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VALIDITY OF THE QUASI STEADY STATE ASSUMPTION FOR ENZYME-CATALYSED REACTIONS WITH COMPETITIVE INHIBITION AND SUBSTRATE INPUT *

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Dedicated to Dr. Dan Tiba on the occasion of his 70^{th} anniversary

Abstract

Enzyme-catalysed reactions are chemical reactions within cells in which the rate of the reaction is significantly increased through the action of enzymes. They are usually part of large and complex biochemical networks, which form the central processing units of the living cell. Enzymatic reactions often operate on multiple time scales, which can be characterized as being either fast or slow. The quasi steady-state approximation (QSSA) utilizes time scale separation to project

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these complex models onto lower-dimensional slow manifolds. In this paper, we investigate the validity of a quasi steady-state assumption for enzyme-catalysed biochemical reactions with competitive inhibition that are subject to a constant substrate input. Necessary and sufficient conditions for the validity of these assumptions were derived and were shown to be dependent, among others, on the substrate input. The validity conditions are numerically verified using the classical Runge-Kutta method.

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1 Introduction

A biochemical reaction is a chemical transformation of one molecule into a different molecule inside a living cell. These reactions are mediated by enzymes, which are biological catalysts (in general, proteins) that help to convert specific molecules (which are called substrates) into products, without being themselves consumed in the process. The main functions of an enzyme are to speed-up and regulate the conversion of substrates into products by lowering the activation free energy of the reaction. An example of enzymatic reaction is the first reaction of the glycolysis (the conversion of glucose into pyruvate), which is catalysed by an enzyme called hexokinase.

Enzyme kinetics is the study of the binding affinities of other molecules (substrates or inhibitors) to enzymes, and the maximal catalytic rates that can be achieved. By understanding the kinetics of an enzyme, one can get insights into the catalytic mechanism of this enzyme, how its activity is controlled in the cell, and how specific drugs and poisons can inhibit its activity.

The kinetics of the enzymes do not follows the mass action kinetics directly, as also observed and studied by Michaelis and Menten in [8]. Their research on enzyme-catalysed reactions led them to propose that the formation of enzyme-substrate complex is a general mechanism of enzyme reactions, which is now known as the Michaelis-Menten formalism. The Michaelis-Menten model (represented in Figure 1) is the one of the simplest and best-known approaches to enzyme kinetics. This formulation considers that a substrate S binds reversibly to an enzyme E to form a complex C. The complex can decay irreversibly to form a product P and the en-

(a) (b)

Figure 1: *The schematic* (*a*) *and the symbolic* (*b*) *representations for the Michaelis-Menten mechanism*

zyme is released, being then able to bind another substrate molecule. Here, k_1, k_{-1} and k_2 are the kinetic parameters associated with the reaction rates. Michaelis and Menten assumed that the substrate concentration greatly exceeds the enzyme concentration ($E_0 \ll S_0$) and, furthermore, the substrate participate in an equilibrium with the enzyme-substrate complex which is not disturbed by product formation during the period that the initial rate of reaction is measured (i.e., $k_2 \ll k_{-1}$). Based on their assumptions, the rate of product formation has the form:

$$\nu_{MM} := \frac{dP}{dt} = \frac{v_{max}\, S}{S + K_S}, \tag{1}$$

where $v_{max} = k_2\, E_0$ is the maximum reaction velocity. Here, $K_S = k_{-1}/k_1$ is the dissociation constant of the enzyme-substrate complex. However, this model cannot be applied in general, as many enzyme-catalyzed reactions are likely to proceed at fast rates, enough to disturb such an equilibrium. One major critique of the Michaelis-Menten approach is that the assumption $k_2 \ll k_{-1}$ is not always valid, and thus the equilibrium is only an approximation. An alternative analysis of the system was undertaken by Briggs and Haldane in 1925 in [3], who introduced a more generally valid assumption. The main idea of their approach is that, after a short transient phase, the complex concentration remains fairly constant on the time-scale of product formation. This assumption is known in literature as *the standard quasi steady-state assumption* (sQSSA, or, simply, QSSA). Briggs and Haldane argued that, when the substrate concentration is much larger than the enzyme concentration, then the enzyme will be saturated with substrate and a complex formation rapidly occurs over a short period of time. The reaction cannot proceed any faster by adding more substrate. Based on their assumption, the production rate becomes

$$\nu_{BH} := \frac{dP}{dt} = \frac{v_{max}\, S}{S + K_M}, \tag{2}$$

where $v_{max} = k_2\, E_0$ is the maximum reaction velocity. Here, $K_M = \frac{k_{-1}+k_2}{k_1}$

is the so called Michaelis-Menten constant. It turns out that the Briggs-Haldane model has a similar form as the one derived by Michaelis and Menten, the only difference being the definition of the constant in the denominator. Therefore, in literature, the equation (2) retained the name of Michaelis-Menten equation, while the dissociation constant K_M is called the Michaelis-Menten constant. We see that the reaction rate v_{BH} increases with the substrate concentration S, and then asymptotically approaches its maximum rate v_{max}, which is attained when all enzyme sites are bound to substrate.

The quasi steady-state approximation for enzyme kinetics has the main advantage of reducing the dimensionality of the system of equations that govern the evolution of the system, and thus the numerical simulations becomes much faster. Moreover, while the kinetic constants are usually not known, finding the kinetic parameters characterizing the QSSA is a standard procedure in vitro biochemistry ([1]). There are also some other types of quasi steady-state approximations for enzyme kinetics that have been introduced in the last two decades, such as: the reversed quasi steady-state approximation (rQSSA, see [11]), which is valid for high enzyme concentrations, and the total quasi steady-state approximation (tQSSA, see [2]), which is valid for a broader range of parameters covering both high and low enzyme concentrations.

In general, the activity of enzymes may also be regulated by cofactors, inhibitors, or activators. Inhibitors are some specific molecules/chemicals that can regulate the action of enzymes by blocking some of their functions. The action of this inhibitors on enzymes is called enzyme inhibition. An inhibitor binds to an active site of an enzyme and decreases its compatibility with substrates, causing the inhibition of the enzyme-substrate complex formation. Enzyme inhibitors can occur naturally of they can be produced as pharmaceutical products. They can serve for a variety of purposes. For example, many inhibitors are used as drugs in medicine to treat diseases (such as allergies, influenza, cancer etc.) or to correct metabolic imbalances (e.g., Angiotensin-Converting Enzyme (ACE) inhibitors used to treat high blood pressure or heart problems). Inhibitors are also found in nature, as various poisons, which have grown to defend a plant or animal against predators, or they can be produced in laboratory to help in agriculture as pesticides and herbicides.

Enzyme inhibition is the reduction in enzyme activity caused by specific binding of a ligand (inhibitor) to a defined binding site at the enzyme, such as a catalytic or regulatory centre. Depending on the specific action of the inhibitor used, most precisely whether the enzyme-inhibitor complex disso-

ciates rapidly or very slowly, the enzyme inhibitions can be either reversible or irreversible. Also, there are three main mechanisms that are most commonly used to describe the binding of an inhibitor to a target enzyme: competitive inhibition, uncompetitive inhibition, and mixed (non-competitive) inhibition.

Competitive inhibition occurs when a substrate (S) and an inhibitor (I) both bind to the same site on the free enzyme (E), competing for the active site of the enzyme. In competitive inhibition it is assumed that all three chemical elements enter simultaneously. The enzyme inhibitor tries to bind to the same active site as the normal enzyme substrate, without undergoing a reaction. The binding of a competitive inhibitor and the binding of substrate are mutually exclusive events, meaning that the substrate molecule cannot enter the active site while the inhibitor is there, and the inhibitor cannot enter the site when the substrate is there. An enzyme-inhibitor complex (EI) is formed, in a similar manner to the enzyme-substrate (ES) complex. The degree of inhibition depends on the relative concentrations of the substrate and the inhibitor. However, the inhibitor does not necessarily have to bind to the same active site that the substrate would bind to. As long as the binding of the inhibitor prevents the binding of the substrate (before it has done so), the inhibition mechanism is competitive. This is also called allosteric inhibition. This can be observed by the binding of an inhibitor to a secondary site on the enzyme causing a conformational change in the structure such that the substrate cannot bind. Any given competitive inhibitor concentration can be overcome by increasing the substrate concentration in which case the substrate will outcompete the inhibitor in binding to the enzyme.

In the case of uncompetitive inhibition, the inhibitor does not combine with the free enzyme or affects its reaction with its normal substrate, but it combines with the enzyme-substrate complex. In this way, an inactive enzyme-substrate-inhibitor complex (ESI) is formed, which cannot undergo further reaction to yield a product.

Finally in the case of non-competitive (mixed) inhibition, the inhibitor (I) can combine with either the free enzyme (E) or the enzyme-substrate (ES) complex. Here, the inhibitor binds to a different site of the enzyme than the active site. For more details about enzymes, substrates and inhibitors one can consult, for example, ([1]).

In this paper, we consider an enzyme-substrate-inhibitor system supplied with a constant influx of substrate, of rate k_0. We assume here only a competitive inhibition mechanism, which means that the inhibitor binds exclusively to the free enzyme. This type of inhibition is the the most

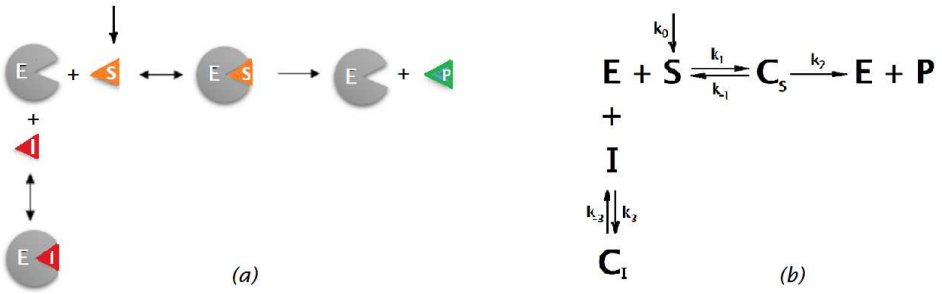

Figure 2: *The schematic* (a) *and the symbolic* (b) *representations for competitive inhibition with substrate input*

common type, in which the inhibitor competes with the natural substrate to overcome binding to the enzyme. An example of a use for a competitive inhibitor is in the treatment of influenza via a neuraminidase inhibitor, such as Tamiflu, Inavir or Relenza.

If we denote by E, S, I, C_S, C_I and P the free enzyme, the free substrate, the free inhibitor, the enzyme-substrate complex, the enzyme-inhibitor complex and the product, respectively, then the schematic representation of this biochemical process is represented in Figure 2, where k_0, k_1, k_{-1}, k_2, k_3 and k_{-3} are constant parameters associated with the rates of the reaction. Note that the concentrations of the reactants are changing in time. The main goals of the present work are to derive conditions that ensure the validity of the sQSSA for an enzyme-catalysed reaction with competitive inhibition and substrate input, and then investigate the effects of substrate input on the nature and validity of the sQSSA. The remaining of the paper is organized as follows. The next section contains a literature review on the validity conditions for various formulations of the quasi steady-state assumption. Section 3 introduces the model under discussion. In Section 4 we introduce and discuss the conditions that will ensure the validity of the sQSSA for the competitive inhibition model with substrate input. Section 5 contains some numerical tests to illustrate the effectiveness of the proposed criteria. Finally, some comments and ideas of future work are presented in the last section of the paper.

2 Short literature review on the validity conditions for sQSSA

The sQSSA is a mathematical technique used to reduce the dimension of the system of differential equations describing some chemical kinetic systems. It assumes that, after an initial fast transient or burst, the concentration of an intermediate molecular species is invariant. In the case of the scheme (1), the invariant species is the enzymesubstrate complex C. Based on this assumption, Briggs and Haldane derived the rate law (2). They also suggested that the criterion for the sQSSA to be valid is that the initial substrate concentration greatly exceeds the initial enzyme concentration, i.e., $E_0 \ll S_0$. Based on this criterion, Heineken et co. ([4]) have used a small parameter,

$$\varepsilon_{HTA} = \frac{E_0}{S_0}, \tag{3}$$

to develop the singular perturbation solution of the Michaelis-Menten rate equations to first order in the small parameter.

Using time scale arguments, Segel has shown in [10] that the sQSSA for the complex concentration C still remains valid under a less restrictive condition. This condition is $E_0 \ll S_0 + K_M$, which emerged from the following two criteria for the validity of the sQSSA:

- there must be only a negligible decrease in substrate concentration S during the duration of the brief transient;
- the brief transient is much shorter when compared to the time during which the substrate changes appreciably.

This new condition was employed in [11] to derive a dimensionless small parameter ε define by

$$\varepsilon_{SS} = \frac{E_0}{S_0 + K_M}, \tag{4}$$

based on which they proved the existence of a sQSS reduction as $\varepsilon_{SS} \to 0$. They have also investigated a new case in which the system of evolution equations can be reduced. It is the case when the enzyme concentration is high and, after a short transient, the substrate S is assumed to be in a quasi steady-state with the complex C. They referred to this situation as the reverse quasi steady-state approximation (rQSSA).

In [2], Borghans et al. were looking to extend the validity of the quasi steady-state approximation by considering the total substrate concentration, $S + C$. Then, by assuming that the complex C in a quasi steady-state,

they showed that the quasi steady-state approximation remains valid for a broader range of parameters, covering both high and low enzyme concentrations. They also suggested a new small parameter, namely

$$\varepsilon_{BBS} = \frac{k_2 E_0}{k_1 (E_0 + S_0 + K_M)^2}, \tag{5}$$

and the condition $\varepsilon_{BBS} \ll 1$ will ensure the validity of the total quasi steady-state assumption. They also argued that, when both the tQSSA and the rQSSA are valid, they yield virtually identical results. The benefit from the tQSSA is that it is valid in a strictly larger parameter domain than the rQSSA.

Starting from the ideas presented by Borghans et al. in [2], Tzafriri rederived in [15] the tQSSA, by retaining terms of order C^2 in the formulation of the time scales, and showed that the validity of the quasi steady-state approximation is much wider than the one obtained by Borghans et al., for both low or high enzyme concentrations. He also proposed a new rather complicated small parameter,

$$\varepsilon_T = \frac{k_2}{2k_1 S_0} \left(\frac{E_0 + S_0 + K_M}{\sqrt{(E_0 + S_0 + K_M)^2 - 4E_0 S_0}} - 1 \right), \tag{6}$$

based on which criteria for the validity of the QSSA can be derived.

The papers [12], [13] considered the Michaelis-Menten reaction mechanism supplied with a constant influx of substrate, I. The main motivation for considering an influx of substrate into the system is that, in general, enzymatic reactions take product molecules from the previous reaction step in a pathway and supply substrate to the next step. In these papers, the authors investigated the effects of a constant substrate input on the derivation and validity of sQSSA and rQSSA. As expected, it was shown that the necessary condition for the validity of the QSSA is relaxed by increasing the amount of substrate input, and the errors in product production rate decrease with increasing input. Based on the small parameter

$$\varepsilon_{SDL} = \frac{E_0}{S_0 + \widehat{S} + K_M}, \tag{7}$$

where $\widehat{S} = \frac{K_M I}{k_2 E_0}$, singular perturbation solutions were constructed and convergence results were established. In [14], this investigation was extended to a time-dependent input, showing that the QSSA established in [12], [13] remains a very good approximation to the full dynamics for a wide range

of input amplitudes and frequencies. This conclusion was also verified in [9] using an artificial neural network approach.

In [5], the authors investigated the conditions under which the stochastic QSSA is valid. They showed that the stochastic QSSA is valid under more restrictive conditions than its deterministic QSSA counterpart. They observed that different types of QSS approximations may provide similar results in the deterministic case, but give completely different results in the stochastic case. Therefore, the stochastic reduction cannot be inferred whenever its deterministic counterpart is valid. They argued that the validity of the stochastic QSSA relies on two assumptions: 1) the separation of time scales between the slow and fast reactions, and 2) the accurate approximation of the stochastic QSS by the deterministic QSS. Starting from some examples that are widely used in biological systems (e.g., the Michaelis-Menten kinetics and the Hill-type kinetics), Kim et al. derived in [6] some conditions for the validity of the stochastic QSSA. They showed that the stochastic QSSA is accurate only when the deterministic QSSA is accurate over a range of initial conditions that cover the most likely states explored by the stochastic system.

3 The competitive inhibition model with substrate input

The temporal behaviour of a reaction network in (2) can be conveniently modelled by a system of nonlinear ordinary differential equations that depend on a set of parameters, which are the kinetic rates. We shall use here the same notation for the chemical species and their corresponding concentrations. By employing the mass action kinetics, we arrive at the following system of equations:

$$\frac{dE}{dt} = -k_1\ E \cdot S + (k_{-1} + k_2)\ C_S - k_3\ E \cdot I + k_{-3}\ C_I, \tag{8}$$

$$\frac{dS}{dt} = k_0 - k_1\ E \cdot S + k_{-1}\ C_S, \tag{9}$$

$$\frac{dC_S}{dt} = k_1\ E \cdot S - (k_{-1} + k_2)\ C_S, \tag{10}$$

$$\frac{dI}{dt} = -k_3\ E \cdot I + k_{-3}\ C_I, \tag{11}$$

$$\frac{dC_I}{dt} = k_3\ E \cdot I - k_{-3}\ C_I, \tag{12}$$

$$\frac{dP}{dt} = k_2\ C_S. \tag{13}$$

The evolution of the concentrations involved in this system of equations is illustrated numerically in Figure 3 for a specific choice of initial data and rate parameters. Using the conservation relations that the chemical concentrations satisfy and some further assumptions, this system will be reduced to a lower dimensional one.
By adding equations (8), (10) and (12) on one side, and then the equations (11) and (12) on the other, we get the following conserved quantities:

$$E(t) + C_S(t) + C_I(t) = E_T, \tag{14}$$

$$I(t) + C_I(t) = I_T, \quad \forall t \geq 0. \tag{15}$$

Here, by E_T and I_T we have denoted the total enzyme and the total inhibitor concentrations. By using these conservation relations, the equations corresponding to the evolution of E and I can be eliminated, and the system can be reduced to a fourth order system of ODEs. Let us denote by $K_M = \dfrac{k_{-1} + k_2}{k_1}$ the affinity of the enzyme for its substrate, and $K_I = \dfrac{k_{-3}}{k_3}$ the affinity of the enzyme for the inhibitor. The reduced system of ODEs is

$$\frac{dS}{dt} = k_0 - k_1(E_T - C_S - C_I) \cdot S + k_{-1}\ C_S, \tag{16}$$

$$\frac{dC_S}{dt} = k_1[(E_T - C_S - C_I) \cdot S - K_M\ C_S], \tag{17}$$

$$\frac{dC_I}{dt} = k_3[(E_T - C_S - C_I) \cdot (I_T - C_I) - K_I\ C_I], \tag{18}$$

together with the uncoupled equation for product formation:

$$\frac{dP}{dt} = k_2\ C_S. \tag{19}$$

The rate of product formation, $\nu := \dfrac{dP}{dt}$ depends on the concentration of the inhibitor I in the following manner (see more details in [1]):

$$\nu = \frac{v_{max}\ S}{S + K_M\left(1 + \frac{I}{K_I}\right)},$$

where $v_{max} = k_2\, E_T$ is the maximum rate of reaction, which is obtained when all the enzyme is bound in the enzyme-substrate complex. We see that

the reaction rate is unaffected by the addition of substrate input, k_0, into the system. On the other side, the increase in the inhibitor concentration reduces the rate of reaction.
The steady states $(\widehat{S},\ \widehat{C}_S,\ \widehat{C}_I)$ of the system (16)−(18) are given by

$$\begin{aligned} k_0 &= k_1(E_T - \widehat{C}_S - \widehat{C}_I)\cdot\widehat{S} - k_{-1}\,\widehat{C}_S, && (20)\\ K_M\,\widehat{C}_S &= (E_T - \widehat{C}_S - \widehat{C}_I)\cdot\widehat{S}, && (21)\\ K_I\,\widehat{C}_I &= (E_T - \widehat{C}_S - \widehat{C}_I)\cdot(I_T - \widehat{C}_I). && (22) \end{aligned}$$

By adding (20) and (21), we get

$$\widehat{C}_S = \frac{k_0}{k_2}. \tag{23}$$

From (21), we get only one biochemically feasible solution (i.e., $0 < \widehat{C}_I < E_T$),

$$\widehat{C}_I = \frac{E_T + I_T + K_I}{2}\cdot\left[1 - \sqrt{1 - 4\frac{E_T\ I_T}{(E_T + I_T + K_I)^2}}\right].$$

(Note that the solution with the "+" sign is larger than E_T.). Then, from (21) we get

$$\widehat{S} = \frac{k_0 K_M}{k_2(E_T - \widehat{C}_I) - k_0}. \tag{24}$$

Thus, we have obtained a unique positive steady state $(\widehat{S},\ \widehat{C}_S,\ \widehat{C}_I)$ for the system (16)−(18). Now, let us choose some initial data for this system. Usually, the initial data for the system (8)−(13) is taken as:

$$E(0) = E_0,\ \ S(0) = S_0,\ \ C_S(0) = 0,\ \ I(0) = I_0,\ \ C_I(0) = 0,\ \ P(0) = 0,$$

but here we shall consider a different one. As also considered in [12], we assume that at first the system (8)−(13) is in its steady state

$$E(0) = E_0,\ \ S = \widehat{S},\ \ C_S = \widehat{C}_S,\ \ I(0) = I_0,\ \ C_I = \widehat{C}_I$$

and, for completeness, we consider that $P(0) = 0$. The reason for considering this initial product is as follows: with a constant, positive input, the product is being continually produced at a rate $k_2 C_S$, but we can assume that this product is being removed from the reaction at an equal rate, and therefore the net product concentration in the reaction at steady state is zero. It is then assumed that a perturbation to this system is made by introducing a further quantity of substrate, denoted by S_0, and dynamics of

the relaxation of the system back to the steady state are investigated. Thus, the appropriate initial data for the system (8)−(13) is

$$E(0) = E_0, \;\; S = S_0 + \widehat{S}, \;\; C_S = \widehat{C}_S, \;\; I(0) = I_0, \;\; C_I = \widehat{C}_I \;\text{ and } P(0) = 0. \tag{25}$$

Consequently, from the conservation relations (14) and (15) written for $t = 0$, we get that

$$E_T = E_0 + \widehat{C}_S + \widehat{C}_I$$

and

$$I_T = I_0 + \widehat{C}_I.$$

By substituting these relations into the system (16)−(18), we arrive at:

$$\frac{dS}{dt} = k_0 - k_1(E_0 + \widehat{C}_S + \widehat{C}_I - C_S - C_I) \cdot S + k_{-1}\, C_S, \tag{26}$$

$$\frac{dC_S}{dt} = k_1[(E_0 + \widehat{C}_S + \widehat{C}_I - C_S - C_I) \cdot S - K_M\, C_S], \tag{27}$$

$$\frac{dC_I}{dt} = k_3[(E_0 + \widehat{C}_S + \widehat{C}_I - C_S - C_I) \cdot (I_0 + \widehat{C}_I - C_I) - K_I\, C_I]. \tag{28}$$

The unique positive steady state $(\widehat{S},\ \widehat{C}_S,\ \widehat{C}_I)$ of the system (26)−(28) becomes:

$$\widehat{S} = \frac{k_0\, K_M}{k_2\, E_0}. \qquad \widehat{C}_S = \frac{k_0}{k_2} \quad \text{and} \quad \widehat{C}_I = \frac{E_0 \cdot I_0}{K_I}. \tag{29}$$

This steady state is asymptotically stable, as the Jacobian matrix evaluated at this state,

$$J(\widehat{S},\ \widehat{C}_S,\ \widehat{C}_I) = \begin{pmatrix} -k_1\, E_0 & k_{-1} & 0 \\ k_1\, E_0 & -k_{-1} - k_2 & 0 \\ 0 & 0 & -k_{-3} \end{pmatrix}$$

is negative definite.

For ease of comparison, we translate the variables such that the only steady state of the system is the trivial solution. Thus, we make the following change of variables:

$$u = S - \widehat{S}, \quad v = C_S - \widehat{C}_S, \quad w = \widehat{C}_I - C_I.$$

The system (26)−(28) becomes:

$$\frac{du}{dt} = -k_1(E_0 - v + w) \cdot u + (k_1\widehat{S} + k_{-1})\, v - k_1\widehat{S}w, \tag{30}$$

$$\frac{dv}{dt} = k_1(E_0 - v + w) \cdot u - k_1(\widehat{S} + K_M)\, v + k_1\widehat{S}w, \tag{31}$$

$$\frac{dw}{dt} = k_3\left[(I_0 + w) \cdot (v - w) - (E_0 + K_I)\, w\right]. \tag{32}$$

The initial condition for this system is

$$u(0) = S_0, \quad v(0) = 0, \quad w(0) = 0.$$

4 Validity conditions for sQSSA

The conditions that we shall introduce in this section are based on estimating relevant time scales for the evolution of the system (30)−(32). In [10] and [11], Segel and Slemrod showed that two time scales are important: $t_1 =$ the time that characterizes the duration of the initial fast transient, during which the initial substrate concentration and the initial inhibitor concentration are practically unaltered but the concentrations for the complexes that involve them go through a rapid change, and $t_2 =$ the time that characterizes the quasi steady-state period. After a fast transient phase, in which the substrate and the inhibitor concentrations are hardly changed from their initial values, the two formed complexes build up quickly and then they remain fairly constant for some time. We say that the system enters in a standard quasi steady-state (sQSS), in which the concentrations of the complexes C_S and C_I are assumed to be in a steady state with respect to the instantaneous substrate and inhibitor concentrations, respectively. In this case, we shall assume that $v' \approx 0$ and $w' \approx 0$. This implies that, in the sQSS, the quantities $v(t)$ and $w(t)$ defined above will be fairly constant, i.e., $v(t) \approx v_{SS}(u)$ and $w(t) \approx w_{SS}(u)$, and the evolution of the system (30) − (32) can be studied by means of the sQSSA

$$\frac{du}{dt} = -k_2 \; v_{SS}(u).$$

In [10], Segel proposed two criteria for the validity of the sQSSA:

(H_1) during the fast transient phase, there are minimal changes in the substrate and the inhibitor concentrations,

(H_2) the time scale for the complexes during the transient phase, t_1, should be much smaller than the time scale for changes in the substrate/inhibitor in the beginning of the quasi steady-state phase, t_2.

(I) the transient phase: During the transient, there are only small (not significant) changes in the initial substrate and inhibitor concentrations, S_0 and I_0, respectively. Thus, we may assume that $u(t) \approx S_0$ and $I(t) \approx I_0$. From the conservation relation for inhibitor, $I_0 + w = I$, we get that w is small. Also, the substrate complex did not change significantly during the

transient, meaning that v is also small at this stage. By setting $u = S_0$ and neglecting the terms involving $v \cdot w$ and w^2 in (31) and (32), we arrive at

$$\frac{dv}{dt} = -k_1 \left[(S_0 + \widehat{S} + K_M)\, v - (S_0 + \widehat{S})\, w - E_0 \cdot S_0 \right], \tag{33}$$

$$\frac{dw}{dt} = k_3 \left[I_0\, v - (E_0 + I_0 + K_I)\, w \right]. \tag{34}$$

To derive the transient time scale, we look at the eigenvalues for this system of equations. The eigenvalues satisfy the following second order algebraic equation:

$$\lambda^2 + \left[k_1 (S_0 + \widehat{S} + K_M) + k_3 (E_0 + I_0 + K_I) \right] \lambda \tag{35}$$
$$+ k_1 k_3 \left[(S_0 + \widehat{S} + K_M) \cdot (E_0 + I_0 + K_I) - I_0 (S_0 + \widehat{S}) \right] = 0.$$

As mentioned in [10], unless $I_0 (S_0 + \widehat{S}) \ll K_M \cdot K_I$, the two eigenvalues are roughly of the same magnitude,

$$\lambda_1 \approx \lambda_2 \approx \lambda = -k_1 (S_0 + \widehat{S} + K_M) - k_3 (E_0 + I_0 + K_I).$$

Thus, assuming that $I_0 (S_0 + \widehat{S})/(K_M \cdot K_I)$ is not too small, one can consider the approximation for the transient time scale to be

$$t_1 \approx -\frac{1}{\lambda} = \frac{1}{k_1 (S_0 + \widehat{S} + K_M) + k_3 (E_0 + I_0 + K_I)}. \tag{36}$$

Note that this time scale decreases (thus, the transient phase will be shorter) with the increase in the substrate input, as $\widehat{S} = \dfrac{k_0\ K_M}{k_2\ E_0}$. Thus, if the initial substrate and the initial inhibitor concentrations are to remain approximately constant during the transient period, then this time period will be a decreasing function of substrate input.

(II) the sQSS phase: At this stage, we assume that $\dfrac{dv}{dt} \approx 0 \approx \dfrac{dw}{dt}$ in equations (31) − (32). We get the $v-$nullcline,

$$v = \frac{(E_0 + w)u + \widehat{S}\, w}{u + \widehat{S} + K_M}, \tag{37}$$

and, respectively, the $w-$nullcline,

$$v = w + (E_0 + K_I)\frac{w}{w + I_0}. \tag{38}$$

These two nullclines are illustrated in Figure 4. By adding the equations (30) and the equation (31) in which $v' = 0$, the evolution equation for u becomes:

$$\frac{du}{dt} = -k_2 \frac{(E_0 + w)u + \widehat{S}\, w}{u + \widehat{S} + K_M}. \tag{39}$$

To estimate the time scales t_2^u, we use an equivalent formula to that proposed in [10], namely,

$$t_2^u = \frac{|u_{max} - u_{min}|}{|\frac{du}{dt}|_{max}^{QSS}},$$

where $|u_{max} - u_{min}| = S_0$ and $\left|\dfrac{du}{dt}\right|_{max}^{QSS}$ is the maximum absolute value in (39). This value is obtained for $u = S_0$ (as $\left|\dfrac{du}{dt}\right|$ is an increasing function with u) and $w = \dfrac{E_0 \cdot I_0}{K_I}$ (as $w < \widehat{C}_I$). Using these, we obtain that

$$t_2^u = \frac{S_0 + \widehat{S} + K_M}{k_2\ E_0 \left(1 + I_0 \cdot \frac{S_0 + \widehat{S}}{S_0\, K_I}\right)}. \tag{40}$$

Note that, when $I_0 = 0$ (no inhibitor), then we rediscover the time scale for the QSS in u (substrate) obtained in [12].
Similarly for t_2^I, we have that

$$t_2^I = \frac{|I_{max} - I_{min}|}{|\frac{dI}{dt}|_{max}^{QSS}},$$

where $|I_{max} - I_{min}| = I_0$ and $\left|\dfrac{dI}{dt}\right|_{max}^{QSS}$ is the maximum absolute change in the inhibitor concentration during the sQSS. Using the fact that $\dfrac{dI}{dt} = -\dfrac{dC_I}{dt} = \dfrac{dw}{dt}$, we find that $\left|\dfrac{dw}{dt}\right|_{max}^{QSS}$ is the maximum absolute value in

$$\frac{dw}{dt} = k_3\ [(I_0 + w) \cdot (v - w) - (E_0 + K_I)w]\,. \tag{41}$$

This value is obtained for $u = S_0$ and $w = \dfrac{E_0 \cdot I_0}{K_I}$. Using these, we obtain that

$$t_2^I = \frac{S_0 + \widehat{S} + K_M}{k_3\ E_0\ S_0}. \tag{42}$$

Let us consider the first condition for the sQSS, namely (H_1). To estimate the relative change in u, i.e. $\left|\frac{\Delta u}{S_0}\right|$ during the transient, we use the following formula

$$\left|\frac{\Delta u}{S_0}\right| \approx t_1 \frac{1}{S_0}\left|\frac{du}{dt}\right|_{max},$$

where $\left|\frac{du}{dt}\right|$ is calculated from formula (30) with $v = w = 0$. We get that

$$\left|\frac{\Delta u}{S_0}\right| \approx \frac{k_1\ E_0}{k_1(S_0 + \widehat{S} + K_M) + k_3(E_0 + I_0 + K_I)}.$$

Therefore, if we require $\left|\frac{\Delta u}{S_0}\right| \ll 1$, then

$$\frac{k_1\ E_0}{k_1(S_0 + \widehat{S} + K_M) + k_3(E_0 + I_0 + K_I)} \ll 1. \tag{43}$$

To estimate the relative change in inhibitor I, i.e. $\left|\frac{\Delta I}{I_0}\right|$ during the transient, we use the following formula

$$\left|\frac{\Delta I}{I_0}\right| \approx t_1 \frac{1}{I_0}\left|\frac{dI}{dt}\right|_{max},$$

where $\left|\frac{dI}{dt}\right| = \frac{dw}{dt}$ is calculated from formula (11) with $C_I = 0$ and $E \approx E_0$. We get that

$$\left|\frac{\Delta I}{I_0}\right| \approx \frac{k_3\ E_0}{k_1(S_0 + \widehat{S} + K_M) + k_3(E_0 + I_0 + K_I)}.$$

Therefore, if we require $\left|\frac{\Delta I}{I_0}\right| \ll 1$, then

$$\frac{k_3\ E_0}{k_1(S_0 + \widehat{S} + K_M) + k_3(E_0 + I_0 + K_I)} \ll 1. \tag{44}$$

So, if we require that the substrate and the inhibitor concentrations are relatively small during the transient, then from (43) and (44) we get that

$$\frac{\max\{k_1,\, k_3\} \cdot E_0}{k_1(S_0 + \widehat{S} + K_M) + k_3(E_0 + I_0 + K_I)} \ll 1. \tag{45}$$

Condition (H_2) requires $t_1 \ll t_2^u$. From (36) and (40) we arrive at

$$\frac{k_1\ E_0}{k_1(S_0+\widehat{S}+K_M)+k_3(E_0+I_0+K_I)} \ll \frac{k_1}{k_2}\frac{S_0+\widehat{S}+K_M}{\left(1+I_0\frac{S_0+\widehat{S}}{K_I\ S_0}\right)}. \tag{46}$$

However, for the usual choices of the kinetic rates and the initial concentrations, the relation (46) is weaker than relation (43), therefore (H_1) and (H_2) are satisfied if (43) is satisfied.
If we require $t_1 \ll t_2^I$, then from (36) and (42) we get

$$\frac{k_3\ E_0}{k_1(S_0+\widehat{S}+K_M)+k_3(E_0+I_0+K_I)} \ll 1+\frac{\widehat{S}+K_M}{S_0}. \tag{47}$$

However, we see that relation (47) is weaker than relation (44), therefore (H_1) and (H_2) are satisfied if (44) is satisfied.
Therefore, we conclude that (45) can be considered as a criterion for the QSSA. Note that when the substrate input rate k_0 is increased, then $\widehat{S}$ becomes larger, and the condition (45) becomes more relaxed. In this situation, the sQSSA is valid for a wider range of parameters and initial data. This situation is confirmed numerically in Figures 6 and 7. A small parameter can be derived from this condition, of the form

$$\varepsilon := \frac{t_1}{\max\{t_2^u,\, t_2^I\}}. \tag{48}$$

If the rates k_1 and k_3 are comparable, then we can rewrite conveniently the criterion (45) in the form

$$1+\frac{I_0+K_I}{E_0}+\frac{S_0+\widehat{S}+K_M}{E_0} \gg 1. \tag{49}$$

We see that there are two different conditions which can guarantee alone that (49) holds. For example, either of the following two conditions,

$$E_0 \ll I_0+K_I \qquad \text{or} \qquad E_0 \ll S_0+\widehat{S}+K_M \tag{50}$$

can guarantee the validity of the sQSSA. Also, bear in mind that the derivation of the criterion (45) was based on the assumption that the quantity $I_0\,(S_0+\widehat{S})/(K_M\cdot K_I)$ is not too small.
Note that the condition $E_0 \ll S_0+\widehat{S}+K_M$ has also been obtained in [12], guaranteeing the validity of the sQSSA when there is a constant substrate input k_0 but no inhibitor present in the system. We see that, if the amount

of substrate input k_0 is increased, then $\widehat{S}$ also increases and the sQSS condition becomes more relaxed, in the sense that it remains valid even if the initial substrate concentration S_0 is not significantly larger than the initial enzyme concentration E_0.
Furthermore, from the first relation in (50), we observe that the initial inhibitor concentration I_0 "supplements" the inhibitor dissociation constant K_I, and the quasi steady-state assumption would also be more accurate in the presence of larger initial concentration of inhibitor, as was also pointed out in [10]. However, the sQSSA may not be more relaxed when only the dissociation constant K_I is increased, as the term $I_0\,(S_0+\widehat{S})/(K_M\cdot K_I)$ could become too small, case in which the criterion (45) (and, subsequently, the condition (49)) might not hold.

5 Numerical results

This section contains numerical approximations for solutions to the system (30)−(32), with the aim of verifying the accuracy of the time scale estimates, the validity of the sQSS approximation and the criterion (45) for sQSSA.

Figure 3 depicts the time evolution of the species concentrations involved in the model (8)−(13) for a range of initial data and kinetic rates for which the condition (45) is satisfied. One can observe that, after a short transient, the two formed complexes, C_S and C_I, enter in a quasi steady-state regime. Finally, when the time goes to infinity, the chemical concentrations converge to a stable steady state.

Figure 4 illustrates the three-dimensional nullcline plot for the reduced model (30)−(32). The blue and the green surfaces show the v and w nullclines, respectively. The blue and the green dotted curves are the projections of the intersection of nullclines on the $u-w$ and $u-v$ planes, respectively. These dotted curves are the corresponding dotted curves drawn in Figure 5. The continuous red curve in Figure 4 represents the numerical solution of (30)−(32). We see that, after a very short transient (not visible from this perspective), this curve follows closely the QSS curve, which is represented by the intersection of the two surfaces. In Figure 4, we have represented by • the approximation of the time scale t_2 for the sQSS. This time scale is an estimate for the start time of the quasi steady-state phase, which seems to be a good approximation.

The plots drawn in Figure 5 illustrate the fact the intersection of the $v-$nullcline and the $w-$nullcline is a good approximation for the solutions to

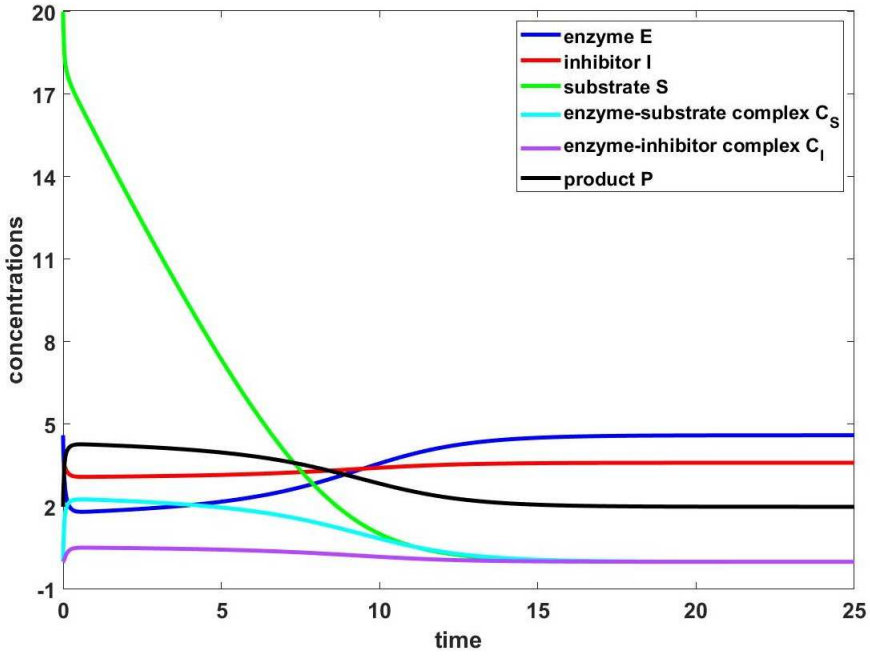

Figure 3: The time evolution of the chemical concentrations involved in the reaction network modelled by the system (8)−(13). Here $E_0 = 2,\ S_0 = 20,\ I_0 = 3$ and $k_0 = k_2 = 2,\ k_{-1} = k_2 = k_3 = 1,\ k_3 = 10$.

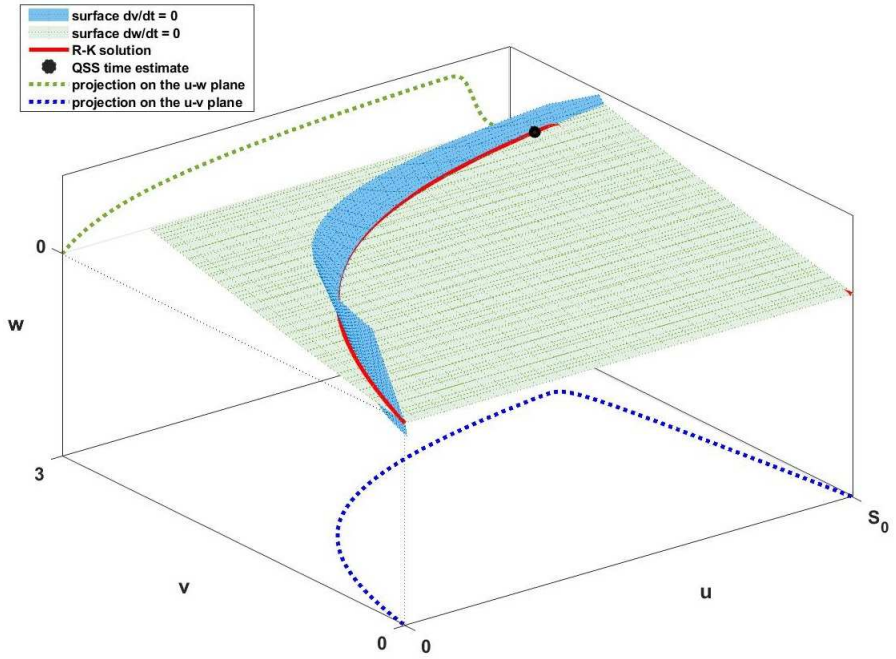

Figure 4: The validity of the standard QSSA for high initial substrate concentration. The continuous red curve represents the numerical solution of (30)−(32), and the two surfaces represent the sQSS nullclines. We indicate by • the estimated time scale t_2 of the QSS. Here $E_0 = 2,\ S_0 = 20,\ I_0 = 3$ and $k_0 = k_2 = 2,\ k_{-1} = k_2 = k_3 = 1,\ k_3 = 10$.

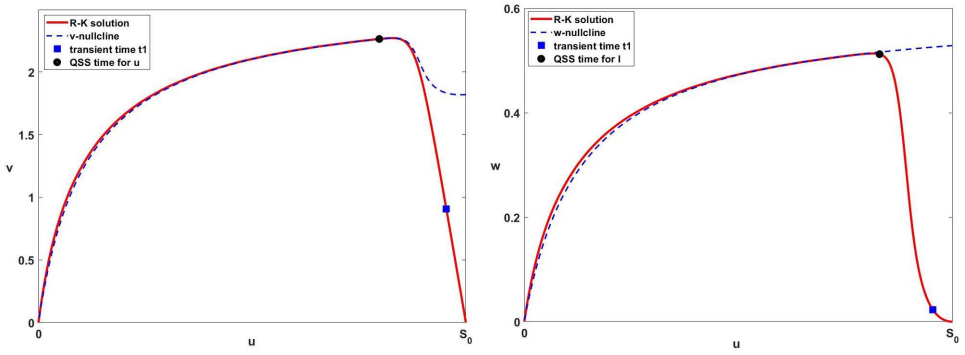

Figure 5: The validity of the standard QSSA for high initial substrate concentration. The continuous red curve represents the numerical solution of (30)−(32), and the dotted curve represents the QSS nullcline. We indicate by the small blue square the estimated time scale t_1 of the transient and by • the estimated time scale t_2 of the sQSS.
Here $E_0 = 2,\ S_0 = 20,\ I_0 = 3$ and $k_0 = k_2 = 2,\ k_{-1} = k_2 = k_3 = 1,\ k_3 = 10$.

the reduced model (30)−(32) at sufficiently large substrate concentrations. The dotted curves in these plots are the projections of the intersection of the nullcline surfaces illustrated in Figure 4. For a numerical validation of the time scales t_1, t_2^u and t_2^I given by the formulae (36), (40) and (42), respectively, we have also displayed their approximations in the figures. As one can observe, these time scales are in concordance with the expectations.

Figures 6 and 7 illustrate the effect of substrate input on the validity of the standard QSSA. Initially (the left plots in each of these two figures), we have chosen parameters such that for $k_0 = 0$ the necessary conditions for the QSSA are not satisfied. Then, a sufficient amount of substrate input is added into the network, and we observe that the solution trajectory will tend to follow closely each of the nullcline surfaces, thus the sQSSA of the system becomes valid. In fact, by increasing the amount of substrate input, the condition for the validity of the sQSSA becomes relaxed, in the sense that will become valid for a wider range of rate parameters. This fact can also be observed from the criterion (45).

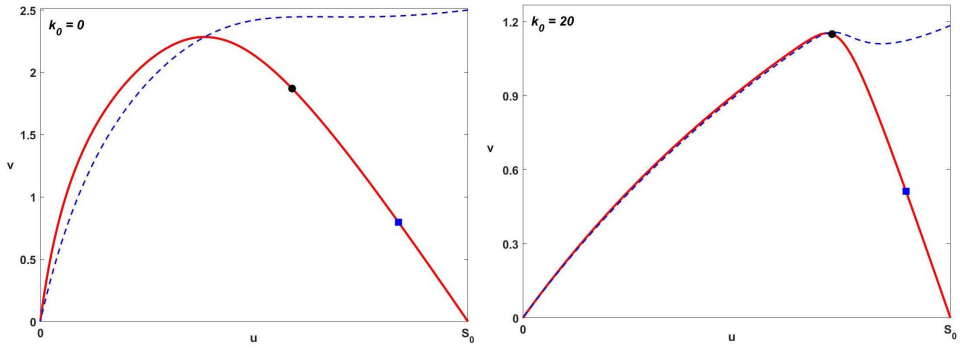

Figure 6: The effect of substrate input on the validity of the standard QSSA. The continuous red curve represents the numerical solution of (30)−(32), and the dotted blue curve is the projected sQSS curve. We indicate by small blue square the estimated time scale t_1 of the transient and by • the estimated time scale t_2 of the sQSS.
Here $E_0 = 3,\ S_0 = 5,\ I_0 = 3$ and $k_1 = 2,\ k_{-1} = k_2 = k_3 = 1,\ k_3 = 10$.

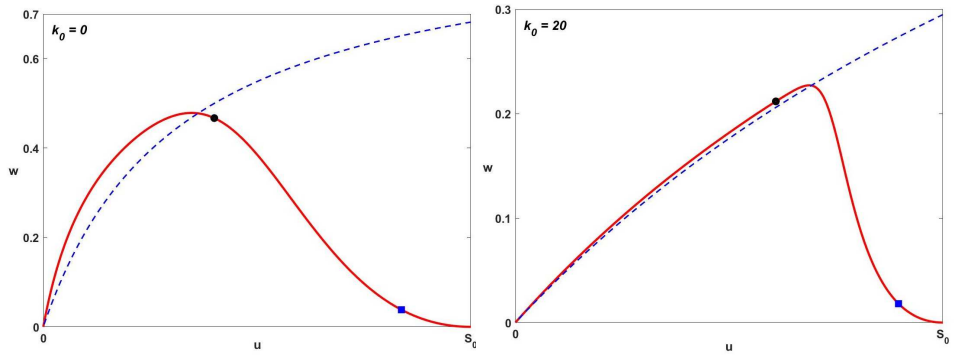

Figure 7: The effect of substrate input on the validity of the standard QSSA. The continuous red curve represents the numerical solution of (30)−(32), and the dotted blue curve is the projected sQSS curve. We indicate by small blue square the estimated time scale t_1 of the transient and by • the estimated time scale t_2 of the sQSS.
Here $E_0 = 3,\ S_0 = 5,\ I_0 = 3$ and $k_1 = 2,\ k_{-1} = k_2 = k_3 = 1,\ k_3 = 10$.

6 Conclusions

This work has concentrated on the derivation of Michaelis-Menten formalism for enzymatic reactions with competitive inhibition and a constant substrate input. The supply of a substrate input into the reaction network is motivated by the fact that, in general, enzymatic reactions take product molecules from the previous reaction step in a pathway and supply substrate to the next step. Therefore, our model will give a better description for the nature and conditions for validity of Michaelis-Menten formalism for enzymatic reactions *in vivo*.

Our investigation has focused on the derivation and the validity of the quasi steady state assumption, and the effects of an added constant substrate input on the sQSSA. The criterion for the sQSS was derived by estimating relevant time scales for the evolution of the system. More precisely, we have estimated the transient and the sQSS time scales corresponding to our model. The accuracy of the sQSSA depends on how well the time scales are estimated. Numerical tests indicate the fact that the time scale estimates are fairly accurate. Using these time scales, we have derived a criterion for the validity of the sQSSA, which was also confirmed numerically. Based on this criterion, we have observed that, by adding a sufficiently large amount of substrate input, the sQSSA becomes a better approximation for the solution trajectories of the model, even when the initial free enzyme and the substrate concentrations are comparable. Also, a good sQSSA for the system of equations can be obtained when either the initial substrate concentration or the initial inhibitor concentration are large enough.

However, our criterion is rather intricate, involving many variables or parameters, which makes it difficult to use the small parameter $\varepsilon := \dfrac{t_1}{\max\{t_2^u,\, t_2^I\}}$ in the approximation of the invariant manifold via perturbation techniques.

Further work can be done for enzyme-catalysed reactions with competitive inhibition and periodic substrate input. Also other inhibition mechanism, either uncompetitive or non-competitive, can be considered for this setting.

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