > 0$, independent of x , such that the relations

$$\|y - \phi(x)\| < \eta, \quad y \neq \phi(x) \quad \text{in} \quad \xi_1 \leq x \leq \xi_2$$

imply

$$g(x, y) \neq 0, \quad \text{in} \quad \xi_1 \leq x \leq \xi_2.$$

The function $\phi(x)$ will be referred to as a root of the equation $g(x, y) = 0$. It is not excluded that $g(x, y) = 0$ may have other roots besides $\phi(x)$. A root $\phi(x)$ that satisfies condition (C) will be called isolated in $\xi_1 \leq x \leq \xi_2$.

Definition 2.1. The system of differential equations

$$(3) \quad \epsilon \frac{dy}{dt} = g(x, y)$$

in which x is a parameter, will be called the boundary layer equation belonging to the system (2). We also assume:

- (D) The singular point $y = \phi(x)$ of the boundary layer equation (3) is asymptotically stable for all $\xi_1 \leq x \leq \xi_2$.

The root $\phi(x)$ will be called, briefly, a stable root in $\xi_1 \leq x \leq \xi_2$, if assumption (D) is satisfied.

In accordance with our previous terminology we refer to the problem consisting of the system (2) together with the initial condition

$$(4) \quad x = \alpha, \quad y = \beta, \quad \text{for } t = 0$$

as the full problem. The reduced problem is here defined by

$$(5) \quad \begin{aligned} \frac{dx}{dt} &= f(x, \phi(x)) \\ y &= \phi(x), \end{aligned}$$

$$(6) \quad x = \alpha, \quad \text{for } t = 0$$

The system (5) is, of course, obtained by setting $\epsilon = 0$ in (2) and choosing the particular root $y = \phi(x)$ of the equation $g(x, y) = 0$. Moreover, we assume:

- (E) The full, as well as the reduced problem has a unique solution in an interval $0 \leq t \leq T$.
 (F) The asymptotic stability of the singular point $y = \phi(x)$ is uniform with respect to x in $\xi_1 \leq x \leq \xi_2$.

For a convenient formulation of Tihonov's Theorem, according to [13], we introduce one more term.

Definition 2.2. A point $(\alpha, \beta) \in \Omega$, $\xi_1 \leq \alpha \leq \xi_2$ is said to lie in the domain of influence of the stable root $y = \phi(x)$ if the solution of the problem

$$dy/d\tau = g(\alpha, y), \quad y(0) = \beta$$

exists and remains in Ω for all $\tau > 0$, and if it tends to $\phi(\alpha)$, as $\tau \rightarrow +\infty$.

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Theorem 2.1. *Let Assumptions (A) to (F) be satisfied and let (α, β) be a point in the domain of influence of the root $y = \phi(x)$. Then the solution $x(t, \epsilon), y(t, \epsilon)$ of the full initial value problem (2), (4) is connected with the solution $x_0(t), y_0(t) = \phi(x_0(t))$ of the reduced problem (5), (6) by the limit relations*

$$(7) \quad \begin{aligned} \lim_{\epsilon \rightarrow 0} x(t, \epsilon) &= x_0(t), & 0 \leq t \leq T_0 \\ \lim_{\epsilon \rightarrow 0} y(t, \epsilon) &= y_0(t) = \phi(x_0(t)) & 0 < t \leq T_0 \end{aligned}$$

Here T_0 is any number such that $y = \phi(x_0(t))$ is an isolated stable root of $g(x_0(t), y) = 0$ for $0 \leq t \leq T_0$. The convergence is uniform in $0 \leq t \leq T_0$, for $x(t, \epsilon)$, and in any interval $0 < t_1 \leq t \leq T_0$ for $y(t, \epsilon)$.

Tihonov's Theorem 2.1 is only the first step in the asymptotic solution of initial value problems of the singular perturbation type. The most natural approach to these problems is to attempt a solution in the form of a series in powers of ϵ :

$$(8) \quad x = \sum_{r=0}^{\infty} x_r(t) \epsilon^r, \quad y = \sum_{r=0}^{\infty} y_r(t) \epsilon^r$$

and to determine the coefficients $x_r(t), y_r(t)$ by means of formal substitution and comparison of coefficients.

It is clear that we have to relate the series (8) to the behavior of the solution of (2) in the boundary layer. For values of t that are small of order $O(\epsilon)$ the solution to our perturbation problem can be found by means of the stretching transformation $t = \tau \epsilon$. Hence, the stretched, or inner, form of the original problem is

$$(9) \quad \begin{aligned} \frac{dx}{d\tau} &= \epsilon f(x, y), & \frac{dy}{d\tau} &= g(x, y), \\ x &= \alpha, & y &= \beta, & \text{for } \tau = 0. \end{aligned}$$

If the functions f and g are analytic the solutions of this problem have convergent expansions in powers of ϵ , say,

$$(10) \quad x(\tau, \epsilon) = \sum_{r=0}^{\infty} u_r(\tau) \epsilon^r, \quad y(\tau, \epsilon) = \sum_{r=0}^{\infty} v_r(\tau) \epsilon^r,$$

and the series reduce to $x = \alpha, y = \beta$, identically in ϵ , for $\tau = 0$, so that

$$(11) \quad \begin{aligned} u_0(0) &= \alpha, & v_0(0) &= \beta, \\ u_r(0) &= 0, & v_r(0) &= 0, & r > 0. \end{aligned}$$

If f and g are only assumed to be infinitely differentiable it is possible to prove that the series (10) calculated by the same formal operations as in the analytic case represent the solution asymptotically, as $\epsilon \rightarrow 0^+$, in every finite τ -interval.

In this paper we consider, as in [11], the case of several parameters $\{\epsilon_j\}_{j=1}^m$ of the same order, namely, $\epsilon_j = \rho_{j-1}\epsilon$ for each $j = 1, \dots, m$ and $\rho_j > 0$ ($\rho_0 = 1$). We obtain a system of the form

$$(12) \quad \begin{aligned} \frac{dx}{dt} &= f(x, y_1, \dots, y_m; t), \\ \epsilon \frac{dy_j}{dt} &= \frac{1}{\rho_{j-1}} g_j(x, y_1, \dots, y_m; t), \quad j = 1, 2, \dots, m, \end{aligned}$$

which becomes, in the vectorial form,

$$(13) \quad \begin{aligned} \frac{dx}{dt} &= f(x, y; t), \\ \epsilon \frac{dy}{dt} &= g(x, y; t), \end{aligned}$$

where

$$y = (y_1, \dots, y_m)^t, \quad g = \left(g_1, \frac{1}{\rho_1} g_2, \dots, \frac{1}{\rho_{m-1}} g_m \right)^t.$$

Then it is possible to apply, for the system (13), Tihonov's Theorem.

2.2. Center Manifold

Let us consider vector fields of the following form

$$(14) \quad \begin{aligned} \dot{x} &= Ax + f(x, y), \\ \dot{y} &= By + g(x, y), \quad (x, y) \in \mathbb{R}^c \times \mathbb{R}^s, \end{aligned}$$

where

$$(15) \quad \begin{aligned} f(0, 0) &= 0, \quad Df(0, 0) = 0, \\ g(0, 0) &= 0, \quad Dg(0, 0) = 0. \end{aligned}$$

where A is a $c \times c$ matrix having eigenvalues with zero real parts, B is an $s \times s$ matrix having eigenvalues with negative real parts, f and g are C^r functions ($r \geq 2$) and D is the jacobian matrix of the system.

Definition 2.3 (Center Manifold [7–9,21]). *An invariant manifold will be called a **center manifold** for (14) if it can locally be represented as follows*

$$W^c(0) = \{(x, y) \in \mathbb{R}^c \times \mathbb{R}^s | y = h(x), |x| < \delta, h(0) = 0, Dh(0) = 0\}$$

for δ sufficiently small.

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To find a center manifold, all we need do is to solve [8,9]:

$$(16) \quad \mathcal{N}(h(x)) \equiv Dh(x)(Ax + f(x, h(x))) - Bh(x) - g(x, h(x)) = 0,$$

and the following theorem gives us a method for computing an approximate solution of (16) to any desired degree of accuracy. Let us remark [8,9,21] the non uniqueness of the center manifold.

Theorem 2.2 (Approximation [7–9]). *Let $\phi : \mathbb{R}^c \rightarrow \mathbb{R}^s$ be a C^1 mapping with $\phi(0) = D\phi(0) = 0$ such that $\mathcal{N}(\phi(x)) = O(|x|^q)$ as $x \rightarrow 0$ for some $q > 1$. Then*

$$|h(x) - \phi(x)| = O(|x|^q), \quad \text{as } x \rightarrow 0.$$

When we consider a system depending on a parameter ϵ

$$(17) \quad \begin{aligned} \dot{x} &= Ax + f(x, y, \epsilon), \\ \dot{y} &= By + g(x, y, \epsilon), \quad (x, y, \epsilon) \in \mathbb{R}^c \times \mathbb{R}^s \times \mathbb{R}, \end{aligned}$$

where

$$(18) \quad \begin{aligned} f(0, 0, 0) &= 0, \quad Df(0, 0, 0) = 0, \\ g(0, 0, 0) &= 0, \quad Dg(0, 0, 0) = 0, \end{aligned}$$

following [8,9,21], we can handle these parameterized systems treating the parameter ϵ as a new dependent variable as follows.

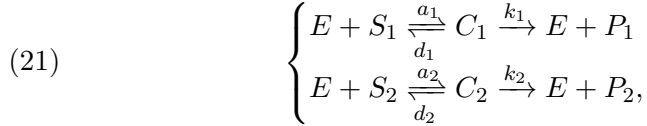
$$(19) \quad \begin{aligned} \dot{x} &= Ax + f(x, y, \epsilon), \\ \dot{\epsilon} &= 0, \\ \dot{y} &= By + g(x, y, \epsilon), \quad (x, y, \epsilon) \in \mathbb{R}^c \times \mathbb{R}^s \times \mathbb{R}. \end{aligned}$$

This system has a fixed point at $(x, y, \epsilon) = (0, 0, 0)$. The matrix associated with the linearization of (19) about this fixed point has $c + 1$ eigenvalues with zero real part and s eigenvalues with negative real part. Let us now apply center manifold theory. Modifying definition 2.3, a center manifold will be represented as a graph over the x and ϵ variables, i.e., the graph of $h(x, \epsilon)$ for x and ϵ sufficiently small. Theorem 2.2 still applies, with the vector field reduced to the center manifold given by

$$(20) \quad \begin{aligned} \dot{u} &= Au + f(u, h(u, \epsilon), \epsilon), \\ \dot{\epsilon} &= 0, \quad (u, \epsilon) \in \mathbb{R}^c \times \mathbb{R}. \end{aligned}$$

3. Mathematical model of the inhibition mechanism

An *inhibition reaction*, where an inhibitor S_2 competes with a substrate S_1 for the same enzyme E , is summarized by the following scheme



where P_1, P_2 are the reaction products and C_1, C_2 the enzyme-substrate complexes. In this kind of reaction, it is clear that S_1 and S_2 have the same role of reciprocal inhibitors (S_1 inhibits S_2 as S_2 inhibits S_1). Introducing the total substrates

$$(22) \quad \overline{S}_1 = S_1 + C_1, \quad \overline{S}_2 = S_2 + C_2,$$

by conservation laws we have

$$(23) \quad \overline{S}_1 + P_1 = S_{1T}, \quad \overline{S}_2 + P_2 = S_{2T}, \quad E + C_1 + C_2 = E_T.$$

Then we can write the following Cauchy Problem:

$$(24) \quad \begin{cases} \frac{d\overline{S}_1}{dt} = -k_1 C_1 \\ \frac{dC_1}{dt} = a_1 [(\overline{S}_1 - C_1)(E_T - C_1 - C_2) - K_{1M} C_1] \\ \frac{d\overline{S}_2}{dt} = -k_2 C_2 \\ \frac{dC_2}{dt} = a_2 [(\overline{S}_2 - C_2)(E_T - C_1 - C_2) - K_{2M} C_2] \\ \overline{S}_1(0) = S_{1T} \quad ; \quad \overline{S}_2(0) = S_{2T} \quad ; \quad C_1(0) = C_2(0) = 0, \end{cases}$$

where

$$(25) \quad K_{1M} = \frac{d_1 + k_1}{a_1}, \quad K_{2M} = \frac{d_2 + k_2}{a_2}$$

are the Michaelis constants. It is easy to see that in this case the origin $X^* := (\overline{S}_1^*, C_1^*, \overline{S}_2^*, C_2^*) = (0, 0, 0, 0)$ is the only fixed point of the system. The linear approximation of the system around X^* is represented by the following system:

$$(26) \quad \begin{cases} \frac{d\overline{S}_1}{dt} = -k_1 C_1 \\ \frac{dC_1}{dt} = a_1 [E_T \overline{S}_1 - E_T C_1 - K_{1M} C_1] \\ \frac{d\overline{S}_2}{dt} = -k_2 C_2 \\ \frac{dC_2}{dt} = a_2 [E_T \overline{S}_2 - E_T C_2 - K_{2M} C_2], \end{cases}$$

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with Jacobian at the origin

$$(27) \quad D(0,0,0,0) = \begin{pmatrix} 0 & -k_1 & 0 & 0 \\ a_1 E_T - a_1(E_T + K_{1M}) & 0 & 0 & 0 \\ 0 & 0 & 0 & -k_2 \\ 0 & 0 & a_2 E_T - a_2(E_T + K_{2M}) & 0 \end{pmatrix},$$

with two blocks. An easy computation shows that

$$|D - \lambda \mathbb{I}| = [\lambda^2 + a_1(E_T + K_{1M})\lambda + k_1 a_1 E_T] [\lambda^2 + a_1(E_T + K_{2M})\lambda + k_2 a_2 E_T],$$

then the eigenvalues are

$$(\lambda_{\pm})_i = \frac{-a_i(E_T + K_{iM}) \pm \sqrt{a_i^2(E_T + K_{iM})^2 - 4a_i k_i E_T}}{2} =$$

$$(28) \quad \frac{a_i(E_T + K_{iM})}{2} \left(-1 \pm \sqrt{1 - \frac{4K_i E_T}{(E_T + K_{iM})^2}} \right),$$

where we have denoted the Van Slyke-Cullen constants by $K_i = \frac{k_i}{a_i}$, $i = 1, 2$. Now it is clear that

$$(29) \quad \frac{4K_i E_T}{E_T + K_{iM}} \ll 1 \implies (\lambda_-)_i \ll (\lambda_+)_i,$$

$i = 1, 2$, i.e., according to Palsson [19], we have a sufficient condition for the separation of time scales.

We could choose

$$\tilde{\epsilon}_i := \frac{K_i E_T}{(E_T + K_{iM})^2},$$

as perturbation parameter. It is easy to show that $\tilde{\epsilon}_i < \frac{1}{4}$ for every choice of data. However, in order to follow the paper [6], it is more convenient to take

$$(30) \quad \epsilon = \max\{\epsilon_i := \frac{K_i E_T}{(E_T + K_{iM} + S_{iT})^2}; i = 1, 2\} < \frac{1}{4}$$

as perturbation parameter (note that $\epsilon_i < \tilde{\epsilon}_i$). Furthermore, thanks to the symmetry of the problem, we can suppose that $\epsilon = \epsilon_1$. With this choice the following change of variables

$$\bar{S}_i = \alpha_i s_i, \quad C_i = \beta_i c_i, \quad t = \gamma \tau,$$

with

$$(31) \quad \alpha_i = S_{iT}, \quad \beta_i = \frac{E_T S_{iT}}{E_T + K_{iM} + S_{iM}}, \quad \gamma = \frac{1}{a_1(E_T + K_{1M} + S_{1M})},$$

$i = 1, 2$, provides an *adimensionalization* of the model equations (see [6]):

$$(32) \quad \begin{cases} \frac{\alpha_1}{\gamma} \frac{d\bar{s}_1}{d\tau} = -k_1 \beta_1 c_1 \\ \frac{\beta_1}{\gamma} \frac{dc_1}{d\tau} = a_1 [\beta_1^2 c_1^2 - (E_T + \alpha_1 \bar{s}_1 - \beta_2 c_2 + K_{1M}) \beta_1 c_1 + \\ \quad - \alpha_1 \bar{s}_1 \beta_2 c_2 + \alpha_1 \bar{s}_1 E_T] \\ \frac{\alpha_2}{\gamma} \frac{d\bar{s}_2}{d\tau} = -k_2 \beta_2 c_2 \\ \frac{\beta_2}{\gamma} \frac{dc_2}{d\tau} = a_2 [\beta_2^2 c_2^2 - (E_T + \alpha_2 \bar{s}_2 - \beta_1 c_1 + K_{2M}) \beta_2 c_2 + \\ \quad - \alpha_2 \bar{s}_2 \beta_1 c_1 + \alpha_2 \bar{s}_2 E_T] \end{cases}$$

thanks to which we obtain the system of equations for the inner solutions:

$$(33) \quad \begin{cases} \frac{d\bar{s}_1}{d\tau} = -\epsilon c_1 \\ \frac{dc_1}{d\tau} = \sigma_1 \eta_1 c_1^2 - (\eta_1 + \kappa_{1M}) c_1 - \sigma_1 \bar{s}_1 c_1 + \sigma_2 \eta_1 c_1 c_2 + \bar{s}_1 - \sigma_2 c_2 \bar{s}_1 \\ \frac{d\bar{s}_2}{d\tau} = -\epsilon \frac{k_2 \eta_2}{k_1 \eta_1} c_2 \\ \frac{dc_2}{d\tau} = \frac{a_2}{a_1} \left[\eta_1 \sigma_2 c_2^2 - \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) c_2 + \right. \\ \quad \left. + \left(\eta_1 \sigma_1 c_1 - \frac{S_{2T}}{S_{1T}} \sigma_1 \bar{s}_2 \right) c_2 + \frac{\eta_1}{\eta_2} \bar{s}_2 - \frac{\sigma_1 \eta_1}{\eta_2} \bar{s}_2 c_1 \right] \end{cases}$$

where

$$\sigma_i = \frac{S_{iT}}{E_T + K_{iT} + S_{iT}}; \quad \eta_i = \frac{E_T}{E_T + K_{iT} + S_{iT}}; \quad \kappa_{iM} = \frac{K_{iM}}{E_T + K_{iT} + S_{iT}}$$

$i = 1, 2$ (note that $\sigma_i + \eta_i + \kappa_{iM} = 1$). We now write the system of equations that gives the outer solutions. To this aim we set

$$(34) \quad \bar{\gamma} := \frac{1}{k_1 \eta_1}$$

and note that putting $T = \frac{t}{\bar{\gamma}}$, we see that

$$T = \frac{\gamma}{\bar{\gamma}} \tau = \epsilon \tau.$$

So we obtain

$$(35) \quad \begin{cases} \frac{d\bar{s}_1}{dT} = -c_1 \\ \epsilon \frac{dc_1}{dT} = \sigma_1 \eta_1 c_1^2 - (\eta_1 + \kappa_{1M}) c_1 - \sigma_1 \bar{s}_1 c_1 + \sigma_2 \eta_1 c_1 c_2 + \bar{s}_1 - \sigma_2 c_2 \bar{s}_1 \\ \frac{d\bar{s}_2}{dT} = -\frac{k_2 \eta_2}{k_1 \eta_1} c_2 \\ \epsilon \frac{dc_2}{dT} = \frac{a_2}{a_1} \left[\eta_1 \sigma_2 c_2^2 - \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) c_2 + \right. \\ \quad \left. + \left(\eta_1 \sigma_1 c_1 - \frac{S_{2T}}{S_{1T}} \sigma_1 \bar{s}_2 \right) c_2 + \frac{\eta_1}{\eta_2} \bar{s}_2 - \frac{\sigma_1 \eta_1}{\eta_2} \bar{s}_2 c_1 \right]. \end{cases}$$

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At this point we can apply Tihonov's Theorem. Putting $\epsilon = 0$, we obtain the reduced system of equations, a system which is both of differential and algebraic type:

$$(36) \quad \begin{cases} \frac{d\bar{s}_1}{dT} = -c_1 \\ \sigma_1 \eta_1 c_1^2 - (\eta_1 + \kappa_{1M})c_1 - \sigma_2 c_2 \bar{s}_1 - \sigma_1 \bar{s}_1 c_1 + \sigma_2 \eta_1 c_1 c_2 + \bar{s}_1 = 0 \\ \frac{d\bar{s}_2}{dT} = -\frac{k_2 \eta_2}{k_1 \eta_1} c_2 \\ \eta_1 \sigma_2 c_2^2 - \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) c_2 - \frac{\sigma_1 \eta_1}{\eta_2} \bar{s}_2 c_1 + \\ + \left(\eta_1 \sigma_1 c_1 - \frac{S_{2T}}{S_{1T}} \sigma_1 \bar{s}_2 \right) c_2 + \frac{\eta_1}{\eta_2} \bar{s}_2 = 0 \end{cases}$$

From the algebraic equations we can obtain cumbersome expressions for $c_1(\bar{s}_1, \bar{s}_2)$ and $c_2(\bar{s}_1, \bar{s}_2)$, which are useful for the numerical integration of the differential equations.

For the sake of clarity we prefer here to show the reverse relations:

$$(37) \quad \bar{s}_1 = \frac{\sigma_1 \eta_1 c_1^2 - (\eta_1 + \kappa_{1M})c_1 + \sigma_2 \eta_1 c_1 c_2}{(\sigma_1 c_1 - \sigma_2 c_2 - 1)}$$

$$(38) \quad \bar{s}_2 = \frac{\eta_1 \sigma_2 c_2^2 - \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) c_2 + \eta_1 \sigma_1 c_1 c_2}{\left(\frac{S_{2T}}{S_{1T}} \sigma_1 c_2 - \frac{\eta_1}{\eta_2} \sigma_1 c_1 - \frac{\eta_1}{\eta_2} \right)}.$$

Figure 1 compares the time behavior of the complexes (left) and of the substrates (right), respectively, obtained as numerical solutions of the system (35) and of their tQSSA (36), in a case where the parameter values are chosen in such a way to obtain a small value of the perturbation parameter ($\epsilon = 0.02$).

Figure 2 compares the time behavior of the complexes (left) and of the substrates (right), respectively, obtained as numerical solutions of the system (35) and of their tQSSA (36), in a case where the parameter values are chosen in such a way to obtain a high value of the perturbation parameter ($\epsilon = 0.23$), very close to its upper bound.

The figures show a very good approximation, even in the stressed case, in accordance with Tihonov's Theorem.

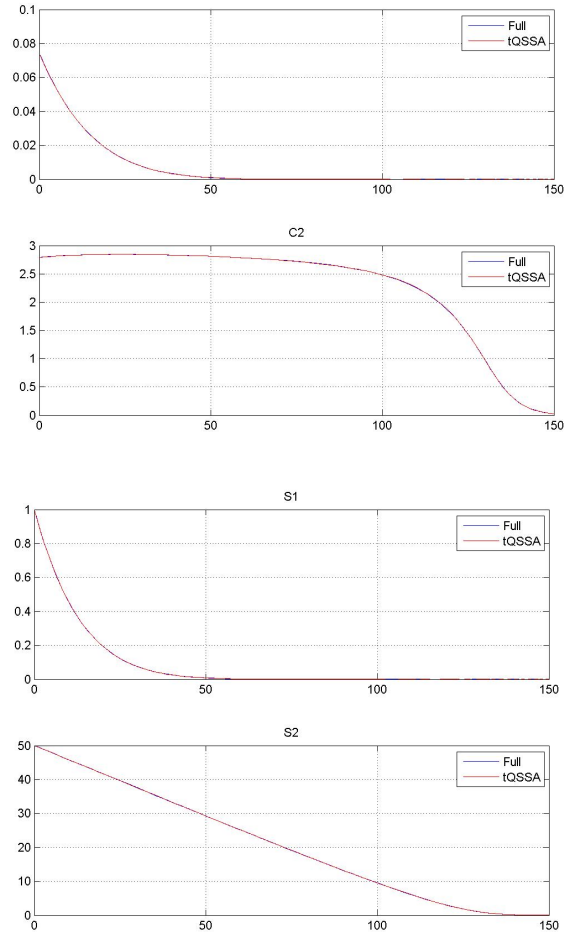


Figure 1. Comparison of the complexes (left) and of the substrates (right), solution of the system (35), with their tQSSA (36). The parameter set is the following: for the first reaction $a_1 = k_1 = 1; d_1 = 0.1; E_T = 0.1; S_1T = 1; K_1M = 1.1; K = 1; \epsilon_1 = 0.02$; for the second reaction $a_2 = 0.1; k_2 = 10; d_2 = 0.01; E_T = 0.1; S_2T = 50; K_2M = 100.1; K = 100; \epsilon_2 = 0.00044$. Thus $\epsilon = 0.02$.

Tihonov theory in inhibitory reactions.

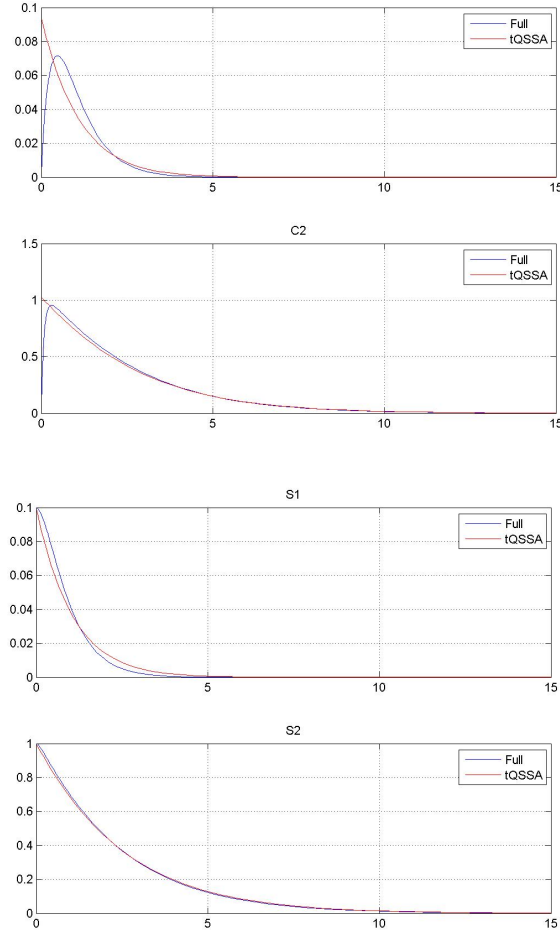


Figure 2. Comparison of the complexes (left) and of the substrates (right), solution of the system (35), with their tQSSA (36). The parameter set is the following: for the first reaction $a_1 = k_1 = 1; d_1 = 0.1; E_T = 1; S_1 T = 0.1; K_1 M = 1.1; K = 1; \epsilon_1 = 0.23$; for the second reaction $a_2 = k_2 = 1; d_2 = 3; E_T = 1; S_2 T = 1; K_2 M = 4; K = 1; \epsilon_2 = 0.03$. Thus $\epsilon = \epsilon_1 = 0.23$. Though the value of ϵ is very close to its bound $1/4$, the approximation is sufficiently satisfactory.

4. The Center Manifold

Let us start again from (33):

$$(39) \quad \begin{cases} \frac{d\bar{s}_1}{d\tau} = -\epsilon c_1, \\ \frac{dc_1}{d\tau} = -(\eta_1 + \kappa_{1M}) c_1 + \bar{s}_1 + \sigma_1 \eta_1 c_1^2 + \\ \quad - \bar{s}_1 (\sigma_2 c_2 + \sigma_1 c_1) + \sigma_2 \eta_1 c_1 c_2, \\ \frac{d\bar{s}_2}{d\tau} = -\epsilon c_2 \frac{k_2 \eta_2}{k_1 \eta_1}, \\ \frac{dc_2}{d\tau} = \frac{a_2}{a_1} \left[-\left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) c_2 + \frac{\eta_1}{\eta_2} \bar{s}_2 \right] + \\ \quad + \frac{a_2}{a_1} \left[\eta_1 \sigma_2 c_2^2 - \sigma_1 \frac{\eta_1}{\eta_2} \bar{s}_2 c_1 - \frac{S_{2T}}{S_{1T}} \sigma_1 \bar{s}_2 c_2 + \sigma_1 \eta_1 c_1 c_2 \right]. \end{cases}$$

The origin $(0, 0, 0, 0)$ is a fixed point of the system (39).

We rewrite (39) in the form (14) and, to this aim, we operate the substitutions

$$(40) \quad y_1 = -(\eta_1 + \kappa_{1M})c_1 + \bar{s}_1 \Leftrightarrow c_1 = \frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}}$$

and

$$(41) \quad y_2 = -\left(\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}\right)c_2 + \frac{\eta_1}{\eta_2}\bar{s}_2 \Leftrightarrow c_2 = \frac{\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}}.$$

From (40) and (41) we have the new equations for y_1 and y_2 :

$$(42) \quad \frac{dy_1}{d\tau} = \frac{d\bar{s}_1}{d\tau} - (\eta_1 + \kappa_{1M})\frac{dc_1}{d\tau} = -\epsilon\frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}} +$$

$$- (\eta_1 + \kappa_{1M}) \left[y_1 + \sigma_1\eta_1 \left(\frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}} \right)^2 - \bar{s}_1 \left(\sigma_2 \frac{\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}} + \right. \right.$$

$$\left. \left. + \sigma_1 \frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}} \right) + \sigma_2\eta_1 \frac{(\bar{s}_1 - y_1) \left(\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2 \right)}{(\eta_1 + \kappa_{1M}) \left(\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M} \right)} \right].$$

$$(43) \quad \frac{dy_2}{d\tau} = \frac{\eta_1}{\eta_2} \frac{d\bar{s}_2}{d\tau} - \left(\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M} \right) \frac{dc_2}{d\tau} = -\frac{k_2}{k_1} \epsilon \frac{\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}} +$$

$$- \left(\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M} \right) \left[\frac{a_2}{a_1}y_2 + \frac{a_2}{a_1}\eta_1\sigma_2 \left(\frac{\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}} \right)^2 + \right.$$

$$- \frac{a_2}{a_1}\sigma_1 \frac{\eta_1}{\eta_2} \bar{s}_2 \frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}} - \frac{a_2}{a_1} \frac{S_{2T}}{S_{1T}} \sigma_1 \bar{s}_2 \frac{\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}} +$$

$$\left. + \frac{a_2}{a_1}\sigma_1\eta_1 \frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}} \frac{\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}} \right].$$

By combining equations (42) and (43) with those in $\frac{dc_1}{d\tau}$ and $\frac{dc_2}{d\tau}$ – where c_1, c_2 are replaced by (40), (41) – and applying the technique described in [8,9,21], the system (39) becomes

$$(44) \quad \begin{cases} \frac{d\bar{s}_1}{d\tau} = -\epsilon \frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}}, \\ \frac{d\bar{s}_2}{d\tau} = -\frac{k_2}{k_1} \frac{\eta_2}{\eta_1} \epsilon \frac{\frac{\eta_1}{\eta_2}\bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M}}, \\ \frac{dy_1}{d\tau} = -(\eta_1 + \kappa_{1M})y_1 + f_1(\bar{s}_1, \bar{s}_2, y_1, y_2, \epsilon), \\ \frac{dy_2}{d\tau} = -\frac{a_2}{a_1} \left(\eta_1 + \frac{K_{2M}}{K_{1M}}\kappa_{1M} \right) y_2 + f_2(\bar{s}_1, \bar{s}_2, y_1, y_2, \epsilon), \\ \frac{d\epsilon}{d\tau} = 0, \end{cases}$$

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where we have isolated the linear part in $(\bar{s}_1, \bar{s}_2, y_1, y_2, \epsilon)$ and

$$(45) \quad f_1(\bar{s}_1, \bar{s}_2, y_1, y_2, \epsilon) := -\epsilon \frac{\bar{s}_1 - y_1}{\eta_1 + \kappa_{1M}} - \sigma_1 \eta_1 \frac{(\bar{s}_1 - y_1)^2}{\eta_1 + \kappa_{1M}} + \\ + (\eta_1 + \kappa_{1M}) \sigma_2 \frac{\bar{s}_1 \left(\frac{\eta_1}{\eta_2} \bar{s}_2 - y_2 \right)}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} + \sigma_1 \bar{s}_1 (\bar{s}_1 - y_1) + \\ - \sigma_2 \eta_1 \frac{(\bar{s}_1 - y_1) \left(\frac{\eta_1}{\eta_2} \bar{s}_2 - y_2 \right)}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}},$$

and

$$(46) \quad f_2(\bar{s}_1, \bar{s}_2, y_1, y_2, \epsilon) := -\frac{k_2}{k_1} \epsilon \frac{\frac{\eta_1}{\eta_2} \bar{s}_2 - y_2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} - \frac{a_2}{a_1} \eta_1 \sigma_2 \frac{\left(\frac{\eta_1}{\eta_2} \bar{s}_2 - y_2 \right)^2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} + \\ + \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) \frac{a_2}{a_1} \sigma_1 \frac{\eta_1 \bar{s}_2 (\bar{s}_1 - y_1)}{\eta_2 (\eta_1 + \kappa_{1M})} + \\ + \frac{a_2}{a_1} \frac{S_{2T}}{S_{1T}} \sigma_1 \bar{s}_2 \left(\frac{\eta_1}{\eta_2} \bar{s}_2 - y_2 \right) - \frac{a_2}{a_1} \sigma_1 \eta_1 \frac{(\bar{s}_1 - y_1) \left(\frac{\eta_1}{\eta_2} \bar{s}_2 - y_2 \right)}{\eta_1 + \kappa_{1M}}.$$

(From here on out we will adopt the notation $\frac{dy}{d\tau} = \dot{y}$.)

Considering the equilibrium point $(0, 0, 0, 0, 0)$, the associated linearized system has a diagonal form and, moreover, the eigenvalues are given by 0 (with multiplicity $c = 3$) and

$$(47) \quad \lambda_1 = -(\eta_1 + \kappa_{1M}), \quad \lambda_2 = -\frac{a_2}{a_1} \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right).$$

All the eigenvalues are ≤ 0 . To find a center manifold, all we need to do is to solve (16) for system (44), employing Theorem 2.2, which gives us a method for computing an approximate solution of (16) to any desired degree of accuracy. So we search for a function $h(x, \epsilon)$ – where $h : \mathbb{R}^3 \rightarrow \mathbb{R}^2$ and $x = (\bar{s}_1, \bar{s}_2)$ – such that

$$h(x, \epsilon) = \begin{pmatrix} h_1(x, \epsilon) \\ h_2(x, \epsilon) \end{pmatrix}$$

and

$$(48) \quad h_1(\bar{s}_1, \bar{s}_2, \epsilon) = \alpha_1 \bar{s}_1^2 + \alpha_2 \bar{s}_2^2 + \alpha_3 \epsilon^2 + \alpha_4 \bar{s}_1 \bar{s}_2 + \alpha_5 \bar{s}_1 \epsilon + \alpha_6 \bar{s}_2 \epsilon + \dots$$

$$(49) \quad h_2(\bar{s}_1, \bar{s}_2, \epsilon) = \beta_1 \bar{s}_1^2 + \beta_2 \bar{s}_2^2 + \beta_3 \epsilon^2 + \beta_4 \bar{s}_1 \bar{s}_2 + \beta_5 \bar{s}_1 \epsilon + \beta_6 \bar{s}_2 \epsilon + \dots$$

Hence, by Definition 2.3, the *center manifold* is:

$$(50) \quad F := \begin{pmatrix} y_1(x, \epsilon) \\ y_2(x, \epsilon) \end{pmatrix} = \begin{pmatrix} h_1(x, \epsilon) \\ h_2(x, \epsilon) \end{pmatrix}.$$

Let $\xi = (\bar{s}_1, \bar{s}_2, \epsilon)$, we have that, from (48), (49) and (50):

$$(51) \quad f_1(\bar{s}_1, \bar{s}_2, \epsilon) = -\frac{\bar{s}_1 \epsilon}{\eta_1 + \kappa_{1M}} - \frac{\sigma_1 \eta_1 \bar{s}_1^2}{\eta_1 + \kappa_{1M}} + \\ + \frac{\frac{\eta_1}{\eta_2} \sigma_2 (\eta_1 + \kappa_{1M}) \bar{s}_1 \bar{s}_2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} + \sigma_1 \bar{s}_1^2 - \frac{\frac{\eta_1^2}{\eta_2} \sigma_2 \bar{s}_1 \bar{s}_2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} + o(|\xi|^2),$$

$$(52) \quad f_2(\bar{s}_1, \bar{s}_2, \epsilon) = -\frac{\frac{k_2}{k_1} \frac{\eta_1}{\eta_2} \bar{s}_2 \epsilon}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} - \frac{\frac{a_2}{a_1} \frac{\eta_1^3}{\eta_2^2} \sigma_2 \bar{s}_2^2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} + \\ + \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) \frac{a_2}{a_1} \frac{\eta_1}{\eta_2} \sigma_1 \frac{\bar{s}_1 \bar{s}_2}{\eta_1 + \kappa_{1M}} + \frac{a_2}{a_1} \frac{\eta_1}{\eta_2} \frac{S_{2T}}{S_{1T}} \sigma_1 \bar{s}_2^2 + \\ - \frac{a_2}{a_1} \sigma_1 \frac{\frac{\eta_1^2}{\eta_2} \bar{s}_1 \bar{s}_2}{\eta_1 + \kappa_{1M}} + o(|\xi|^2).$$

for $\xi \rightarrow 0$.

By definition of center manifold and from (47), we calculate

$$(53) \quad \dot{y}_1 = D_x h_1(x, \epsilon) \dot{x} + D_\epsilon h_1(x, \epsilon) \underbrace{\dot{\epsilon}}_{=0} = \lambda_1 y_1 + f_1(x, y_1, y_2)$$

and

$$(54) \quad \dot{y}_2 = D_x h_2(x, \epsilon) \dot{x} + D_\epsilon h_2(x, \epsilon) \underbrace{\dot{\epsilon}}_{=0} = \lambda_2 y_2 + f_2(x, y_1, y_2),$$

which are a consequence of (16).

We start from (53). It can be shown that

$$D_x h_1(x, \epsilon) \dot{x} = \frac{\partial h_1}{\partial \bar{s}_1} \dot{\bar{s}}_1 + \frac{\partial h_1}{\partial \bar{s}_2} \dot{\bar{s}}_2 = o(|\xi|^2)$$

for $\xi \rightarrow 0$, then (53) becomes

$$(55) \quad \lambda_1 y_1 + f_1(x, y_1, y_2) = o(|\xi|^2)$$

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or, from (48), (49),(50) and more explicitly:

$$\begin{aligned}
& - (\eta_1 + \kappa_{1M}) (\alpha_1 \bar{s}_1^2 + \alpha_2 \bar{s}_2^2 + \alpha_3 \epsilon^2 + \alpha_4 \bar{s}_1 \bar{s}_2 + \alpha_5 \bar{s}_1 \epsilon + \\
& + \alpha_6 \bar{s}_2 \epsilon + o(|\xi|^2)) - \frac{\bar{s}_1 \epsilon}{\eta_1 + \kappa_{1M}} - \frac{\sigma_1 \eta_1 \bar{s}_1^2}{\eta_1 + \kappa_{1M}} + \\
& + \frac{\frac{\eta_1}{\eta_2} \sigma_2 (\eta_1 + \kappa_{1M}) \bar{s}_1 \bar{s}_2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} + \sigma_1 \bar{s}_1^2 - \frac{\frac{\eta_1^2}{\eta_2} \sigma_2 \bar{s}_1 \bar{s}_2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} + o(|\xi|^2) = o(|\xi|^2).
\end{aligned}$$

Equating to zero terms of the same powers gives $\alpha_2 = \alpha_3 = \alpha_6 = 0$ and:

$$\begin{aligned}
& - (\eta_1 + \kappa_{1M}) \alpha_1 - \frac{\sigma_1 \eta_1}{\eta_1 + \kappa_{1M}} + \sigma_1 = 0 \\
& - (\eta_1 + \kappa_{1M}) \alpha_4 + \frac{\frac{\eta_1}{\eta_2} \sigma_2 (\eta_1 + \kappa_{1M})}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} - \frac{\frac{\eta_1^2}{\eta_2} \sigma_2}{\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M}} = 0 \\
& (\eta_1 + \kappa_{1M}) \alpha_5 + \frac{1}{\eta_1 + \kappa_{1M}} = 0,
\end{aligned}$$

from which

$$(56) \quad \alpha_1 = \frac{\kappa_{1M} \sigma_1}{(\eta_1 + \kappa_{1M})^2} \quad ; \quad \alpha_4 = \frac{\frac{\eta_1}{\eta_2} \sigma_2 \kappa_{1M}}{(\eta_1 + \kappa_{1M}) \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right)} \quad ; \quad \alpha_5 = -\frac{1}{(\eta_1 + \kappa_{1M})^2}.$$

Similar argumentations can be used to handle (54). At the end, we obtain *the center manifold* of (44)

$$\begin{aligned}
(57) \quad y_1(\bar{s}_1, \bar{s}_2, \epsilon) &= h_1(\bar{s}_1, \bar{s}_2, \epsilon) = \frac{\kappa_{1M} \sigma_1}{(\eta_1 + \kappa_{1M})^2} \bar{s}_1^2 + \\
& + \frac{\frac{\eta_1}{\eta_2} \sigma_2 \kappa_{1M}}{(\eta_1 + \kappa_{1M}) \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right)} \bar{s}_1 \bar{s}_2 - \frac{1}{(\eta_1 + \kappa_{1M})^2} \bar{s}_1 \epsilon + o(|\xi|^2).
\end{aligned}$$

(58)

$$\begin{aligned}
y_2(\bar{s}_1, \bar{s}_2, \epsilon) &= h_2(\bar{s}_1, \bar{s}_2, \epsilon) = \frac{\eta_1 \frac{S_{2T}}{S_{1T}} \sigma_1 \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) - \frac{\eta_1^2}{\eta_2} \sigma_2}{\eta_2 \left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right)^2} \bar{s}_2^2 + \\
& + \frac{\frac{\eta_1}{\eta_2} \sigma_1 \frac{K_{2M}}{K_{1M}} \kappa_{1M}}{\left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right) (\eta_1 + \kappa_{1M})} \bar{s}_1 \bar{s}_2 - \frac{\frac{a_1 k_2 \eta_1}{a_2 k_1 \eta_2}}{\left(\eta_1 + \frac{K_{2M}}{K_{1M}} \kappa_{1M} \right)^2} \bar{s}_2 \epsilon + o(|\xi|^2).
\end{aligned}$$

Figures 3 and 4 show the behavior of the center manifold and of the trajectories of the solutions and their tQSSAs in the phase space $((\bar{s}_1, \bar{s}_2, c_1)$ on the left and $(\bar{s}_1, \bar{s}_2, c_2)$ on the right), for $\epsilon = 0.23$. In Figure 4 we have rotated the center manifold, in order to show more clearly the asymptotic equivalence of the manifold and of the trajectories. While in the plots on the left we can observe that the trajectories of the points $(\bar{s}_1, \bar{s}_2, c_1)$ are asymptotically equivalent to the center manifold, as expected from the theory, in the plots on the right the comparison is absolutely unsatisfactory.

In [16] it was shown that for a single enzyme reaction the tQSSA is asymptotically equivalent to the center manifold of the system. For more complex reactions we still lack a mathematical justification of this fact which, however, seems to be a reasonable result, confirmed by the plots on the left.

We can however explain the apparently contradictory behavior in the plots on the right with the fact that in this case the value of ϵ is not sufficiently small to guarantee the applicability of the theory.

Presumably, the computation of corrections of higher order in ϵ could improve our results.

5. Conclusion and perspectives

In this paper we have applied Palsson theory related to the separation of time scales in linear enzyme reactions to the case of the inhibition mechanism. We were able to state sufficient conditions for the separation of variables in this case, too, determining an appropriate perturbation parameter ϵ , which becomes crucial for the application of Tihonov's Theorem. We have also explicitly computed the center manifold for this kind of systems and compared it with the trajectories of the solutions and of their tQSSAs. We observed that, even for high values of ϵ , the tQSSA well approximates the solution of the differential system governing the reactions. We related this fact to the consideration that the tQSSA can be seen as the application, under suitable hypotheses, of Tihonov theory for differential systems characterized by the presence of small parameters. As already observed in other papers (see, for example, [1,4–6]), any QSSA can be interpreted as the leading order term of an asymptotic expansion of the solutions with respect of an appropriate parameter. The unsatisfactory behavior of the center manifold and of the tQSSA with respect to the solution of the differential system can be corrected determining the successive terms at order ϵ . This will be subject of a future work. We will also study the bifurcation analysis of the differential system associated to the inhibition mechanism.

Palsson theory seems very promising in order to handle more complex

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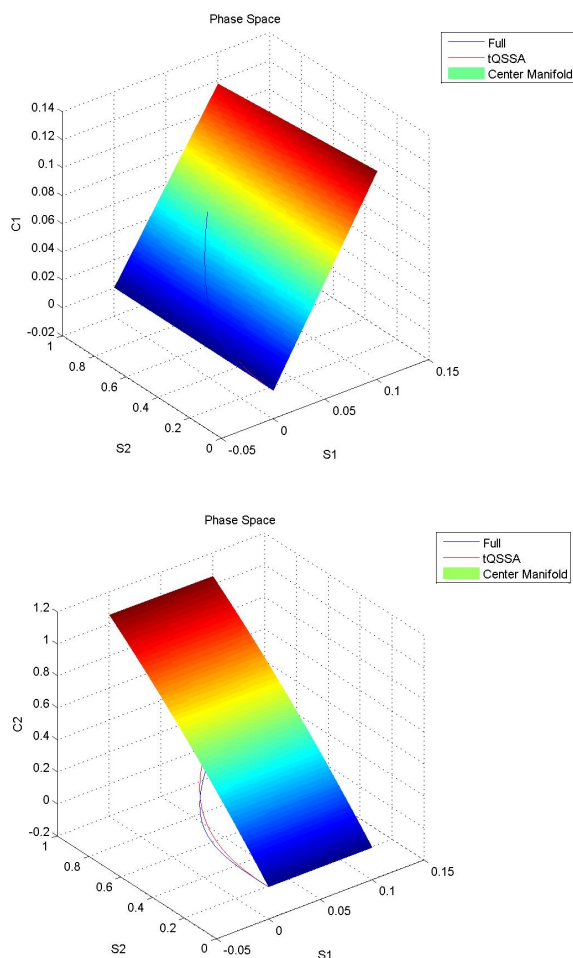


Figure 3. Comparison among the behavior of the center manifold, the trajectory of the solutions of the system (24) and of their tQSSAs for $(\bar{s}_1, \bar{s}_2, c_1)$ (left) and $(\bar{s}_1, \bar{s}_2, c_2)$ (right), in the stressed case $\epsilon = 0.23$. The center manifold is here clearly shown.

mechanisms by means of Tihonov theory and Center Manifold Theory. In a work in preparation, we are trying to apply these techniques to several mechanisms of phosphorylation and dephosphorylation, including cycles of single and double phosphorylation.

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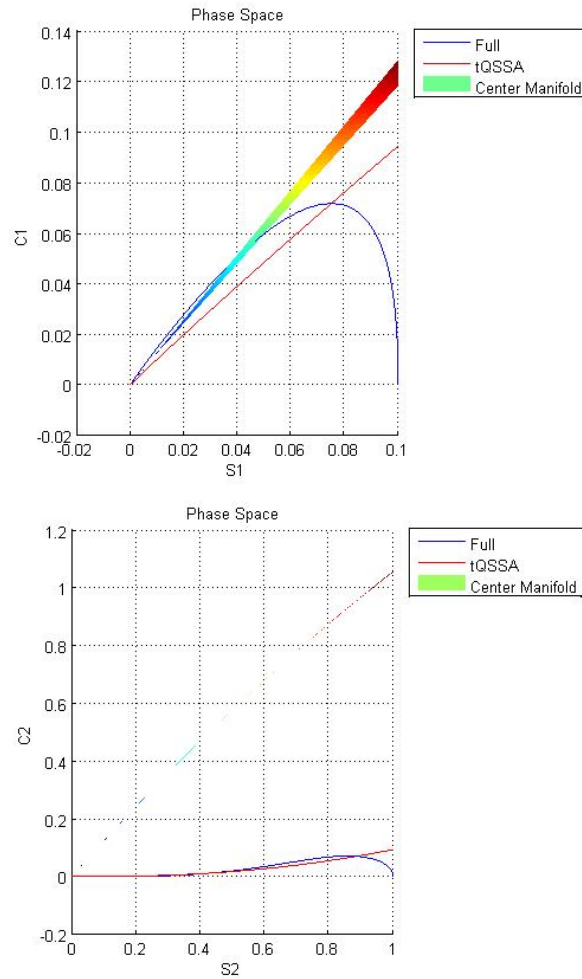


Figure 4. Comparison among the behavior of the center manifold, the trajectory of the solutions of the system (24) and of their tQSSAs for $(\bar{s}_1, \bar{s}_2, c_1)$ (left) and $(\bar{s}_1, \bar{s}_2, c_2)$ (right), in the stressed case $\epsilon = 0.23$. The plots are here rotated in order to show more clearly the trajectories. While in the plot on the left we can appreciate the convergence of the trajectories to the center manifold, in the plot on the right there are evident differences, which can be explained with the high value of the parameter ϵ .

(Olomouc, Czech Republic) for their translations of the papers [10–12] and some precious clarifications concerning some passages of the papers.

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