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Enzyme Kinetics at High Enzyme Concentration

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We re-visit previous analyses of the classical Michaelis–Menten substrate–enzyme reaction and, with the aid of the reverse quasi-steady-state assumption, we challenge the approximation $\mathrm{d}[C]/\mathrm{d}t \approx 0$ for the basic enzyme reaction at high enzyme concentration. For the first time, an approximate solution for the concentrations of the reactants uniformly valid in time is reported. Numerical simulations are presented to verify this solution. We show that an analytical approximation can be found for the reactants for each initial condition using the appropriate quasi-steady-state assumption. An advantage of the present formalism is that it provides a new procedure for fitting experimental data to determine reaction constants. Finally, a new necessary criterion is found that ensures the validity of the reverse quasi-steady-state assumption. This is verified numerically.

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1. INTRODUCTION

Biochemists usually analyse enzyme kinetics parameters within the Michaelis and Menten (1913) framework (Alberty, 1956, 1959; Hearon *et al*., 1959; Segel, 1975; Fersht, 1985; Schulz, 1994). In particular, the reversible reaction between enzyme E and substrate S, giving the enzyme–substrate complex C, which irreversibly yields product P

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

has been extensively studied, based on the standard quasi-steady-state assumption (sQSSA, Briggs and Haldane, 1925), resulting in the MM equation,

$$v = \frac{v_{\max}[S]}{K_M + [S]} \tag{2}$$

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which leads to the double-reciprocal linear plot (Haldane and Stern, 1932; Lineweaver and Burk, 1934)

$$\frac{1}{v} = \frac{1}{v_{\max}} \left(1 + \frac{K_M}{[S]}\right) \tag{3}$$

and allows one to estimate the reaction parameters, namely the *MM constant* $K_M \equiv (k_{-1} + k_2)/k_1$ and the *maximum velocity* $v_{\max} \equiv k_2[E_0]$.

More recently, based on the sQSSA, Schnell and Mendoza (1997) have derived for the basic enzyme reaction (1) a closed form solution for the total time evolution of the substrate:

$$[S'](t) = W\left([S'_0] \exp(-kt + [S'_0])\right). \tag{4}$$

In this expression $[S'] \equiv [S]/K_M$ is the *reduced concentration* and $k \equiv v_{\max}/K_M$ is the *first-order rate constant*. The innovative aspect of this solution is the relatively unknown *omega function* W (Wright, 1959; Fritsch *et al*., 1973; Barry *et al*., 1995a,b; Corless *et al*., 1996) which satisfies the transcendental equation $W(x)\exp(W(x)) = x$, and allows the complete characterization of the system, i.e., the time evolution of all the reactant concentrations and their derivatives. This result also allows for a novel procedure for fitting experimental data to determine reaction constants based on concentrations rather than velocities. The effectiveness of this procedure has been recently verified by Goudar *et al*. (1999).

The actual validity of the quasi-steady-state (QSS) approximation was first discussed by Laidler (1955) who found that the substrate concentration ($[S]$) has to greatly exceed that of the enzyme ($[E]$)

$$\frac{[E_0]}{[S_0]} \ll 1, \tag{5}$$

where the subscript 0 denotes initial concentration. Using the early analog computers, Hommes (1962), Walter and Morales (1964) and Walter (1966) mapped the range of validity of the sQSSA, showing notable shortcomings for the case with large reverse bimolecular velocity constants. Wong (1965) made an attempt to develop a continuous description of the transient-state and the steady-state phase, and concluded that the transient must be brief for the sQSSA to be applicable. Stayton and Fromm (1979) found the sQSSA to generally hold for substrate–enzyme ratios greater than 100 by means of simulation modelling on a digital computer, and by considering the time-dependent process, Schauer and Heinrich (1979) gave a detailed analysis of the errors resulting from the sQSSA. More recently, Segel (1988) and Segel and Slemrod (1989) showed that a more general condition for the sQSSA to be valid is

$$\frac{[E_0]}{K_M + [S_0]} \ll 1. \tag{6}$$

In the case of most *in vitro* assays, condition (6) is satisfied easily for the basic enzyme reaction (1). It is normally assumed that the formation of the enzyme–substrate complex C does not diminish significantly the concentration of the substrate S. Thus, the purpose of assumption (6) is to guarantee that there is not a significant fraction of the substrate bound to the enzyme during the assay (Reiner, 1969; Segel, 1975, 1988; Schulz, 1994). According to Schulz (1994), the presumption is not that the enzyme must be saturated with substrate, this is a misinterpretation of the assumption. Although, this condition implies that the concentration of the intermediate complex is in a QSS with regard to the substrate and the product, due to enzyme saturation (Segel, 1988; Segel and Slemrod, 1989; Schnell and Mendoza, 1997).

However, the sQSSA condition breaks down in *in vivo* conditions (Sols and Marco, 1970). Intra-cellular concentrations of enzyme are usually higher or at least of the same magnitude as their substrates and, consequently, a significant fraction of S can be bound as C complexes. Substrate concentration within the cells are in the neighbourhood of their K_M values (these values range from about 10^{-6} to 10^{-2} M), otherwise the full potential of the enzyme would not be realized (Goldstein, 1944; Srere, 1967; Cha, 1970; Segel, 1975). Furthermore, it is also recognized that high affinity of an enzyme for a substrate may lead to binding of a significant proportion of substrate to the enzyme.

Under this situation, the MM equation (2), its double reciprocal plot (3) and the Schnell and Mendoza (1997) equation (4) become increasingly invalid (Straus and Goldstein, 1943; Cha, 1970; Segel, 1988; Schnell and Mendoza, 1997). Some expressions have been developed that allow the determination of the equilibrium constants for high enzyme concentration or high affinity of an enzyme for a substrate (Goldstein, 1944; Dixon, 1972; Henderson, 1973). The equation most widely used is the generalized rate equation for the formation of product derived by Goldstein (1944), Cha and Cha (1965) and Reiner (1969)

$$v = \frac{k_2}{2}\left((K_M + [E_0] + [\widetilde{S}]) - \sqrt{(K_M + [E_0] + [\widetilde{S}])^2 - 4[\widetilde{S}][E_0]}\right), \qquad (7)$$

where $[\widetilde{S}]$ is a new variable, the total substrate concentration, given by what is called the substrate mass balance (Reiner, 1969; Segel, 1975; Schulz, 1994)

$$[\widetilde{S}] = [S] + [C]. \qquad (8)$$

In spite of these attempts to study the enzyme kinetics at high enzyme concentrations, the latter rate equation (7) has been developed in accordance with the sQSSA for the complex C without examining if the sQSSA holds for this case. Lim (1973) showed that expression (8) is not the substrate mass balance, it is only a definition for the sum of C and S. The correct conservation law is

$$[\widetilde{S}] = [S] + [C] = [S_0] - [P]. \qquad (9)$$

Substituting (9) reduces expression (7) to (2) (see Lim, 1973, p. 660). Therefore, the general rate equation is equivalent to the MM equation. In addition, Lim (1973) has analysed the discrepancies between the numerical solution of S and the sQSSA solution. The agreement between the sQSSA solution and the numerical solution is quite good when $[E_0] \leq 0.01[S_0]$. However, when $[E_0]/[S_0]$ becomes large, the error of the sQSSA solution becomes intolerably high. Furthermore, the author illustrates that the error involved is particularly high during the initial stages of the reaction. These results suggest that the assumption $\mathrm{d}[C]/\mathrm{d}t \approx 0$ of the sQSSA could be inappropriate at high enzyme concentration.

Recently, Borghans *et al*. (1996) re-examined the problem. In their paper, by changing variables from free substrate S to total substrate $\widetilde{S}$, they extend the domain of parameters for which it is permissible to employ the classical assumption $\mathrm{d}[C]/\mathrm{d}t \approx 0$, with the following condition

$$\frac{k_2[E_0]}{k_1(K_M + [S_0] + [E_0])^2} \ll 1. \tag{10}$$

Recalling Segel (1988), they explain that the essential reason why the basic assumptions hold is that the QSS variable C has a fast intrinsic rate of change compared with the non-QSS variable: total substrate $\widetilde{S}$. This variable changes very much more slowly than S, and therefore the sQSSA is improved. This new condition for the total substrate system $\widetilde{S}$ is called the total quasi-steady-state assumption (tQSSA). It is important to note that Borghans *et al*. (1996) neither challenged the basic assumption $\mathrm{d}[C]/\mathrm{d}t \approx 0$ nor found a rate equation for the substrate or the product at high enzyme concentration. They employed a different approach by changing variables and extending the sQSSA.

The aim of the present work is to extend the formalism of Schnell and Mendoza (1997) to the basic enzyme reaction at high enzyme concentration. In Section 2 we show how to derive an approximate solution to describe the variations of the reactant concentrations during the complete span of the enzyme reaction, based on the reverse quasi-steady-state assumption (rQSSA, Segel and Slemrod, 1989). This solution simplifies analysis and provides a new procedure for fitting experimental data. In pursuing this goal, we challenge the assumption $\mathrm{d}[C]/\mathrm{d}t \approx 0$ for the basic reaction at high enzyme concentration. In Section 3 we investigate the conditions under which the rQSSA is valid. In Section 4 we study the domains of validity of the rQSSA and the sQSSA. Crucially, we find that the union of these two domains covers the whole positive $[S_0]$–$[E_0]$ plane. This means that we can find an analytic approximation for the reactant concentration profiles for any initial substrate and enzyme concentrations. Finally, in Section 5 we summarize the main conclusions and discuss the advantages of the present approach.

2. The Reverse Quasi-steady-state Assumption

In the general reference for the sQSSA as a case study in perturbation, Segel and Slemrod (1989) proposed a rQSSA in which the substrate S is in a QSS with respect to the enzyme–substrate complex C. The derivation of the rQSSA is considered to further test the principles of their scaling concepts to discover new aspects of the QSS. However, the full potential of the rQSSA has not been exploited.

Our aim is to challenge the basic assumption $\mathrm{d}[C]/\mathrm{d}t \approx 0$ with the aid of the rQSSA when the enzyme reaction (1) occurs at high enzyme concentration. The paper of Segel and Slemrod (1989) examines the sQSSA, showing that it holds if $[S_0] + K_M \gg [E_0]$. From a biophysical point of view, it seems reasonable to state that the enzyme–substrate complex C is in a QSS when the concentration of the substrate S is high enough, because the free enzyme E will immediately combine with another molecule of S. However, when there is an excess of enzyme E, this condition does not hold (Segel and Slemrod, 1989; Borghans *et al*., 1996). In the latter case, all the molecules of substrate S will immediately combine with the molecules of E. This implies that the substrate will be depleted, and the approximation $\mathrm{d}[S]/\mathrm{d}t \approx 0$ can be valid for a considerable period of time.

Therefore, instead of C being in a QSS with respect to S, at high enzyme concentration it seems to be more reasonable to propose that S is in QSS with respect to C. To further elucidate the principles of the rQSSA, we firstly derive the governing equations for the system.

By applying the law of mass action, the time evolution of reaction (1) can be described completely by the following pair of coupled non-linear differential equations

$$\frac{\mathrm{d}[S]}{\mathrm{d}t} = -k_1([E_0] - [C])[S] + k_{-1}[C] \tag{11}$$

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k_1([E_0] - [C])[S] - (k_{-1} + k_2)[C], \tag{12}$$

together with the uncoupled equation

$$\frac{\mathrm{d}[P]}{\mathrm{d}t} = k_2[C] \tag{13}$$

and the enzyme and substrate conservation laws

$$[E_0] = [E](t) + [C](t) \tag{14}$$

$$[S_0] = [S](t) + [C](t) + [P](t), \tag{15}$$

with initial conditions at $t = 0$

$$([S], [E], [C], [P]) = ([S_0], [E_0], 0, 0). \tag{16}$$

In this system, the parameters k_1, k_{-1} and k_2 are positive rate constants for each reaction.

The phase plane curves or phase trajectories for the system are the solutions of the following expression obtained on dividing (12) by (11),

$$\frac{\mathrm{d}[C]}{\mathrm{d}[S]} = \frac{k_1([E_0]-[C])[S]-(k_{-1}+k_2)[C]}{-k_1([E_0]-[C])[S]+k_{-1}[C]}. \tag{17}$$

This equation can be rearranged to give

$$[C]([S]) = \frac{[E_0][S]}{K_S + \dfrac{K}{1+\mathrm{d}[C]/\mathrm{d}[S]} + [S]}, \tag{18}$$

where $K_S = k_{-1}/k_1$ is the *equilibrium dissociation constant* of S from C, and $K = k_2/k_1$, which we call the *Van Slyke–Cullen constant* (van Slyke and Cullen, 1914).

Further simplification can be introduced to this system by assuming the rQSS. We consider that the rQSS is based on the following two assumptions:

(1) After the initial transient, $t > t_S$ say, the substrate concentration has been depleted and approaches zero. Therefore, in the slow time regime

$$\frac{\mathrm{d}[S]}{\mathrm{d}t} \approx 0. \tag{19}$$

Substituting this condition into (18) leads to

$$[C] = \frac{[E_0][S]}{K_S + [S]} \qquad (t > t_S). \tag{20}$$

If $k_2 \ll k_{-1}$, this equation will be indistinguishable from the corresponding sQSSA equation obtained by substituting $\mathrm{d}[C]/\mathrm{d}t \approx 0$ into (18), $[C] = ([E_0][S])/(K_M + [S])$, where K_M, the MM constant, is equal to $K_S + K$.

Solving (20) for S, we obtain

$$[S] = \frac{K_S[C]}{[E_0]-[C]} \qquad (t > t_S), \tag{21}$$

which substituted into (12) leads to the decoupled differential equation

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = -k_2[C], \tag{22}$$

with initial condition $[C](t \to t_S) = c_0$, where c_0 is a constant to be determined by matching with the solution for $t < t_S$.

(2) During the initial transient, that is for $t < t_S$, a negligible amount of the initial enzyme E_0 is used to form the enzyme–substrate complex C. This assumption implies that the complex concentration is negligible with respect to the initial enzyme concentration. Therefore, for $t < t_S$, it may be assumed that

$$[E](t) \approx [E_0] \Rightarrow \frac{[C]}{[E_0]} \ll 1 \qquad (t < t_S). \tag{23}$$

This assumption has an important implication for system (11)–(12), because it enables the following simplification to be made for the fast transient,

$$\frac{\mathrm{d}[S]}{\mathrm{d}t} = -k_1[E_0][S] \tag{24}$$

$$\frac{\mathrm{d}[C]}{\mathrm{d}t} = k_1[E_0][S], \tag{25}$$

with initial conditions $([S], [C]) = ([S_0], 0)$ at $t = 0$. Solving these equations, we obtain for $t < t_S$

$$[S](t) = [S_0] \exp(-k_1[E_0]t) \tag{26}$$

$$[C](t) = [S_0] \left(1 - \exp(-k_1[E_0]t)\right). \tag{27}$$

To match the enzyme–substrate complex (C) solutions for $t < t_S$ and $t > t_S$, we calculate the limit of equation (27) as $t \to \infty$. We find this limit ($[S_0]$) is the initial condition c_0 for $[C]$ in (22). With this, equation (22) yields

$$[C](t) = [S_0] \exp(-k_2 t) \qquad (t > t_S). \tag{28}$$

Now, we can obtain a uniform approximation for the total time evolution ($0 < t < \infty$) of the reactant concentration. For $[C]$, we add the solution for $t < t_S$ and $t > t_S$ and then subtract their common part, which is given by the limit previously estimated ($[S_0]$). For $[S]$, the matching procedure is not quite as straightforward as it is for $[C]$. We find that we need to go to higher-order terms in the small parameter given by (23) to obtain a uniformly valid solution that is non-trivial in the outer time scale. If $k_1[E_0] \gg k_2$ (see next section) this may be approximated by

$$[S](t) = [S_0] \exp(-k_1[E_0]t) + \frac{K_S[S_0]}{[E_0]} (\exp(-k_2 t) - \exp(-k_1[E_0]t)) \tag{29}$$

$$[C](t) = [S_0](\exp(-k_2 t) - \exp(-k_1[E_0]t)). \tag{30}$$

In Fig. 1, these approximations are shown (solid curve) along with the solutions for $t < t_S$ (dashed curve) and $t > t_S$ (dotted curve), and the numerical solutions

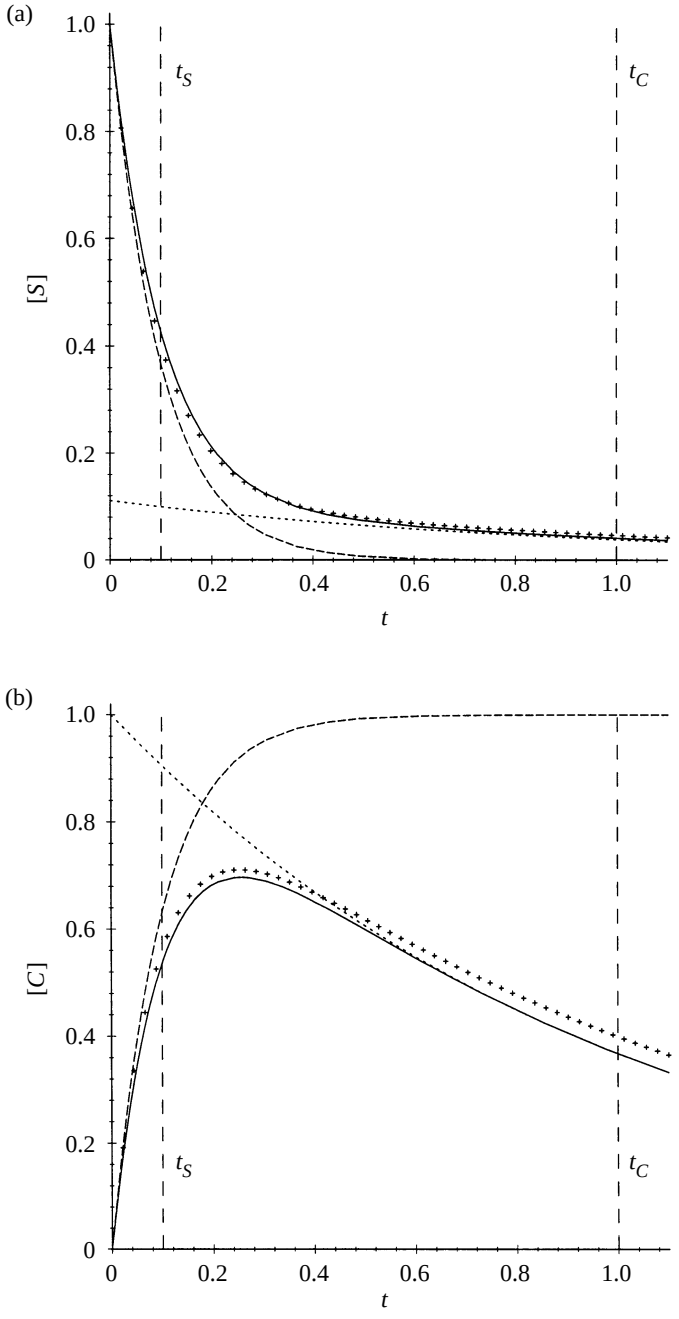

Figure 1. Graph of the numerical solution (cross symbols), the uniform approximation (solid curve), the solutions for $t < t_S$ (dashed curve) and $t > t_S$ (dotted curve) for the time-dependent behaviour of (a) the substrate concentrations ($[S]$) and (b) the enzyme–substrate complex ($[C]$) in the basic reaction (1) at high enzyme concentration. The fast, t_S, and the slow, t_C, time-scales are also shown as vertical dashed lines. Initial conditions are: $[E_0] = 10$ and $[S_0] = 1$; parameter values are $k_1 = 1$, $k_{-1} = 1$ and $k_2 = 1$.

(cross symbols) for $[S_0]/[E_0] = 0.1$ and $K_M = 2$. Note that the approximations are in very close agreement with the numerical solutions. The approximate and numerical solutions would be almost indistinguishable over most of the interval if the $[S_0]/[E_0]$ ratio is decreased.

It will be shown in the following section (Section 3) under what circumstances we can use the rQSSA. Of great interest is that the $[S_0]/[E_0]$ ratio needs to be small as a necessary condition for the validity of the rQSSA.

3. CONDITIONS FOR THE VALIDITY OF rQSSA

The selection of appropriate time-scales is the basis for deriving the necessary conditions for the validity of quasi-steady-state assumptions. In the context of the rQSS, Segel and Slemrod (1989) suggested two time-scales: (i) the time related to the duration of the initial fast transient, t_S, which is the time taken for a significant change in S concentration, and (ii) the time that characterizes the rQSS period, t_C, which is the time taken for a significant change in C concentration. Using the criteria proposed by Segel (1984) and Lin and Segel (1988), we can determine the time-scales for the rQSSA. From (26), the fast time-scale for the initial transient is derived as

$$t_S = \frac{1}{k_1[E_0]}, \tag{31}$$

and the slow time-scale for a significant change in C concentration, t_C, is determined from equation (28) as

$$t_C = \frac{1}{k_2}. \tag{32}$$

The intrinsic time-scale for the substrate S differs from that quoted by Segel and Slemrod (1989) who found that the fast time-scale is $(k_1([E_0] - [C]))^{-1}$ during the test of their methods when S is considered in QSS.

On the other hand, in agreement with Segel and Slemrod (1989), if we derived the condition for the fast time-scale t_S to be indeed much smaller than the slow time-scale t_C, that is

$$t_S \ll t_C, \tag{33}$$

we find the criterion

$$\frac{k_2}{k_1[E_0]} \ll 1. \tag{34}$$

This condition can also be expressed as

$$[E_0] \gg K, \tag{35}$$

which can be written, if we restrict $k_{-1} \ll k_2$, as

$$[E_0] \gg K_M. \tag{36}$$

We now derive another necessary condition. An essential feature for the rQSSA, stated in (23), is that a negligible amount of the initial enzyme E_0 is used to form the enzyme–substrate complex C during the initial transient. This is ensured by demanding that the fractional change of the free enzyme is small during the fast transient, that is,

$$\left|\frac{\Delta[E]}{[E_0]}\right| \approx \frac{t_S}{[E_0]}\left|\frac{\mathrm{d}[E]}{\mathrm{d}t}\right|_{\max} \ll 1. \tag{37}$$

From the conservation law (14), it follows that,

$$\frac{\mathrm{d}[E]}{\mathrm{d}t} = -\frac{\mathrm{d}[C]}{\mathrm{d}t}. \tag{38}$$

Using equation (25) with $[S] = [S_0]$ to determine $\Delta[C]$ leads to

$$\left|\frac{\mathrm{d}[E]}{\mathrm{d}t}\right|_{\max} = |-k_1[E_0][S_0]|, \tag{39}$$

and with definition (31) of t_S, it follows that

$$\left|\frac{\Delta[E]}{[E_0]}\right| \approx \frac{[S_0]}{[E_0]}. \tag{40}$$

Therefore, we find the additional necessary condition for the validity of the rQSSA

$$\frac{[S_0]}{[E_0]} \ll 1. \tag{41}$$

In Fig. 2, to check that conditions (34) and (41) are necessary for the validity of the rQSSA, we compare the approximation (20) for the rQSS (dashed curve) with the numerical solution plotted in the phase plane (solid curve) for different values of k_1, k_2 and $[E_0]$, with initial conditions $[S_0] = 1$ and $k_{-1} = 1$. The initial transient t_S holds for substrate concentrations greater than those indicated by the point r.

We expect the rQSSA to be valid when the substrate concentration is depleted during the initial transient ($t < t_S$) and the rQSS solution (20) to approach the

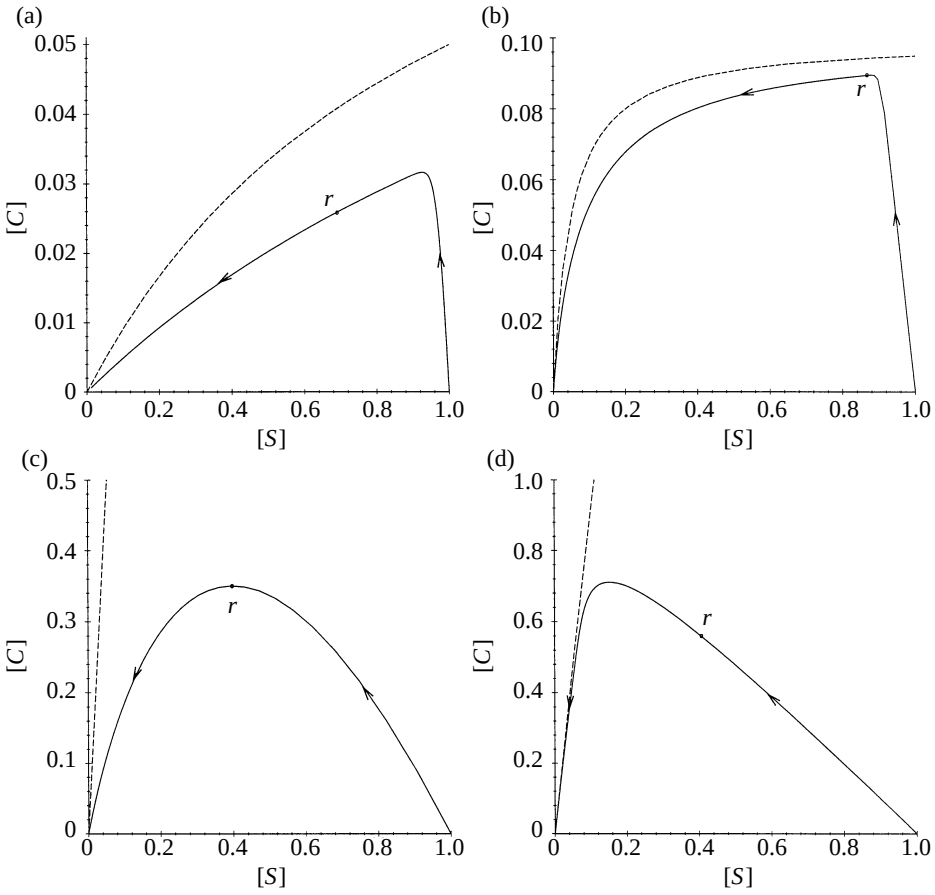

Figure 2. Comparison between the phase plane rQSS approximation (20) (dashed curve) and the numerical solution (solid curve) for $k_{-1} = 1$ and the initial conditions $[S_0] = 1$. The initial transient, t_S, holds for all substrate concentrations above those indicated by the point r in the graph. In: (a) conditions (34) and (41) are invalid ($[E_0] = 0.1$, $k_1 = 1$ and $k_2 = 1$), (b) condition (34) is satisfied, but new criterion (41) is not valid ($[E_0] = 0.1$, $k_1 = 20$ and $k_2 = 1$), (c) criterion (34) is not valid, but condition (41) is satisfied ($[E_0] = 10$, $k_1 = 1$ and $k_2 = 20$) and (d) both conditions are valid ($[E_0] = 10$, $k_1 = 1$ and $k_2 = 1$). Note that when (41) is satisfied, the substrate concentration is depleted during the initial transient. If the two conditions of the rQSSA are valid, then the rQSS approximation approaches the numerically computed phase plane trajectory.

numerical solution in the phase plane after the initial transient ($t > t_S$). This happens when conditions (34) and (41) are satisfied [Fig. 2(d)]. If these conditions are not satisfied [see Fig. 2(a)], the substrate concentration hardly changes during the initial transient and the rQSS approximation does not approach the numerical solution. Note that the change in substrate concentration is small during the initial transient ($t < t_S$), when the initial portion of the numerically computed trajectory is nearly vertical in the phase plane, and occurs when condition (41) is not satisfied [Fig. 2(a) and (b)]. If this condition is satisfied, the substrate is depleted during the initial transient [see Fig. 2(c) and (d)]. This confirms that (41) is also a necessary condition for the rQSSA. Additionally, if (34) is not satisfied [Fig. 2(a) and (c)], the rQSS approximation (20) does not approach the numerically calculated phase plane trajectory after the initial transient ($t > t_S$).

Note that there is an additional important finding related to the discovery of condition (41). We observe that the general condition for the validity of the sQSSA (6) and the second necessary condition for the rQSSA (41) are both valid in a domain which depends on K_M. In Section 4, we shall compare the sQSSA and rQSSA, and it will be shown that the rQSSA may extend the range for which the QSSA can be applied to solve the enzyme reaction (1) completely in the positive $[S_0]$–$[E_0]$ plane.

4. Comparison Between sQSSA and rQSSA

In Fig. 3(a), the condition for the validity of the sQSSA (6) and the second condition for the validity of the rQSSA (41) are plotted in $[S_0]$–$[E_0]$ space to compare their regions of validity. Note that, strictly speaking, the rQSSA can be valid only when both criteria (34) and (41) are satisfied. Therefore, to make a fair comparison, we have chosen parameter values that satisfy the first condition (34) for the rQSSA.

Under such conditions, we find three regions in the positive $[S_0]$–$[E_0]$ plane for the fixed parameters $k_1 = 2$, $k_{-1} = 1$ and $k_2 = 1$ ($K_M = 1$). In region A, only the rQSS approximation is valid, that is

$$\frac{[E_0]}{K_M + [S_0]} > 1. \tag{42}$$

On the other hand, in region C, the only approximation valid is the sQSS, which is given by

$$\frac{[E_0]}{[S_0]} < 1. \tag{43}$$

Finally, the sQSSA and rQSSA are both valid in B, which is defined as

$$\frac{[E_0]}{K_M + [S_0]} < 1 \qquad \text{and} \qquad \frac{[E_0]}{[S_0]} > 1. \tag{44}$$

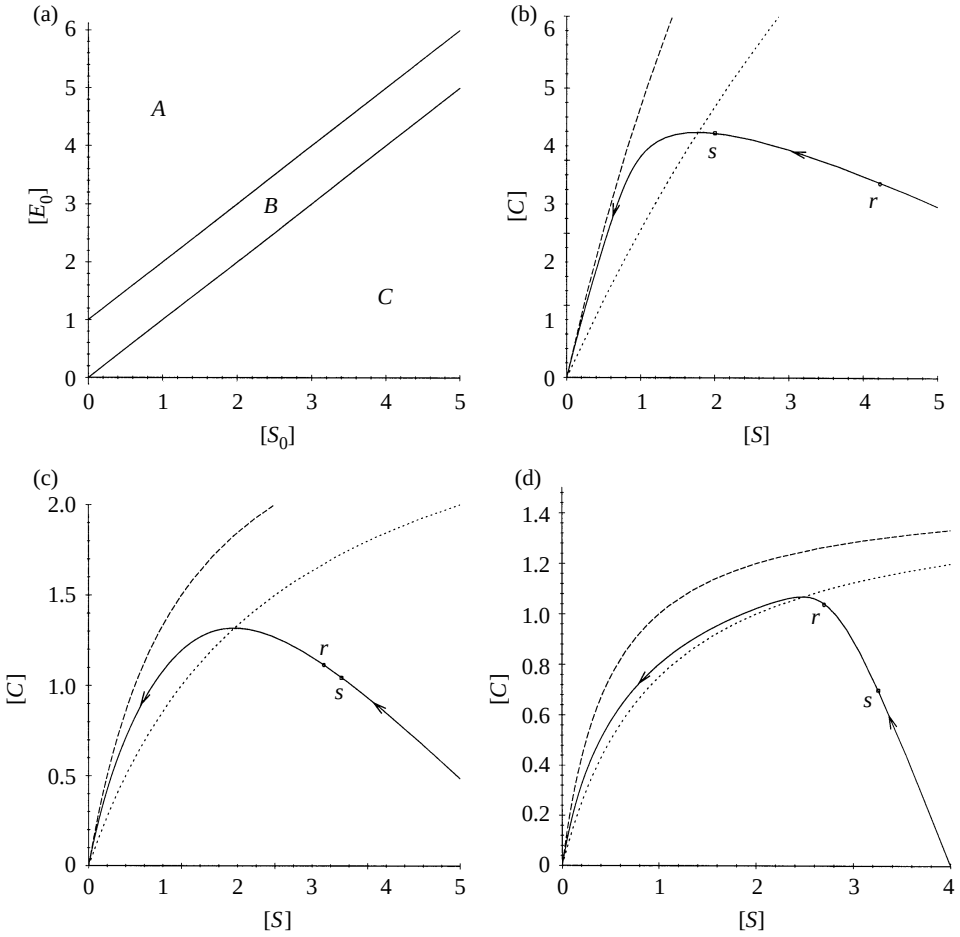

Figure 3. (a) Validity of the sQSSA and the rQSSA for the enzyme reaction (1) for the case when condition (34) is satisfied. Parameter values are $k_1 = 2$, $k_{-1} = 1$ and $k_2 = 1$ ($K_M = 1$). There are three regions: A where the rQSSA is the better approximation, B where rQSSA and sQSSA are both good approximations and C where the sQSSA is the better approximation. In (b), (c) and (d) we compare the rQSS (dashed curve) and sQSS (dotted curve) approximations with the numerical solutions (solid curve) for the three regions. In addition, the initial transient for the rQSSA and the sQSSA are indicated, respectively, as the points r and s. In (b) the rQSS approximation approaches the numerical solution ($[S_0] = 1.0$, $[E_0] = 4.5$); in (c) both the rQSS and sQSS approximations approach the numerical solution ($[S_0] = 2.5$, $[E_0] = 3.0$) and in (d) the sQSS approximation approaches the numerical solution ($[S_0] = 4.5$, $[E_0] = 1.0$).

Note that the common region of validity for both is a function of K_M, and would increase as K_M increases.

To illustrate this dynamics, we plot the phase trajectories from the numerical solution of (18) (solid curve) and its approximate solution (20) for the rQSSA (dashed curve) and for the sQSSA, which is obtained by substituting $\mathrm{d}[C]/\mathrm{d}t \approx 0$ into (18), $[C] = ([E_0][S])/(K_M + [S])$ (dotted curve). Additionally, we indicate by r and s, respectively, the points on the trajectory corresponding to the end of the initial transients for the rQSSA (31) and the sQSSA, $t_c = [k_1(K_M + [S_0])]^{-1}$ [see, equation (13) of Segel and Slemrod (1989)]. In Fig. 3(b), we plot the behaviour of the approximations and numerical solution for the initial conditions $[S_0] = 1.0$ and $[E_0] = 4.5$. Note that the rQSSA represents a better approximation than the sQSSA. In Fig. 3(d), we illustrate the reverse case, where $[S_0] = 4.5$ and $[E_0] = 1.0$, and the sQSSA is indeed much better. Finally, we consider a point in space where both approximations are valid ($[S_0] = 2.5$, $[E_0] = 3.0$).

It is important to note that the sQSSA and the rQSSA span all the positive $[S_0]$–$[E_0]$ plane. Thus, the results in this paper, combined with those in Schnell and Mendoza (1997), show that analytical approximations can be derived for system (11)–(12) for any initial conditions ($[S_0]$, $[E_0]$).

5. DISCUSSION

The main contribution of the present work is to demonstrate that the rQSSA is the appropriate approximation for the study of the basic reaction (1) at high enzyme concentrations. This result challenges the basic assumption $\mathrm{d}[C]/\mathrm{d}t \approx 0$. In addition, we derived a new criterion for the validity of the rQSSA, namely $[E_0] \gg [S_0]$. Note that in using the rQSSA, Segel and Slemrod (1989) assumed as a special case that $[E_0] \gg [S_0]$. In Section 3, we claimed that this is a necessary condition (see Fig. 2).

We also take the opportunity to emphasize that, subject to the constraints of the rQSSA, an approximate solution for the reactant concentrations is derived valid uniformly in time. This uniform approximate solution is not the exact solution but should be accurate for $[S_0]/[E_0] \ll 1$. It is possible to improve on these approximations in a systematic manner, by considering, in the usual way, series expansions for the solutions (Lin and Segel, 1988; Segel and Slemrod, 1989).

We have shown (see Fig. 3) that the domain of validity of the QSSA (standard + reverse) covers completely the positive $[S_0]$–$[E_0]$ plane. This improves on previous studies in which all this plane could not be covered by analytic approximations to the reactant concentrations [see Borghans *et al*. (1996), Fig. 1(b), p 51]. Note that this framework can be extended to other enzymatic modes of action.

Furthermore, in the light of the results of Schnell and Mendoza (1997), the present formalism brings forth new perspectives in the implementation of experimental techniques to determine kinetics parameters. This new experimental ap-

proach, in practice, would consist of fitting the experimentally determined substrate concentration decay profile as a function of time with the uniformly valid approximate solution (29) to obtain k_1, k_{-1} and k_2. In addition, approximation (30) to the complex concentration could also be fitted to experimental data.

Finally, it is important to note that after the initial transient ($t > t_S$), when the rQSS has been reached, we can deduce an equation for the velocity of product formation by substituting (20) into (13), $\mathrm{d}[P]/\mathrm{d}t = v_{\max}[S]/(K_S + [S])$, which can be integrated to give $[P](t) = [S_0](1 - \exp(-k_2 t))$, and again we can use this for fitting the concentration of product formation after the initial transient.

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