The Total Quasi-Steady-State for Multiple Alternative Substrate Reactions

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Abstract

The Michaelis-Menten-Brigs-Haldane approximation and its extension, the total quasi-steady-state approximations (tQSSA) are famous assumptions for simplifications of mathematical modeling of enzyme-substrate reactions. These approximations and their validity conditions are well studied for a single substrate reaction system. However, the extension of these studies for the tQSSA of the general case of multiple substrate reactions is yet to be performed precisely due to the consequent non-linear expressions for tQSSA. In this paper, we introduce a linearization method for equations governing the tQSSA of multiple substrate reactions to obtain an analytical solution for the evolution of concentrations of reactants that is valid throughout the whole time period. In addition, we provide the validity conditions of the tQSSA for multiple substrate reaction systems using the singular perturbation analysis method.

Keywords: Singular perturbation analysis; Linearization; Taylor series; Matrix methods ; Enzyme kinetics; Approximate solutions.

1 Introduction

The most well known enzyme-substrate reaction in biochemistry is the catalytic conversion of the substrate, S, into a product, P. This conversion happens through a system of reactions consisting of a reversible formation of enzyme-substrate complex, C, from the substrate and the enzyme, E, and irreversible decay of the complex to the product and the enzyme. The schematic representation of this reaction system is

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \overset{m_1}{\to} P + E. \tag{1}$$

where the mass-action kinetic parameters of the reactions in the scheme are denoted by k_1, k_{-1}, and m_1. Also, parameter $K_m = \frac{k_{-1}+m_1}{k_1}$ is usually called the Michaelis constant. The dynamic behavior of this network of reactions can be modeled by the application of the corresponding governing laws. The most common governing law for chemical reaction networks is the mass action kinetics principle, according to which, the rate of each reaction is proportional to product of the instantaneous concentrations of the substrates. Using conservation laws, the mathematical model for

the system of reactions (1) governed by mass action kinetics could be reduced to a set of coupled differential equations with two variables: the concentrations of the substrate (S) and the substrate-enzyme complex (C). However, the consequent mathematical equations are complicated to be solved analytically and require appropriate approximations for ease of analysis.

The hypothesis of quasi-steady-state approximation (QSSA) is a famous and standard simplification approach for the system of reactions (1). In this approximation scheme, under specific validity conditions, one of the variables in the differential equations is considered to be at a steady state after a small initial transient. There are different kinds of quasi-steady-state approximations that are based on the variable that is assumed to reach a quick steady state.

There are three commonly accepted quasi-steady-state approximations in the literature for the system of reactions (1). They are standard QSSA (sQSSA), reverse QSSA (rQSSA), and total QSSA (tQSSA). Each one of these approximations leads to a first order differential equation in one of the variables. The assumption in sQSSA is that the concentration of enzyme-substrate complex is assumed to be approximately constant after an initial fast transient time period. This approximation is traditionally accepted when the initial substrate concentration is high compared with the total enzyme concentration, i.e. $\mathtt{e_t} \ll \mathtt{s}$, where $\mathtt{e_t}$ is the total amount of enzyme, $\mathtt{s}$ is the initial value of the substrate concentration variable. However, Segel and Slemrod [1] showed that the traditional validity condition is too strong and in fact sQSSA is valid if $\mathtt{e_t} \ll \mathtt{s} + K_m$. In this approximation, it is assumed that the free enzyme is always saturated with high enough amount of substrate, that leads the substrate to be the only variable of the mathematical model. In the case of sQSSA the concentration of enzyme-substrate complex changes rapidly while concentration of free substrate variable remains constant in the initial fast transient period. This approximation is usually called Michaelis-Menten approximation since it was systemized by Michaelis and Menten in 1913 [2], and further developed by Briggs and Haldane [3]. In rQSSA [4], the hypothesis is the constancy of substrate concentration after an initial fast transient, and it is accepted when the enzyme amount is high compared with the substrate, i.e. $\mathtt{s} \ll \mathtt{e_t}$.

The total QSSA is an extension of sQSSA which is proven to expand the domain of parameters where it is valid [5]. In the case of tQSSA, it is assumed that in the initial fast transient period, the concentration of the enzyme-substrate complex (C) changes rapidly while concentration of total substrate, which is the summation of the free substrate (S) and the part engaged in the enzyme-substrate complex (C), remains constant. In the post-transient period, the concentration of the complex C is assumed to remain at a steady state as in the case of sQSSA. This approximation was initially developed in [5] by linearization of the mathematical model, however further developed in [6] using more accurate solutions of the mathematical equations, and in [7] providing a uniform solution based on the singular perturbation analysis in a total substrate framework.

In order to determine, which QSSA is the best approximation for the model, it is important to determine the precise range of parameters for which each of the three approximations is valid. The three quasi-steady-state approximations are valid after an initial fast transient time for three different ranges of parameters [6]. A key step to determine this range of parameters is to estimate the time scale of fast transient duration, t_{pre}, and the time scale of post-transient period, t_{post}. Then, the validity conditions of the approximation are obtained by imposing $t_{\text{pre}} \ll t_{\text{post}}$ [8]. This condition implies that in order to have validity of QSSA in the whole time period, the initial induction period prior to the steady state has to be much shorter than the time scale of depletion of the reactant that has very low concentration variation in the initial fast transient period.

The tQSSA is well studied for a single dimensional system (1) in terms of derivation of a mathematical model and its validity conditions [6]. These studies could be extended for higher dimensional systems: for example the simplest extension is for a system with two substrates which is called fully competitive enzyme reaction network with the

schematic representation of the corresponding mechanism given by

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \overset{m_1}{\rightarrow} P_1 + E$$
$$E + I \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} C_2 \overset{m_2}{\rightarrow} P_2 + E \tag{2}$$

The dynamic behavior of the higher dimensional models corresponding to this network is less studied due to its complicated mathematical description. For example in [9], the mathematical model of the system of reactions (2) is developed only for special ranges of parameters where analytical solutions are possible. This study was followed by [10], wherein the authors introduced a suitable perturbation parameter for the singular perturbation analysis of the system of reactions (2) and derived an explicit expression for an equivalent system of non-dimensional equations. Further they obtained the center manifold for the system of reactions (2) and provided sufficient conditions under which the tQSSA asymptotically approaches the obtained center manifold of the system. Additionally, in [11] utilizing the same perturbation parameter as introduced in [10], the authors extended the uniform solution of the singular perturbation analysis to the first order. Furthermore, these techniques have been applied to other mechanisms of double substrate systems of reactions such as Goldbeter-Koshland switch [12] and auxiliary enzyme reactions [13].
The system of reactions (1) could be further extended to a general n-dimensional system which is called the multiple alternative substrate system with the schematic representation of the corresponding mechanism given by

$$E + S_i \underset{k_{-i}}{\overset{k_i}{\rightleftharpoons}} C_i \overset{m_i}{\rightarrow} P_i + E; \qquad i = 1, \ldots, n. \tag{3}$$

In this article, a framework for the mathematical modeling of tQSSA is developed for a multiple alternative substrate system of reactions (3). In addition, the validity condition of tQSSA approximation is determined in terms of the parameters of the system of reactions (3). We use the singular perturbation analysis (SPA) method [14] for the determination of the validity conditions. In this method, we divide the time period of the dynamics of the system of reactions (3) into the initial fast transient part, and the succeeding slow post-transient part. The fast transient part is assumed to be a boundary layer of the time period, therefore it is called the inner part, and the time period after that is called the outer part. After derivation of a set of differential equations that govern the dynamics of the system of reactions (3), for each part of the time period we choose an appropriate time scale and a set of dimensionless variables to obtain the corresponding set of differential equations. We solve these differential equations separately with the initial condition of the system as the initial condition of the inner part, and the extreme value of the solution of the inner part as the initial condition of the outer part. Then we match these solutions in the boundary layer, and we combine the solutions of the inner part and the outer part to obtain a uniform solution that would be valid throughout the whole time period. For more detailed exposition on the singular perturbation analysis method, the reader is referred to [14].
The paper is organized as follows: In section 2, we provide the mathematical framework and previous studies with regard to single substrate reactions (1) and present the validity conditions for the tQSSA of system (1). In section 3, we derive the differential equations governing the multiple alternative substrate systems of reactions (3) and we determine the validity conditions of the tQSSA for the system of reactions (3) by application of the singular perturbation analysis method. In section 4, we perform simulations on mass action kinetics and tQSSA model of a triple substrate system of reactions and compare these simulations in order to investigate the obtained validity conditions of the tQSSA. In section 5, we compare the results of our method with the formerly published validity conditions of the tQSSA of multiple alternative substrate system, and we discuss the advantages of our approach in the derivation of the validity conditions of the tQSSA.

2 Preliminaries

In this section we explain the framework of the mathematical modeling and determination of tQSSA validity condition of the system (1) which is developed in [5]. To construct the mathematical model for the system of reactions (1), let e, s, c, p respectively denote the concentrations of E, S, C, P at any instant of time. Then, the governing equations based on mass action kinetics are

$$\begin{aligned} \frac{de}{dt} &= -k_1 es + k_{-1} c + m_1 c \\ \frac{ds}{dt} &= -k_1 es + k_{-1} c \\ \frac{dc}{dt} &= k_1 es - k_{-1} c - m_1 c \\ \frac{dp}{dt} &= m_1 c \end{aligned} \tag{4}$$

where the initial conditions of the variables are $e(0) = \mathtt{e_t}$, $s(0) = \mathtt{s}$, $c(0) = 0$ and $p(0) = 0$. The conservation law for the system (4) is given by $e + c = \mathtt{e_t}$. Using this conservation law, the system (4) can be reduced to

$$\begin{aligned} \frac{ds}{dt} &= -k_1(\mathtt{e_t} - c)s + k_{-1} c \\ \frac{dc}{dt} &= k_1(\mathtt{e_t} - c)s - (k_{-1} + m_1)c \end{aligned} \tag{5}$$

At this stage, we could apply the sQSSA, $\frac{dc}{dt} \approx 0$, which leads to a differential equation in the variable s and an expression for c in terms of s given by

$$\begin{aligned} \frac{ds}{dt} &= -\frac{m_1 \mathtt{e_t} s}{K_m + s} \\ c(s) &= \frac{\mathtt{e_t} s}{K_m + s} \end{aligned}$$

where $K_m = \frac{k_{-1}+m_1}{k_1}$. For derivation of tQSSA from equations (5) where the variables are s and c, we consider $\bar{s} = s + c$ and c as variables of interest leading to the differential equations

$$\begin{aligned} \frac{d\bar{s}}{dt} &= -m_1 c \\ \frac{1}{k_1}\frac{dc}{dt} &= c^2 - (\mathtt{e_t} + K_m + \bar{s})c + \mathtt{e_t}\bar{s} \end{aligned} \tag{6}$$

The second equation in (6) could be written in the following form

$$\frac{dc}{dt} = k_1(c - c_+(\bar{s}))(c - c_-(\bar{s})) \tag{7}$$

where

$$c_\pm(\bar{s}) = \frac{\mathtt{e_t} + K_m + \bar{s}}{2}\left(1 \pm \sqrt{1 - \frac{4\mathtt{e_t}\bar{s}}{(\mathtt{e_t} + K_m + \bar{s})^2}}\right) \tag{8}$$

are roots of the following quadratic equation in c

$$c^2 - (\mathtt{e_t} + K_m + \bar{s})c + \mathtt{e_t}\bar{s} = 0 \tag{9}$$

With the assumptions that during the initial transient period the substrate concentration remains at the initial condition, i.e. $\bar{s} \approx \mathtt{s}$, and if the time scale of the initial transient period is much smaller than that of the post-transient

period, the approximate solution of equation (7) for the whole time period, as mentioned in [6] and [7], is

$$c \approx c_-(\bar{s}) \left(\frac{1 - e^{-\frac{t}{t_c}}}{1 - \frac{c_-(\mathtt{s})}{c_+(\mathtt{s})} e^{-\frac{t}{t_c}}} \right)$$

where

$$t_c = \frac{1}{k_1(c_+(\mathtt{s}) - c_-(\mathtt{s}))} \tag{10}$$

Alternatively, the equations (6) could be simplified by the application of the tQSSA assumption, $\frac{dc}{dt} \approx 0$, which gives equation (9). Then, tQSSA approximation is

$$\frac{d\bar{s}}{dt} = -m_1 c_-(\bar{s}) \tag{11}$$

2.1 Validity conditions of tQSSA

The time scale t_s for the dynamics of the post-transient part of the system of reactions (1) can be approximated as follows

$$t_s \approx \frac{\bar{s}|_{\mathtt{max}} - \bar{s}|_{\mathtt{min}}}{\frac{d\bar{s}}{dt}|_{\mathtt{max}}}. \tag{12}$$

Since the maximum possible value of the substrate concentration is its initial value, from the first part of the equation (6), it follows that

$$t_s = \frac{\mathtt{s}}{m_1 c_-(\mathtt{s})} \tag{13}$$

Equations (8) and (10) can be further expanded to

$$\begin{aligned} c_+ &= (\mathtt{e_t} + K_m + \bar{s})[(1 - \frac{r(\bar{s})}{4}) + O(r^2(\bar{s}))] \\ c_- &= \frac{\mathtt{e_t}\bar{s}}{\mathtt{e_t} + K_m + \bar{s}} + O(r^2(\bar{s}))(\mathtt{e_t} + K_m + \bar{s}) \\ t_c &= \frac{1}{k_1(\mathtt{e_t} + K_m + \mathtt{s})(1 - \frac{r(\mathtt{s})}{2})} + O(r^2(\mathtt{s})) \end{aligned} \tag{14}$$

where $r(\bar{s}) = \frac{4\mathtt{e_t}\bar{s}}{(\mathtt{e_t} + K_m + \bar{s})^2}$. From (14), it follows that when $r \ll 1$, the expression for c_- is equivalent to the solution of (9) after linearization by ignoring c^2 term. With this condition, the tQSSA model in equation (11) becomes

$$\frac{d\bar{s}}{dt} = -\frac{m_1 \mathtt{e_t}\bar{s}}{\mathtt{e_t} + K_m + \bar{s}} \tag{15}$$

Also, from equations (13) and (14) when $r \ll 1$, the validity condition of tQSSA, $t_c \ll t_s$, gives

$$\frac{m_1 \mathtt{e_t}}{k_1(\mathtt{e_t} + K_m + \mathtt{s})^2} \ll 1 \tag{16}$$

Equation (16) can be rewritten in the form

$$\left(1 + \frac{\mathtt{e_t} + \mathtt{s}}{\frac{m_1}{k_1}} + \frac{k_{-1}}{m_1}\right)\left(1 + \frac{\mathtt{s} + K_m}{\mathtt{e_t}}\right) \gg 1 \tag{17}$$

From equation (17), the following sub-conditions could be derived, any of which guarantees validity of tQSSA.

$$\mathtt{e_t} + \mathtt{s} \gg \frac{m_1}{k_1}, \qquad k_{-1} \gg m_1, \qquad \mathtt{s} + K_m \gg \mathtt{e_t}.$$

Therefore, it appears that the tQSSA is valid for a range of parameters considerably larger than the sQSSA [5].

3 tQSSA for multiple substrate systems

For the system of reactions (3), if e, s_i, c_i respectively denote the concentrations of E, S_i, C_i at any instant of time, the following conservation law is valid for this network

$$e + \sum_{i=1}^{n} c_i = \mathtt{e_t} \tag{18}$$

where e_t is a constant. Then, the equations describing the dynamics of the system (3), using mass action kinetics and the conservation law (18) are

$$\begin{aligned}
\frac{ds_i}{dt} &= -k_i \left(\mathtt{e_t} - \sum_{j=1}^{n} c_j \right) s_i + k_{-i} c_i \\
\frac{dc_i}{dt} &= k_i \left(\mathtt{e_t} - \sum_{j=1}^{n} c_j \right) s_i - (k_{-i} + m_i) c_i
\end{aligned}$$

The equations with $\bar{s}_i = s_i + c_i$, and c_i as the variables of interest may be derived as follows

$$\begin{aligned}
\frac{d\bar{s}_i}{dt} &= -m_i c_i \\
\frac{dc_i}{dt} &= k_i \left(\bar{s}_i - c_i \right) \left(\mathtt{e_t} - \sum_{j=1}^{n} c_j \right) - (k_{-i} + m_i) c_i
\end{aligned} \tag{19}$$

For the application of SPA method, we choose $t_s = \alpha^{-1}$ as the time scale of the slow dynamics and $t_c = \beta^{-1}$ as the time scale of the fast dynamics; Thus let $\tau := \beta t$ and $T := \alpha t$ denote the non-dimensional time variables for the inner and outer solutions of SPA, respectively. We further non-dimensionalize the other variables by scaling using appropriate constants; Let $K_i := \frac{k_{-i}+m_i}{k_i}$ denote the Michaelis constant of the i^{th} reaction in (3). Define $s_i^* := \frac{\bar{s}_i}{K_i}$, $c_i^* := \frac{c_i}{\mathtt{e_t}}$ and $\epsilon := \frac{t_c}{t_s} = \frac{\alpha}{\beta}$. Using these new variables, the governing equations for the initial fast transient part of the multiple alternative substrate system are

$$\begin{aligned}
\frac{ds_i^*}{d\tau} &= -\epsilon \frac{m_i}{K_i} \frac{\mathtt{e_t}}{\alpha} c_i^* \\
\frac{dc_i^*}{d\tau} &= \frac{k_i K_i}{\beta} \left[\left(s_i^* - \frac{\mathtt{e_t}}{K_i} c_i^* \right) \left(1 - \sum_{j=1}^{n} c_j^* \right) - c_i^* \right],
\end{aligned} \tag{20}$$

and the equations for post-transient part are

$$\begin{aligned}
\frac{ds_i^*}{dT} &= -\frac{m_i}{K_i} \frac{\mathtt{e_t}}{\alpha} c_i^* \\
\epsilon \frac{dc_i^*}{dT} &= \frac{k_i K_i}{\beta} \left[\left(s_i^* - \frac{\mathtt{e_t}}{K_i} c_i^* \right) \left(1 - \sum_{j=1}^{n} c_j^* \right) - c_i^* \right].
\end{aligned} \tag{21}$$

Consider the solution of (20) to be of the form

$$\begin{aligned}
\mathcal{S}_i &= \mathcal{S}_{i,0} + \epsilon \mathcal{S}_{i,1} + \epsilon^2 \mathcal{S}_{i,2} + \cdots \\
\mathcal{C}_i &= \mathcal{C}_{i,0} + \epsilon \mathcal{C}_{i,1} + \epsilon^2 \mathcal{C}_{i,2} + \cdots
\end{aligned} \tag{22}$$

and the solution of (21) to be of the form

$$\begin{aligned}
\mathfrak{s}_i &= \mathfrak{s}_{i,0} + \epsilon \mathfrak{s}_{i,1} + \epsilon^2 \mathfrak{s}_{i,2} + \cdots \\
\mathfrak{c}_i &= \mathfrak{c}_{i,0} + \epsilon \mathfrak{c}_{i,1} + \epsilon^2 \mathfrak{c}_{i,2} + \cdots .
\end{aligned} \tag{23}$$

We make the following assumptions in order to ensure that equations (20) and (21) are the governing equations for the inner and outer solutions of tQSSA:
(i): variables $\mathcal{S}_{i,j}$ and $\mathcal{C}_{i,j}$ are regular functions of τ, and $\mathfrak{s}_{i,j}$ and $\mathfrak{c}_{i,j}$ are infinitely differentiable functions of T.
(ii): we are looking for a continuous function $\alpha(\mathtt{e_t})$ such that $\lim\limits_{\mathtt{e_t}\to 0}\dfrac{\alpha}{\mathtt{e_t}} = L_1$ where $0 < L_1 < \infty$.
(iii): we are looking for a continuous function $\beta(\mathtt{e_t})$ such that $\lim\limits_{\mathtt{e_t}\to 0}\beta = L_2$ where $0 < L_2 < \infty$.
From the above assumptions, it follows that $\lim\limits_{\mathtt{e_t}\to 0}\alpha = 0$ and $\lim\limits_{\mathtt{e_t}\to 0}\epsilon = 0$. Since enzyme is a catalyst for the slow dynamics involving the ultimate conversion of the substrate to product, assumption (ii) has been made to suggest that the time scale of the slow dynamics increases as the total enzyme concentration, which is the same as the initial enzyme concentration, decreases. Assumption (iii) is made to suggest that the initial fast transient that involves the complex C attaining a steady state, has a time scale that is more or less a constant for very low values of initial enzyme concentration.

We now determine expressions for $\mathcal{S}_{i,0}$ and $\mathcal{C}_{i,0}$. In order to do so, we substitute (22) in (20), and take limit as $\mathtt{e_t} \to 0$. This gives us the following equations

$$\begin{aligned} \mathcal{S}_{i,0} &= \mathfrak{s}_i^* \\ \frac{d\mathcal{C}_{i,0}}{d\tau} &= \frac{k_i K_i}{\beta}\left[\left(\mathfrak{s}_i^* - \frac{\mathtt{e_t}}{K_i}\mathcal{C}_{i,0}\right)\left(1 - \sum_{j=1}^{n}\mathcal{C}_{j,0}\right) - \mathcal{C}_{i,0}\right], \end{aligned} \tag{24}$$

where $\mathfrak{s}_i^*$ is value of $\mathcal{S}_{i,0}$ at time $t = 0$. This agrees with the assumption of tQSSA mentioned in the introduction according to which the total substrate concentrations remain constant in the initial fast transient period. Similarly, in order to determine expressions for $\mathfrak{s}_{i,0}$ and $\mathfrak{c}_{i,0}$, substitute (23) in (21), and take limit as $\mathtt{e_t} \to 0$. This gives us the following equations

$$\begin{aligned} \frac{d\mathfrak{s}_{i,0}}{dT} &= -\frac{m_i}{K_i}\frac{\mathtt{e_t}}{\alpha}\mathfrak{c}_{i,0} \\ \left(\mathfrak{s}_{i,0} - \frac{\mathtt{e_t}}{K_i}\mathfrak{c}_{i,0}\right)\left(1 - \sum_{j=1}^{n}\mathfrak{c}_{j,0}\right) - \mathfrak{c}_{i,0} &= 0, \end{aligned} \tag{25}$$

in agreement with the assumption of tQSSA according to which the concentrations of intermediate complexes remain at a steady state in the post-transient period. The second set of equations (25) is a set of nonlinear equations. By ignoring higher powers of the enzyme-substrate complex concentration, it is possible to linearize the equations and solve them using matrix methods. Note that the linearization is valid if $\sum\limits_{j=1}^{n}\mathfrak{s}_{i,0}\mathfrak{c}_{j,0}$ is very large in magnitude when compared with $\sum\limits_{j=1}^{n}\dfrac{\mathtt{e_t}}{K_i}\mathfrak{c}_{i,0}\mathfrak{c}_{j,0}$, i.e. if

$$\frac{\mathtt{e_t}}{K_i}\mathfrak{c}_{i,0} \ll \mathfrak{s}_{i,0}; \qquad i = 1, \ldots, n. \tag{26}$$

After linearization, the second set of equations in (25) can be rewritten as

$$\mathfrak{s}_{i,0} - \frac{\mathtt{e_t}}{K_i}\mathfrak{c}_{i,0} - \mathfrak{s}_{i,0}\sum_{j=1}^{n}\mathfrak{c}_{j,0} = \mathfrak{c}_{i,0} \tag{27}$$

Defining $\xi := 1 - \sum\limits_{j=1}^{n}\mathfrak{c}_{j,0}$ and substituting in (27), we get $\sum\limits_{j=1}^{n}\mathfrak{c}_{j,0} = \xi\sum\limits_{j=1}^{n}\dfrac{\mathfrak{s}_{j,0}}{1+\frac{\mathtt{e_t}}{K_j}}$. Since $\xi + \sum\limits_{j=1}^{n}\mathfrak{c}_{j,0} = 1$, the solution

of equations (27) is

$$\mathfrak{c}_{i,0} = \frac{\frac{\mathfrak{s}_{i,0}}{1+\frac{\mathfrak{e}_\mathfrak{t}}{K_i}}}{1+\sum_{j=1}^{n}\frac{\mathfrak{s}_{j,0}}{1+\frac{\mathfrak{e}_\mathfrak{t}}{K_j}}}, \tag{28}$$

for $i = 1, \ldots, n$. Substituting in (26), the validity condition for linearization becomes

$$\max_{i=1}^{n}\left(\frac{\frac{1}{1+\frac{K_i}{\mathfrak{e}_\mathfrak{t}}}}{1+\sum_{j=1}^{n}\frac{K_j\mathfrak{s}_{j,0}}{\mathfrak{e}_\mathfrak{t}+K_j}}\right) \ll 1, \tag{29}$$

Also, the first set of equations in (25) can now be written as

$$\frac{d\mathfrak{s}_{i,0}}{dT} = -\frac{\frac{m_i}{\alpha}}{1+\frac{K_i}{\mathfrak{e}_\mathfrak{t}}}\frac{\mathfrak{s}_{i,0}}{1+\sum_{j=1}^{n}\frac{\mathfrak{s}_{j,0}}{1+\frac{\mathfrak{e}_\mathfrak{t}}{K_j}}} \tag{30}$$

Define $\delta_i := \frac{\frac{m_i}{\alpha}}{1+\frac{K_i}{\mathfrak{e}_\mathfrak{t}}}$ and $\delta_{ij} := \frac{\delta_i}{\delta_j}$. From equation (30), it follows that

$$\frac{d\mathfrak{s}_{i,0}}{d\mathfrak{s}_{j,0}} = \delta_{ij}\frac{\mathfrak{s}_{i,0}}{\mathfrak{s}_{j,0}} \tag{31}$$

Equation (31) is integrable to

$$\frac{\mathfrak{s}_{j,0}}{\mathfrak{s}_j^*} = \left(\frac{\mathfrak{s}_{i,0}}{\mathfrak{s}_i^*}\right)^{\delta_{ij}} \tag{32}$$

By substitution of (32) in (30), as explained in [15], we get

$$\frac{d\mathfrak{s}_{i,0}}{dT} = \frac{-\delta_i\mathfrak{s}_{i,0}}{\left(1+\sum_{j=1}^{n}\mathfrak{s}_j^*\left(\frac{\mathfrak{s}_{i,0}}{\mathfrak{s}_i^*}\right)^{\delta_{ij}}\right)} =: g(\mathfrak{s}_{i,0}), \tag{33}$$

where g is a regular function of $\mathfrak{s}_{i,0}$. Differentiating the above equation with respect to T gives

$$\frac{d^2\mathfrak{s}_{i,0}}{dT^2} = g'(\mathfrak{s}_{i,0})\frac{d\mathfrak{s}_{i,0}}{dT}$$

where $g' := \frac{dg}{d\mathfrak{s}_{i,0}}$. By successively differentiating the above equation with respect to T, it can be verified that $\frac{d\mathfrak{s}_{i,0}}{dT}$ is a factor of $\frac{d^k\mathfrak{s}_{i,0}}{dT^k}$ for every natural number k. This fact can be used to construct the formal Taylor series expansion for $\mathfrak{s}_{i,0}$ given by $\mathfrak{s}_{i,0} = \mathfrak{s}_{i,0}\Big|_{T=0} + \sum_{j=1}^{\infty}\frac{d^j\mathfrak{s}_{i,0}}{dT^j}\Big|_{T=0}T^j$, by noting that $\mathfrak{s}_{i,0}$ is an infinitely differentiable function of T. Since $\mathfrak{s}_{i,0}(0) = \mathfrak{s}_i^*$, we obtain

$$\mathfrak{s}_{i,0} = \mathfrak{s}_i^* - \frac{\delta_i\mathfrak{s}_i^*}{1+\sum_{j=1}^{n}\frac{\mathfrak{s}_j^*}{1+\frac{\mathfrak{e}_\mathfrak{t}}{K_j}}}T\left(1+\sum_{k=1}^{\infty}\eta_{ik}T^k\right)$$

where $\eta_{ik} \in \mathbb{R}$ for $k = 1, \ldots, \infty$ must be determined. Thus the formal solution of the system (28) - (30), obtained by linearizing the second set of equations in (25), are

$$\begin{aligned} \mathfrak{s}_{i,0} &= \mathtt{s}_i^* \left[1 - \frac{m_i}{1 + \frac{K_i}{\mathtt{e_t}}} \frac{1}{\left(1 + \sum_{j=1}^{n} \frac{\mathtt{s}_j^*}{1 + \frac{\mathtt{e_t}}{K_j}} \right)} \frac{T}{\alpha} \left(1 + \sum_{k=1}^{\infty} \eta_{ik} T^k \right) \right] \\ \mathfrak{c}_{i,0} &= \frac{\frac{\mathfrak{s}_{i,0}}{1 + \frac{\mathtt{e_t}}{K_i}}}{1 + \sum_{j=1}^{n} \frac{\mathfrak{s}_{j,0}}{1 + \frac{\mathtt{e_t}}{K_j}}} \end{aligned} \tag{34}$$

A similar linearization can be carried out in the second set of equations (24) by ignoring $\frac{\mathtt{e_t}}{K_i} \mathcal{C}_{i,0} \mathcal{C}_{j,0}$. As shown earlier, this linearization is valid under condition (29). After carrying out the linearization, the second set of equations in (24) may be written as

$$\frac{d\mathcal{C}_{i,0}}{d\tau} = \frac{k_i K_i}{\beta} \left[\left(1 - \sum_{j=1}^{n} \mathcal{C}_{j,0} \right) \mathtt{s}_i^* - \left(1 + \frac{\mathtt{e_t}}{K_i} \right) \mathcal{C}_{i,0} \right], \tag{35}$$

which is a system of linear constant coefficients differential equations in $\mathcal{C}_{i,0}$. Define $\mu_i := k_i(\mathtt{e_t} + K_i) = k_i \mathtt{e_t} + k_{-i} + m_i$, $a_i := k_i K_i \mathtt{s}_i^*$, $\nu_i = k_i K_i$, $\mathcal{C}_0 := \mathrm{col}(\mathcal{C}_{1,0}, \mathcal{C}_{2,0}, \ldots, \mathcal{C}_{n,0})$; $\mathcal{S}_0 := \mathrm{col}\big(\mathcal{S}_{1,0}, \mathcal{S}_{2,0}, \ldots, \mathcal{S}_{n,0}\big)$; $\mathtt{s}^* := \mathrm{col}\big(\mathtt{s}_1^*, \mathtt{s}_2^*, \ldots, \mathtt{s}_n^*\big)$; $\boldsymbol{D} := \mathrm{diag}\ (\nu_1, \nu_2, \ldots, \nu_n)$ and

$$\boldsymbol{M} := \begin{bmatrix} a_1 + \mu_1 & a_1 & \cdots & a_1 \\ a_2 & a_2 + \mu_2 & \cdots & a_2 \\ \vdots & \vdots & \ddots & \vdots \\ a_n & a_n & \cdots & a_n + \mu_n \end{bmatrix} \tag{36}$$

Then the equation (35) may be rewritten as follows

$$\frac{d\mathcal{C}_0}{d\tau} = \frac{1}{\beta} \boldsymbol{D} \mathtt{s}^* - \frac{1}{\beta} \boldsymbol{M} \mathcal{C}_0 \tag{37}$$

Let λ_i, $i = 1, \ldots, n$ denote the distinct eigenvalues of $\boldsymbol{M}$ and let n_i denote the algebraic multiplicity of the eigenvalue λ_i. It will be shown later that all eigenvalues of $\boldsymbol{M}$ are real and positive. The solution of the linear system of differential equations (37) is of the form

$$\mathcal{C}_0 = \boldsymbol{A} + \sum_{i=1}^{n} \sum_{j=0}^{n_i - 1} \boldsymbol{B}_{ij} \tau^j \exp\left(-\frac{\lambda_i \tau}{\beta} \right),$$

where $\boldsymbol{A}, \boldsymbol{B}_{ij} \in \mathbb{R}^n$. Thus the first term in the asymptotic expansion of the inner solution is given by the following equations

$$\begin{aligned} \mathcal{S}_0 &= \mathtt{s}^* \\ \mathcal{C}_0 &= \boldsymbol{A} + \sum_{i=1}^{n} \sum_{j=0}^{n_i - 1} \boldsymbol{B}_{ij} \tau^j \exp\left(-\frac{\lambda_i \tau}{\beta} \right) \end{aligned} \tag{38}$$

The next step to complete the SPA solution is to obtain the uniform solution valid for all time points. For this purpose, we now need to match the fast-transient solution with the post-transient solution at the boundary layer between the two time scales. In fact, each part is a reasonable approximation to the true solution, therefore the two approximations

must reasonably be required to agree in an overlapping region. In order to facilitate this matching, we introduce a function $\psi(\epsilon)$, such that the following conditions hold

$$\lim_{\epsilon\to 0}\psi(\epsilon)=0; \qquad \lim_{\epsilon\to 0}\frac{\psi(\epsilon)}{\epsilon}=\infty$$

One example of such a function is $\psi(\epsilon)=\epsilon^{\kappa}$, where $0<\kappa<1$. Note that when $T=\psi(\epsilon)$, $\tau=\dfrac{\psi(\epsilon)}{\epsilon}$. The matching conditions are

$$\lim_{\epsilon\to 0}\mathcal{S}_{i,0}\Big|_{\tau=\frac{\psi(\epsilon)}{\epsilon}}=\lim_{\epsilon\to 0}\mathfrak{s}_{i,0}|_{T=\psi(\epsilon)} \tag{39}$$

$$\lim_{\epsilon\to 0}\mathcal{C}_{i,0}\Big|_{\tau=\frac{\psi(\epsilon)}{\epsilon}}=\lim_{\epsilon\to 0}\mathfrak{c}_{i,0}|_{T=\psi(\epsilon)} \tag{40}$$

for $i=1,\ldots,n$. These conditions impose that the limit (when $T\to 0$) of the outer solution is equal to the limit (when $\tau\to\infty$) of the inner solution. From solutions for the total substrate concentration (34) and (38), the matching condition (39) is valid if

$$\lim_{\epsilon\to 0}\left[\left(\frac{m_i}{1+\frac{K_i}{\mathtt{e_t}}}\right)\frac{1}{\left(1+\sum_{j=1}^{n}\frac{\mathtt{s}_j^*}{1+\frac{\mathtt{e_t}}{K_j}}\right)}\frac{\psi(\epsilon)}{\alpha}\right]=0 \tag{41}$$

for $i=1,\ldots,n$. Since $\lim_{\epsilon\to 0}\psi(\epsilon)=0$, the condition (41) is guaranteed to be valid if $\left(\dfrac{\frac{1}{\alpha}}{1+\sum_{j=1}^{n}\frac{\mathtt{s}_j^*}{1+\frac{\mathtt{e_t}}{K_j}}}\right)\max_{i=1}^{n}\left(\frac{m_i}{1+\frac{K_i}{\mathtt{e_t}}}\right)$ is of the order of 1. We can thus take

$$\alpha=\left(\frac{1}{1+\sum_{j=1}^{n}\frac{\mathtt{s}_j^*}{1+\frac{\mathtt{e_t}}{K_j}}}\right)\max_{i=1}^{n}\left(\frac{m_i}{1+\frac{K_i}{\mathtt{e_t}}}\right) \tag{42}$$

for the matching condition on the substrates to be valid. This also ensures that α is a function of $\mathtt{e_t}$ with $0<\lim_{\mathtt{e_t}\to 0}\dfrac{\alpha}{\mathtt{e_t}}<\infty$ as assumed earlier.

For the matching condition on the intermediate complexes given by equation (40), we observe that

$$\lim_{\epsilon\to 0}\mathfrak{c}_{i,0}|_{T=\psi(\epsilon)}=\frac{\frac{\mathtt{s}_i^*}{1+\frac{\mathtt{e_t}}{K_i}}}{1+\sum_{j=1}^{n}\frac{\mathtt{s}_j^*}{1+\frac{\mathtt{e_t}}{K_j}}};$$

which is a constant for $i=1,\ldots,n$. Since $\lim_{\epsilon\to 0}\dfrac{\psi(\epsilon)}{\epsilon}=\infty$, the constant value on the right hand side of the above equation is guaranteed to match with $\lim_{\epsilon\to 0}\mathcal{C}_{i,0}\Big|_{\tau=\frac{\psi(\epsilon)}{\epsilon}}$, if $\min_{i=1}^{n}\lambda_i$ is at least of the order of β. $\min_{i=1}^{n}\lambda_i$ can be determined from the following Theorem.

Theorem 1 *Given positive values of μ_i and $\mathtt{s}_i^*$ for $i=1,\ldots,n$, all the eigenvalues of $\boldsymbol{M}$ defined by equation (36) are real and positive . Furthermore, each eigenvalue λ_i $(i=1,\ldots,n)$ satisfies*

$$\lambda_i\geq\min_{p=1}^{n}\mu_p$$

Proof: See [16, Appendix A].

It follows that we can take

$$\beta = \min_{p=1}^{n}(k_p \mathtt{e_t} + k_{-p} + m_p) \tag{43}$$

so that the matching condition on the intermediate complexes is valid. With this expression for β, we get

$$\lim_{\epsilon \to 0} \mathcal{C}_{i,0}\Big|_{\tau = \frac{\psi(\epsilon)}{\epsilon}} = \boldsymbol{A}_i = \frac{\frac{\mathtt{s}_i^*}{1 + \frac{\mathtt{e_t}}{K_i}}}{1 + \sum_{j=1}^{n} \frac{\mathtt{s}_j^*}{1 + \frac{\mathtt{e_t}}{K_j}}}.$$

Equation (43) ensures that β is a function of $\mathtt{e_t}$ with $0 < \lim_{\mathtt{e_t} \to 0} \beta < \infty$ as assumed earlier.

Thus, after matching solutions the validity condition of the tQSSA, $\epsilon = \dfrac{\alpha}{\beta} \ll 1$, becomes

$$\left(\frac{\mathtt{e_t}}{1 + \sum_{j=1}^{n} \frac{\mathtt{s}_j}{\mathtt{e_t} + K_j}} \right) \max_{i=1}^{n} \left(\frac{m_i}{\mathtt{e_t} + K_i} \right) \ll \min_{p=1}^{n}(k_p \mathtt{e_t} + k_{-p} + m_p) \tag{44}$$

Also, when $t \to \infty$, substrates S_i will completely convert to products P_i, therefore the minimum value of $\displaystyle\sum_{j=1}^{n} \frac{K_j \mathfrak{s}_{j,0}}{\mathtt{e_t} + K_j}$ in the linearization condition (29) occurs when substrates are completely depleted. This implies that the linearization condition (29) reduces to

$$\mathtt{e_t} \quad \ll \quad \min_{i=1}^{n}(K_i), \tag{45}$$

The final step to provide a solution for the linearized form of equations (19) is to combine the inner solution (38) and the outer solution (34) to obtain a uniform approximation which would be valid throughout the whole time period. This uniform solution can be obtained by subtraction of the common term from the summation of solutions of the inner part and outer part. The common part of solutions is the limit of the inner solution at the end of the boundary layer between the inner and outer parts. Therefore, the uniform solution expressed in terms of the time variable T can be calculated by

$$\begin{aligned} \bar{s}_i^u(T) &= \mathfrak{s}_i(T) + \mathcal{S}_i\left(\frac{T}{\epsilon}\right) - \mathfrak{s}_i(0) = \sum_{j=0}^{\infty} \left(\mathfrak{s}_{i,j}(T) + \mathcal{S}_{i,j}\left(\frac{T}{\epsilon}\right) - \mathfrak{s}_{i,j}(0) \right) \epsilon^j \\ c_i^u(T) &= \mathfrak{c}_i(T) + \mathcal{C}_i\left(\frac{T}{\epsilon}\right) - \mathfrak{c}_i(0) = \sum_{j=0}^{\infty} \left(\mathfrak{c}_{i,j}(T) + \mathcal{C}_{i,j}\left(\frac{T}{\epsilon}\right) - \mathfrak{c}_{i,j}(0) \right) \epsilon^j \end{aligned} \tag{46}$$

where $\bar{s}_i^u$ and c_i^u denote the uniform solutions for the i^{th} non-dimensional total substrate concentration variable and i^{th} non-dimensional substrate-enzyme complex concentration variable, respectively. Define

$$\bar{s}_{i,0}^u(T) := \mathfrak{s}_{i,0}(T) + \mathcal{S}_{i,0}(\frac{T}{\epsilon}) - \mathfrak{s}_{i,0}(0),$$

$$c_{i,0}^u(T) := \mathfrak{c}_{i,0}(T) + \mathcal{C}_{i,0}(\frac{T}{\epsilon}) - \mathfrak{c}_{i,0}(0),$$

and

$$\gamma_i := \frac{\left(\frac{m_i}{1 + \frac{K_i}{\mathtt{e_t}}} \right)}{\max_{j=1}^{n} \left(\frac{m_j}{1 + \frac{K_j}{\mathtt{e_t}}} \right)},$$

Also, note that because of matching conditions

$$\lim_{\epsilon\to 0}\bar{s}_i^u(T)=\lim_{\epsilon\to 0}\bar{s}_{i,0}^u(T)=\mathtt{s}_{i,0}(T)$$

and

$$\lim_{\epsilon\to 0}c_i^u(T)=\lim_{\epsilon\to 0}c_{i,0}^u(T)=\mathtt{c}_{i,0}(T).$$

For the i^{th} non-dimensional total substrate concentration variable, the common term of solutions is $\mathtt{s}_i^*$, and for the i^{th} non-dimensional enzyme-substrate complex concentration variable, the common term is $\boldsymbol{A}_i$. Therefore, the zero-th order expansion of the uniform solution for the linearized form of equations (19) is given by

$$\begin{aligned}\bar{s}_{i,0}^u(T) &= \mathtt{s}_i^*\left[1-\gamma_i T-\sum_{k=1}^{\infty}\eta_{ik}T^{k+1}\right]\\ c_{i,0}^u(T) &= \frac{\left(\frac{\bar{s}_{i,0}^u(T)}{1+\frac{\mathtt{e_t}}{K_i}}\right)}{1+\sum_{j=1}^{n}\left(\frac{\bar{s}_{j,0}^u(T)}{1+\frac{\mathtt{e_t}}{K_j}}\right)}+\sum_{k=1}^{n}\sum_{j=0}^{n_k-1}(\boldsymbol{B}_{kj})_i\left(\frac{T}{\epsilon}\right)^j e^{-\left(\frac{\lambda_k}{\min_{k=1}^n\lambda_k}\right)\left(\frac{T}{\epsilon}\right)}\end{aligned}\tag{47}$$

When $\epsilon\to 0$, the uniform solution approaches the outer solution which is the tQSSA model. By using the non-scaled form of the total substrate and complex concentration variables in equations (25) and (28), the tQSSA approximation model in terms of the time variable t with validity conditions (45) and (44) is

$$\begin{aligned}\frac{d\bar{s}_i}{dt} &= -m_i c_i(t),\\ c_i(t) &= \frac{\frac{\mathtt{e_t}\bar{s}_i(t)}{\mathtt{e_t}+K_i}}{1+\sum_{j=1}^{n}\frac{\bar{s}_j(t)}{\mathtt{e_t}+K_j}};\qquad i=1,\ldots,n.\end{aligned}\tag{48}$$

Since ϵ is dimensionless, $\epsilon\ll 1$ leading to (44) may be taken as a validity condition for the tQSSA model.

4 Simulations

We perform two sets of numerical simulations in order to investigate the validity condition (45) for linearization given by $\mathtt{e_t}\ll\min_{i=1}^n(K_i)$ and the validity condition (44) for tQSSA given by $\epsilon\ll 1$. In each of these two sets of simulations, we compare the trajectories of the original mass action kinetics model (19) with those of the tQSSA model (48). For the demonstration of condition (44), we choose three sets of parameters that correspond to different values of ϵ (0.017, 0.17, 1.7), while condition (45) for linearization is valid in all the three cases. For the demonstration of condition (45), we choose another two sets of parameters such that $\epsilon\ll 1$ for both the sets, while condition (45) is valid for only one of the two sets. The results of the simulations demonstrating the validity of conditions (44) and (45) are shown in Figures 1 and 2 respectively, in which the evolution of the total substrate concentrations predicted by the mass action kinetics model (19) is compared with the corresponding evolution as predicted by the tQSSA model (48). All simulations are performed using Matlab Simulink software.

The result of the first set of simulations is shown in Figure 1. As mentioned earlier, three specific cases A, B and C corresponding to three different sets of parameters are considered. Some of the parameter values are common for all the three cases A, B and C. These common parameters are $\mathtt{e_t}=0.1$, $k_1=1$, $k_{-1}=1$, $m_1=0.1$, $k_2=0.1$, $m_2=0.1$, $k_{-2}=0.05$, $k_3=10$ and $\mathtt{s}_1=\mathtt{s}_2=\mathtt{s}_3=1$. The remaining parameters that are different for the three different cases

are given by (i) Case A: $k_{-3} = 9.9$ and $m_3 = 0.1$; (ii) Case B: $k_{-3} = 9$ and $m_3 = 1$; (iii) Case C: $k_{-3} = 0.1$ and $m_3 = 9.9$. These parameters lead to common values of Michaelis constants K_1, K_2 and K_3 for all the three cases given by $K_1 = 1.1$, $K_2 = 1.5$ and $K_3 = 1$. The condition for linearization $\mathsf{e_t} \ll \min_{i=1}^{n}(K_i)$ is thus satisfied in all the three cases. Also, we obtain three different values 0.017, 0.17, 1.7 for ϵ for cases A, B and C respectively. From Figure 1, it can be seen that increasing the value of ϵ leads to a difference between the graphs of $\bar{s}_3$ obtained using the mass action model (19) and the tQSSA model (48).

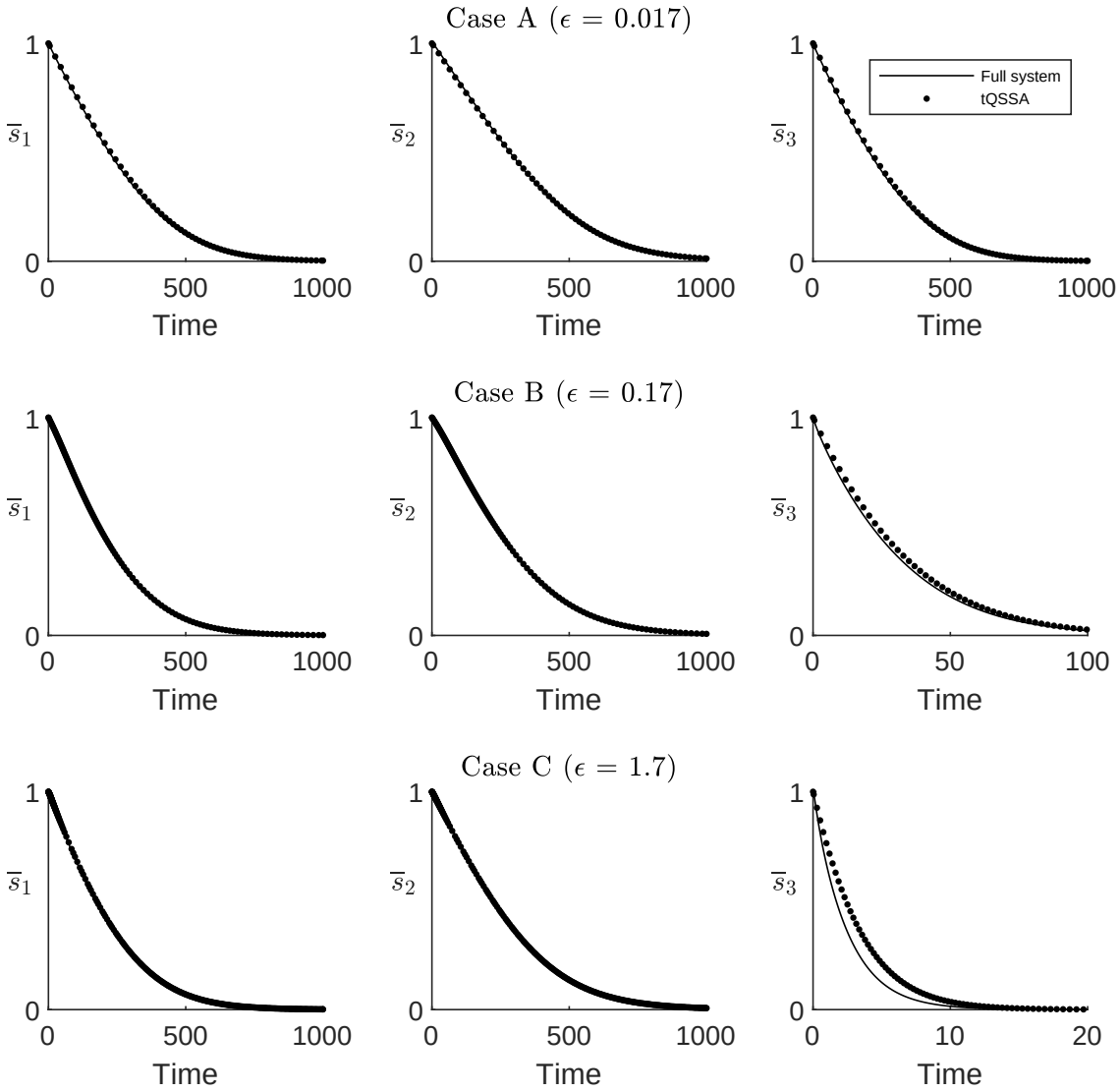

Figure 1: Comparison plots for demonstration of validity condition (44). Full line correspond to mass action kinetics model (19) and points correspond to tQSSA model (48).

The result of the second set of simulations is shown in Figure 2. As mentioned earlier, two specific cases D and E corresponding to two different sets of parameters are considered. Some of the parameters are common for the two cases D and E. These common parameters are $k_1 = 1$, $k_{-1} = 1$, $m_1 = 0.1$, $k_2 = 0.1$, $m_2 = 0.1$, $k_{-2} = 0.05$, $k_3 = 10$,

$k_{-3} = 9.9$, $m_3 = 0.1$ and $\mathtt{s}_1 = \mathtt{s}_2 = \mathtt{s}_3 = 1$. These common parameters lead to common values of Michaelis constants K_1, K_2 and K_3 for the two cases D and E given by $K_1 = 1.1$, $K_2 = 1.5$ and $K_3 = 1$. The remaining parameter that is different for the cases D and E is $\mathtt{e_t}$ and its values for the two cases are given by (i) case D: $\mathtt{e_t} = 0.1$; (ii) case E: $\mathtt{e_t} = 1$. These parameters lead to different values of ϵ for the two cases given by $\epsilon = 0.084$ for case D, and $\epsilon = 0.017$ for case E. However condition (44) may be considered to be valid for both the cases. Note that $\left(\frac{\mathtt{e_t}}{\min_{i=1}^{3}(K_i)}\right)$ has different values 0.1 and 1 for cases D and E respectively. From Figure 2 it can be seen that increasing the value of $\left(\frac{\mathtt{e_t}}{\min_{i=1}^{3}(K_i)}\right)$ leads to a difference between the graphs obtained by the mass action model (19) and the tQSSA model (48) corresponding to all three total substrate concentrations. This difference is due to the deviation from the linearization condition (45) that is shown to be essential for tQSS approximation.

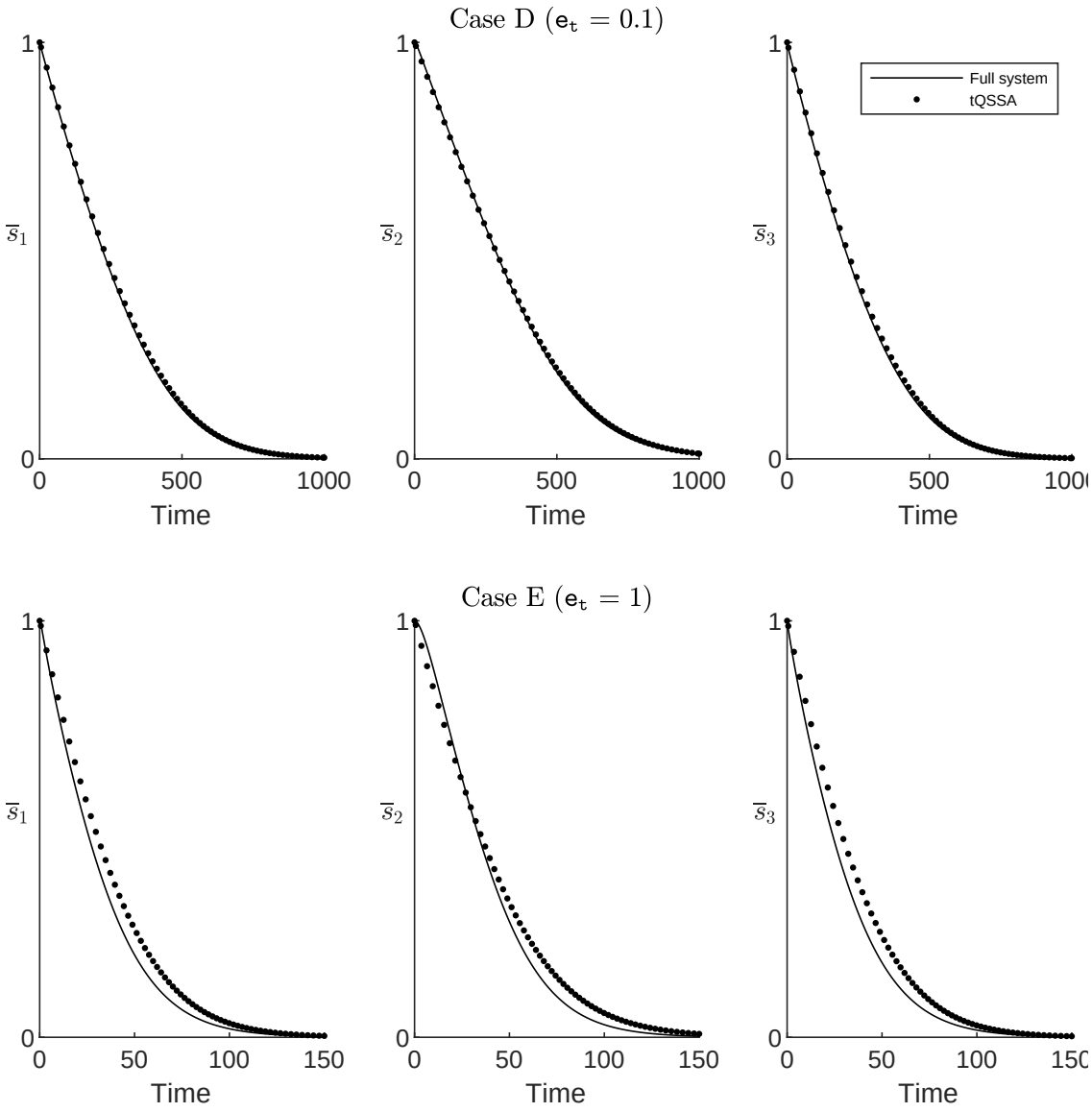

Figure 2: Comparison plots for demonstration of validity condition (45). Full line correspond to mass action kinetics model (19) and points correspond to tQSSA model (48).

5 Discussion and conclusion

We derived the tQSSA model, equation (48), and its corresponding validity conditions, equations (44) and (45) for the system of reactions (3). The key step that enables us to provide this framework of the solution is the linearization of governing equations (24) and (25) by ignoring terms that are non-linear in the non-dimensional enzyme-substrate complex concentration variables, $\mathcal{C}_{i,0}$ and $\mathfrak{c}_{i,0}$, under the validity condition (45). This condition is inspired by the linearization used in [5] for the single substrate system of reactions (1), which is explained in the preliminaries section. The alternative solution of equations (24) and (25), instead of using linearization, is solving the Riccati equation which is proposed in [6] for the system of reactions (1). However, using this method for the system of reactions (3) ends up in a set of complicated mathematical equations for which it is not possible to derive closed-form solutions. In contrast, by using the singular perturbation method, we have been able to obtain a closed-form solution for tQSSA for the system of reactions (3) and validity conditions for the same.

As a special case, we examine our result for the single substrate system of reactions (1). In equations (48) by using only $i = 1$, we obtain tQSSA model for the system of reactions (1) that is in accordance with the equation (15) explained in the preliminaries section. Also, the solution of equation (35) for $i = 1$ is

$$\mathcal{C}(\tau) \approx \frac{\mathtt{s}}{\mathtt{e_t} + K_m + \mathtt{s}} \left(1 - e^{-\dfrac{k_1(\mathtt{e_t} + K_m + \mathtt{s})\tau}{\beta}} \right), \tag{49}$$

therefore, the time scale of the initial fast transient period, $\beta = k_1(\mathtt{e_t} + K_m + \mathtt{s})$, and the time scale of post-transient part given in equation (42), $\alpha = \dfrac{m_1 \mathtt{e_t}}{\mathtt{e_t} + K_m + \mathtt{s}}$, regenerate the validity condition in equation (16) by using our general framework.

For a fully competitive system of reactions (2), to the best of our knowledge, an attempt to provide the solution of tQSSA model and to determine its validity condition is reported in [9], [10] and [11]. In [9], to determine the validity condition of tQSSA, they have generalized the condition $t_c \ll t_s$ as

$$\max_{i=1,2} \frac{t_{c_i}}{t_{s_i}} = \frac{\max(t_{c_1}, t_{c_2})}{\max(t_{s_1}, t_{s_2})} \quad \ll \quad 1, \tag{50}$$

We explain the notation used in the above condition. Let $\bar{s}_i$ and c_i for $i = 1, 2$ denote the total substrate concentrations and enzyme-substrate complex concentrations respectively in the system of reactions (2). Then $\frac{d\bar{s}_i}{dt}|_{\mathtt{max}}$ and $\frac{dc_i}{dt}|_{\mathtt{max}}$ can be estimated from equations (19) for $i = 1, 2$ in the fast transient period when substrates S and I may be approximately assumed to have constant concentrations $\mathtt{s}_1$ and $\mathtt{s}_2$ respectively. In [9], separate time scales t_{s_i} and t_{c_i} have been considered for the depletion of the i^{th} total substrate and the rapid change of the i^{th} complex concentration respectively. These have been estimated as follows

$$\begin{aligned} t_{s_i} &\approx \frac{\bar{s}_i|_{\mathtt{max}} - \bar{s}_i|_{\mathtt{min}}}{\frac{d\bar{s}_i}{dt}|_{\mathtt{max}}} = \frac{\mathtt{s}_i}{m_i c_i(\mathtt{s}_1, \mathtt{s}_2)} \\ t_{c_i} &\approx \frac{c_i|_{\mathtt{max}} - c_i|_{\mathtt{min}}}{\frac{dc_i}{dt}|_{\mathtt{max}}} = \frac{c_i(\mathtt{s}_1, \mathtt{s}_2)}{k_i \mathtt{e_t} \mathtt{s}_i}. \end{aligned} \tag{51}$$

In [10] and [11], the following variable is obtained as a small parameter of the singular perturbation analysis.

$$\epsilon := \max_{i=1,2} \left(\frac{m_i \mathtt{e_t}}{k_i(\mathtt{e_t} + K_i)^2} \right) \tag{52}$$

This parameter has been obtained by first linearizing the original mass action kinetics model (19) around its only equilibrium point that corresponds to all the substrate as well as complex concentrations being equal to zero. This

equilibrium point corresponds to the state at which all the substrates and intermediate complexes are completely exhausted to form the product, in other words, the state of the end of the slow post-transient period. It is shown that linearization around this equilibrium point leads to a decoupling of the dynamics of the different enzyme-substrate reactions in the scheme (3). Imposing the condition for time scale separation for each of the resulting decoupled enzyme-substrate reactions in the linearized model leads to ϵ given by equation (52). On the contrary, our scheme of linearization of the model (19) does not lead to a decoupling of the enzyme-substrate reactions in the scheme (3) since we do not linearize about an equilibrium point and instead only ignore the nonlinear terms in the right hand side of equations (19). We subsequently determine the time scales of the fast and slow dynamics of the resulting model using known mathematical results and techniques (Theorem 1 and Taylor series expansion). While the linearization carried out in this manuscript is valid at all time points as long as condition (45) is satisfied, the linearization carried out in [10] and [11] is valid only around the equilibrium point of the system.

The other difference between our approach for obtaining a validity condition for tQSSA as compared with previously known approaches ([9], [10] and [11]) is that we begin with unknown values of the time scales of the slow and fast dynamics (α^{-1} and β^{-1}) and then determine those using the balancing condition in the SPA method, while in previously known approaches ([9], [10] and [11]), a small parameter is first chosen based on time scales separation arguments and it is then shown that it leads to a validity condition for tQSSA. A major issue in the approaches of [9], [10] and [11] is that the analytical solutions of differential equations for variables c_i and $\bar{s}_i$ are only possible for a small range of parameters due to their complicated mathematical equations. In contrast, our approach provides a general framework of solution for the n-substrate system of reactions (3) that could be applied for fully competitive enzyme reactions (2) as well. This approach is based on the simplification of differential equations by using linearization condition given by equation (45). According to this condition, the total enzyme concentration must be very low compared with the minimum of Michaelis constants of reactions in the system (3) which is stronger than the traditional validity condition of sQSSA, $\mathtt{e_t} \ll \mathtt{s} + K_m$. The simulations performed in section 4 for a sample system of reactions, also numerically approves the obtained validity conditions of the tQSSA and the utilized linearization method.

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