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# Enzyme Kinetics Far From the Standard Quasi-Steady-State and Equilibrium Approximations

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**Abstract**—Analytic approximations of the time-evolution of the single enzyme-substrate reaction are valid for all but a small region of parameter space in the positive initial enzyme-initial substrate concentration plane. We find velocity equations for the substrate decomposition and product formation with the aid of the total quasi-steady-state approximation and an aggregation technique for cases where neither the more normally employed standard nor reverse quasi-steady-state approximations are valid. Applications to determining reaction kinetic parameters are discussed. 

**Keywords**—Total quasi-steady-state approximation, Fitting procedure, Aggregation technique, Singular perturbation.

## 1. INTRODUCTION

One of the most widely studied reactions in enzyme kinetics is the single enzyme-substrate reaction

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P, \tag{1}$$

where $S$, $E$, $C$, and $P$ represent substrate, enzyme, complex, and product, respectively, and $k_1$, $k_{-1}$, and $k_2$ are reaction rate constants. The time evolution of reaction (1) can be described completely by the following pair of coupled nonlinear differential equations:

$$\frac{\mathrm{d}[S]}{\mathrm{d}t} = k_1[([C] - [E_0])[S] + K_S[C]], \tag{2}$$

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k_1[([E_0] - [C])[S] - K_M[C]], \tag{3}$$

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together with the uncoupled equation

$$\frac{\mathrm{d}[P]}{\mathrm{d}t} = V = k_2[C] \tag{4}$$

and the enzyme and substrate conservation laws $[E_0] = [E](t)+[C](t)$, $[S_0] = [S](t)+[C](t)+[P](t)$ with initial conditions $([S],[E],[C],[P])(0) = ([S_0],[E_0],0,0)$, where the square brackets denote concentrations. In this system, $K_S = k_{-1}/k_1$ is the equilibrium dissociation constant of the complex and $K_M = (k_{-1}+k_2)/k_1$ the Michaelis-Menten constant [1].

From (3) and (2), it follows that:

$$[C]([S]) = \frac{[E_0][S]}{K_S + (K/(1+\mathrm{d}[C]))/\mathrm{d}[S] + [S]}, \tag{5}$$

where $K = k_2/k_1$ is the Van Slyke-Cullen constant [2].

Substituting (5) in (4) yields the general velocity of product formation

$$V = \frac{v_{\max}[S]}{\phi + [S]}, \tag{6}$$

where $v_{\max} = k_2[E_0]$ is the maximum velocity and

$$\phi = K_S + \frac{K}{1+\mathrm{d}[C]/\mathrm{d}[S]}. \tag{7}$$

Under certain conditions, one can use (6),(7) to derive simpler velocity equations to estimate the reaction parameters, namely $v_{\max}$, $K_S$, and $K$. First, under the condition that the sum of initial substrate concentration ($[S_0]$) and $K_M$ greatly exceeds the initial enzyme concentration ($[E_0]$), that is,

$$\frac{[E_0]}{K_M + [S_0]} \ll 1, \tag{8}$$

biochemists, with the aid of the standard quasi-steady-state assumption (sQSSA), study the long time behaviour of reaction (1) [3,4]. Setting $\frac{\mathrm{d}[C]}{\mathrm{d}t} \approx 0$, implies $\frac{\mathrm{d}[C]}{\mathrm{d}[S]} \to 0$ and $\phi = K_M$ in the sQSSA velocity equation.

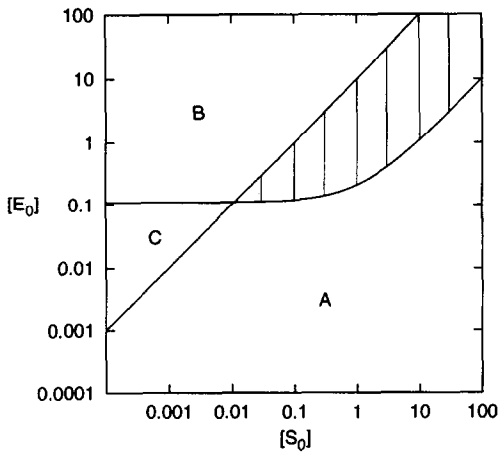
Second, for conditions in which the initial enzyme concentration greatly exceeds that of the substrate, that is,

$$\frac{[E_0]}{[S_0]} \gg 1, \tag{9}$$

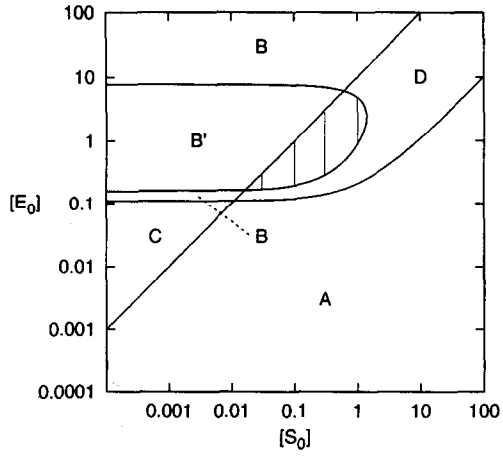
it can be shown that the sQSSA is invalid [2]. In this case, the appropriate assumption is the reverse quasi-steady-state assumption (rQSSA) [5] or equilibrium approximation ($\frac{\mathrm{d}[S]}{\mathrm{d}t} \approx 0$) and the velocity equation for the long time behaviour is of the form (6) with $\phi = K_S$.

Segel and collaborators [3,5] have shown that the sQSSA can provide a good approximation even when $[S_0] \approx [E_0]$ as long as $[E_0]$ is small compared to $K_M$. The positive $[S_0]$-$[E_0]$ plane can be divided into regions in which these approximations hold, but in certain circumstances, there remains a region where neither holds. We illustrate this in Figure 1a, from which we note that in the shaded region, when both the sQSSA and rQSSA are invalid, the initial enzyme and substrate concentrations are comparable. Recently, Borghans and collaborators [6] re-examined the problem when there is an excess of enzyme and $K_M$ is small, so that (8) does not hold. They introduced a new variable, the total substrate concentration $[\tilde{S}] = [S] + [C]$, to extend the parameter domain for which it is permissible to employ the classical assumption $\frac{\mathrm{d}[C]}{\mathrm{d}t} \approx 0$, with the following condition:

$$\frac{K[E_0]}{(K_M + [S_0] + [E_0])^2} \ll 1. \tag{10}$$




(a). Regions of validity of the sQSSA and rQSSA for the enzyme-substrate reaction (1) plotted using conditions (8) and (9). There are four regions: A where only the sQSSA is valid, B where only the rQSSA is valid, C where both assumptions are valid, and the shaded region where both are invalid. Note that in the latter region, the initial enzyme and substrate concentrations are comparable.




(b). Regions of validity of the sQSSA, rQSSA, and tQSSA plotted using conditions (8)–(10). The positive $[S_0]$–$[E_0]$ plane is now divided into six regions. The sQSSA, rQSSA, and tQSSA are not valid in the shaded region. In region B', the sQSSA and tQSSA are both invalid, but the rQSSA is valid. In region D, only the tQSSA is valid. The regions A, B, and C are as in (a), but here the tQSSA is also valid. Parameter values used are $k_1 = 10$, $k_{-1} = 1$, $k_2 = 10$ ($K = 1$, $K_S = 0.1$, $K_M = 1.1$).

Figure 1.

This new condition is called the total quasi-steady-state assumption (tQSSA). By including the tQSSA in the plot (see, Figure 1b), the positive $[S_0]$–$[E_0]$ plane is divided into six regions. It can be seen that the region in which none of the assumptions are valid is reduced considerably due to the tQSSA. However, this does not provide a practical advantage to biochemists, because the velocity equations for the substrate or product, used to determine the kinetic parameters, have not been derived into this region.

The aim of this letter is to obtain velocity equations of substrate decomposition and product formation for reaction (1) with the aid of the tQSSA and a singular perturbation method for an aggregated variable. These equations allow biochemists to determine kinetics parameters under conditions in which neither the sQSSA nor the rQSSA are valid.

## 2. THE TOTAL QUASI-STEADY-STATE ASSUMPTION AS AN AGGREGATION TECHNIQUE

The total substrate concentration ($[\tilde{S}]$) is an aggregated or lumped variable. Aggregation or lumping techniques have been used in a number of areas to reduce systems of equations. This approach was initiated in chemistry by Wei and Kuo [7] and their work has been extended by Li and collaborators [8]. Aggregation techniques have been applied in ecology, population dynamics, and also in more general systems [9,10].

We nondimensionalise the system (2),(3) with the aid of the tQSSA [6] which provides the appropriate dimensionless variables for the application of an aggregation technique. Substituting

$$s = \frac{[S]}{[S_0]}, \qquad c = \frac{K_M + [E_0] + [S_0]}{[E_0][S_0]}\,[C], \qquad T = \frac{v_{\max}}{K_M + [E_0] + [S_0]}\,t, \tag{11}$$

into (2) and (3) and rearranging the terms, we define the dimensionless parameters

$$\epsilon = \frac{K[E_0]}{(K_M + [E_0] + [S_0])^2}, \qquad \sigma = \frac{[S_0]}{K_M + [E_0] + [S_0]},$$
$$\kappa = \frac{K}{K_M + [E_0] + [S_0]}, \qquad \kappa_m = \frac{K_M}{K_M + [E_0] + [S_0]}, \tag{12}$$
$$\kappa_s = \frac{K_S}{K_M + [E_0] + [S_0]}.$$

This yields the following nondimensional governing equations for reaction (1):

$$\frac{ds}{dT} = \frac{1}{\kappa}\left[(\sigma c - 1)s + \kappa_s c\right], \tag{13}$$
$$\epsilon \frac{dc}{dT} = (1 - \sigma c)s - \kappa_m c, \tag{14}$$

together with the uncoupled velocity equation of product formation

$$\frac{dp}{dT} = v = c \tag{15}$$

and the enzyme and substrate conservation laws with initial conditions $(s, c)(0) = (1, 0)$. Equation (13) can also be written

$$\frac{ds}{dT} = \frac{1}{\kappa}(\sigma c - 1)s + \mu c, \tag{16}$$

where $\mu = \kappa_s/\kappa = k_{-1}/k_2$ is the ratio of the fission constants for the enzyme-substrate complex.

We can now employ the singular perturbation method introduced by Li and collaborators [11] to determine an approximate aggregated differential equation for the substrate decomposition. We first define a purely fast variable by separating the fast and slow variables as follows; let

$$\bar{s} = c + h[s], \tag{17}$$

where $h[s(T)]$ is a first-order differentiable variable. Multiplying by $\epsilon$ and differentiating, we have

$$\epsilon \frac{d\bar{s}}{dT} = \epsilon \frac{dc}{dT} + \epsilon \frac{dh[s]}{ds}\frac{ds}{dT}, \tag{18}$$

which, after substitution from (16) and (14), becomes

$$\epsilon \frac{d\bar{s}}{dT} = (1 - \sigma c)s - \kappa_m c + \epsilon \frac{dh[s]}{ds}\left[\frac{1}{\kappa}(\sigma c - 1)s + \mu c\right]. \tag{19}$$

Employing (17) to eliminate $c$, after some algebra we find that

$$\begin{aligned} \epsilon \frac{d\bar{s}}{dT} = &-\left[\kappa_m + \sigma s - \epsilon \frac{dh[s]}{ds}\left(\mu + \frac{\sigma}{\kappa}s\right)\right]\bar{s} + s \\ &+(\kappa_m + \sigma s)h[s] - \epsilon \frac{dh[s]}{ds}\left[\frac{1}{\kappa}s + \left(\mu + \frac{\sigma}{\kappa}s\right)h[s]\right]. \end{aligned} \tag{20}$$

As $\epsilon \to 0$, we obtain the purely fast differential equation,

$$\epsilon \frac{d\bar{s}}{dT} = -\left[\kappa_m + \sigma s - \epsilon \frac{dh[s]}{ds}\left(\mu + \frac{\sigma}{\kappa}s\right)\right]\bar{s} = -\gamma(s, \epsilon)\bar{s}, \tag{21}$$

provided that $h[s]$ satisfies the following equation:

$$\epsilon \frac{dh[s]}{ds}\left[\frac{1}{\kappa}s + \left(\mu + \frac{\sigma}{\kappa}s\right)h[s]\right] - (\kappa_m + \sigma s)h[s] - s = 0. \tag{22}$$

Let us assume that $h[s]$ and $\frac{dh[s]}{ds}$ have expansions of the form

$$h[s] = \sum_{i=0}^{\infty} \epsilon^i h_i[s], \tag{23}$$

$$\frac{dh[s]}{ds} = \sum_{i=0}^{\infty} \epsilon^i \frac{dh_i[s]}{ds}. \tag{24}$$

Substituting (23) and (24) into (22), and equating powers of $\epsilon$, allows us to determine $h_i[s]$ and $\frac{dh_i[s]}{d}$ $s$. We only take the first two terms of the expansion of $h[s]$ and $\frac{dh[s]}{ds}$ as higher-order terms rapidly become messy and, for our purposes, the first two terms provide sufficient accuracy. In summary, we obtain

$$h[s] = -\frac{s}{\kappa_m + \sigma s} - \epsilon \frac{\kappa_m s}{(\kappa_m + \sigma s)^4} + \cdots, \tag{25}$$

$$\frac{dh[s]}{ds} = -\frac{\kappa_m}{(\kappa_m + \sigma s)^2} - \epsilon \frac{\kappa_m(\kappa_m - 3\sigma s)}{(\kappa_m + \sigma s)^5} + \cdots. \tag{26}$$

Li and collaborators [11] solved the differential equation (21) using a singular perturbation method. Considering $\bar{s}$ as a scalar function, without expanding it, they found that the solution for $\bar{s}$ only consists of an initial layer expansion and has the form

$$\bar{s}(T) = \bar{s}(0) \exp\left(-\gamma[s(0), \epsilon] \frac{T}{\epsilon}\right), \tag{27}$$

with the initial condition $\bar{s}(0) = c(0) + h[s(0)] = h[s(0)]$. Notice that $\bar{s}(T)$ is an exponential function which approaches 0 after the initial transient, that is $T > \epsilon$.

The value of $c(T)$ can be obtained by substituting (27) into (17),

$$c(T) = h[s(0)] \exp\left(-\gamma[s(0), \epsilon] \frac{T}{\epsilon}\right) - h[s(T)]. \tag{28}$$

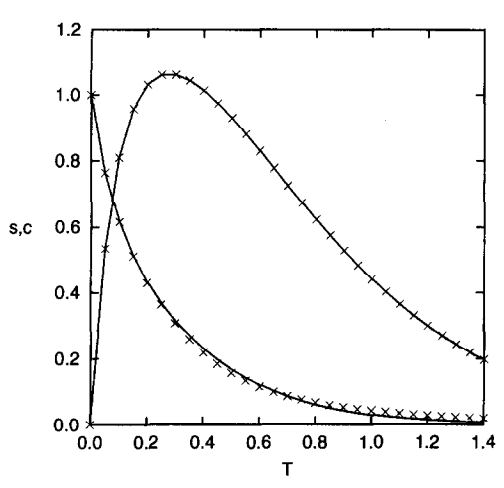
Now, we can obtain the aggregated velocity equation for substrate decomposition by substituting (28) into (16),

$$\frac{ds}{dT} = -\frac{1}{\kappa} s - \left(\mu + \frac{\sigma}{\kappa} s\right) \left[h[s] - h[s(0)] \exp\left(-\gamma[s(0), \epsilon] \frac{T}{\epsilon}\right)\right]. \tag{29}$$

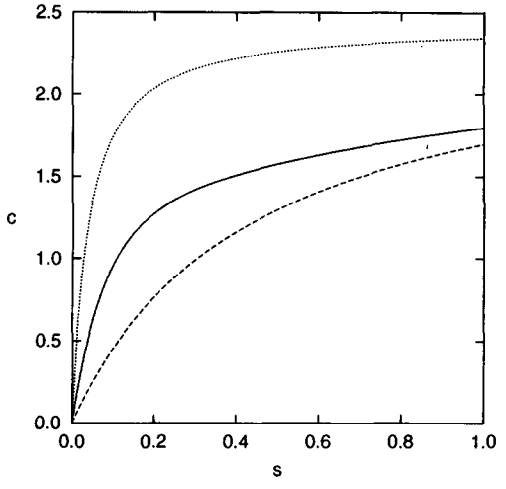
In Figure 2a, the numerical solutions of the approximations (28),(29) are shown (cross symbols) along with the numerical solutions of system (13),(14) (solid curve) for an illustrative example. The approximations are indistinguishable during the initial transient ($T < \epsilon$) and are in close agreement with the numerical solutions for the slow transient. We also find that the velocity equation for substrate decomposition is in close agreement with the numerical solutions for conditions in which the sQSSA or rQSSA are valid. In some cases, higher-order approximations are almost indistinguishable over most of the interval. However, under certain conditions, i.e., larger values of $\epsilon$, higher-order approximations cannot provide a good approximation after the initial transient. This situation can be corrected by expanding the singular perturbation solution of $\bar{s}$ (equation (27)) and matching appropriately. Hence, in principle, the kinetic parameters can be determined from progress curves by numerically integrating (29). This has been done previously for the Michaelis-Menten equation [12].

We can also determine a velocity equation for product formation. After an initial transient, that is $T > \epsilon$, $\bar{s} \approx 0$. The inertial manifold which attracts all solution trajectories can then be obtained from (17) as

$$c = -h[s]. \tag{30}$$




(a). Graph of the numerical solutions (solid curve) and approximate solutions obtained with the aggregation technique (cross symbols) for the reaction (1).




(b). Representation of the sQSSA (dashed curve) and rQSSA (dotted curve) velocity equations for product formation obtained from the general velocity equation (6) along with the tQSSA velocity equation for product formation (solid curve, (31)]). Initial conditions $[S_0] = 2.5$, $[E_0] = 2.5$; $\kappa = 0.0901$, $\kappa_s = 0.0090$, $\kappa_m = 0.0991$, $\epsilon = 0.8115 \times 10^{-5}$, $\mu = 0.1000$, $\sigma = 0.9008$. With these parameter values, $K = 1$, $K_S = 0.1$, $K_M = 1.1$, and only the tQSSA is valid.

Figure 2.

Therefore, the aggregated velocity equation of product formation can be derived from (15) by substituting (25) and (30) yielding

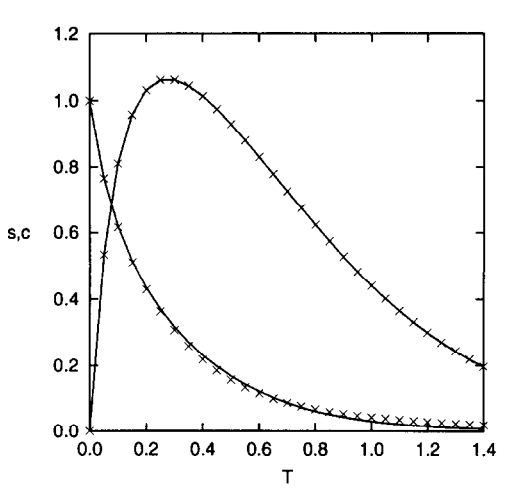
$$v = \frac{s}{\kappa_m + \sigma s} + \epsilon \frac{\kappa_m s}{(\kappa_m + \sigma s)^4} + \cdots . \tag{31}$$

We call this equation the tQSSA velocity equation. This equation can also be derived from (28) by ignoring the contribution of the term $h[s(0)] \exp(-\gamma[s(0), \epsilon]\,(T/\epsilon))$. Notice that this can only be a good approximation after the initial transient, that is $T > \epsilon$. In Figure 2(b), the tQSSA (31), sQSSA and rQSSA velocity equations of product formation are plotted in conditions when the sQSSA and rQSSA are not valid, but the tQSSA is valid. It can be seen that the tQSSA velocity curve lies between the sQSSA and rQSSA velocity curve when the initial enzyme and substrate concentrations are comparable and the sQSSA is not valid.

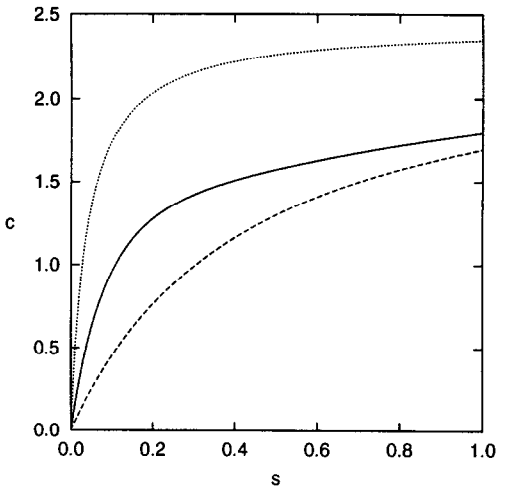
To illustrate the extended range of validity of the tQSSA velocity equation for product formation, we compare it with the sQSSA and rQSSA velocity equations for product formation under conditions in which the sQSSA is valid (see, Figure 3a) or the rQSS is valid (see, Figure 3b). If the sQSSA is valid, the tQSSA velocity equation for product formation is almost indistinguishable from the sQSSA over most of the interval (Figure 3a). If the rQSSA is valid, the tQSSA velocity equation for product formation is in very close agreement with the rQSSA velocity equation (Figure 3b). In this plot, the parameters and initial values chosen provide a representative example of the ranges of validity of the sQSSA and rQSSA.

## 3. DISCUSSION

One of the key practical problems associated with the single enzyme-substrate reaction (1) is identifying parameter regimes in which various analytical approximations hold, as these are then used to calculate kinetic parameters from experimental data. We have derived velocity equations for substrate decomposition and product formation with the aid of the total quasi-steady-state




(a). Initial conditions $[S_0] = 10$, $[E_0] = 0.001$; $\kappa = 0.0901$, $\kappa_s = 0.0090$, $\kappa_m = 0.0991$, $\epsilon = 0.8115 \times 10^{-5}$, $\mu = 0.1000$, $\sigma = 0.9008$. With these parameter values, $K = 1$, $K_S = 0.1$, $K_M = 1.1$, and both the sQSSA and tQSSA are valid.




(b). Initial conditions $[S_0] = 0.001$, $[E_0] = 10$; $\kappa = 0.0901$, $\kappa_s = 0.0090$, $\kappa_m = 0.0991$, $\epsilon = 0.0812$, $\mu = 0.1000$, $\sigma = 0.0001$. With these parameter values, $K = 1$, $K_S = 0.1$, $K_M = 1.1$, and both the rQSSA and tQSSA are valid.

Figure 3. Representation of the sQSSA (dashed curve) and rQSSA (dotted curve) velocity equations for product formation obtained from the general velocity equation (6) along with the tQSSA velocity equation for product formation (cross symbols, (31)). Parameter values are $k_1 = 10$; $k_{-1} = 1$, $k_2 = 10$.

assumption. This allows us to enhance the regions in parameter space for which analytical approximations are valid. In some cases, the tQSSA provides a good approximation in regions where either one or other of the standard quasi-steady-state assumption and the reverse quasi-steady-state (or equilibrium) assumption hold. Additionally, a fitting procedure can now be used to determine the kinetic parameters far from the standard quasi-steady-state and equilibrium. This method would fix experimental data on the variation of substrate or product concentration as a function of time with numerical simulations of the velocity equations derived in this manuscript.

The analytic framework presented here can be easily extended to other enzymatic modes of action which may be more realistic than (1), and therefore, our results may have broader application.

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