



Michaelis–Menten Kinetics at High Enzyme Concentrations

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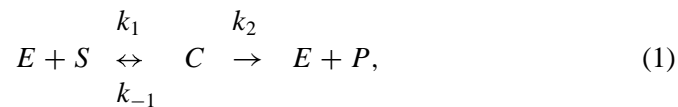
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The total quasi-steady state approximation (tQSSA) for the irreversible Michaelis–Menten scheme is derived in a consistent manner. It is found that self-consistency of the initial transient guarantees the uniform validity of the tQSSA, but does not guarantee the validity of the linearization in the original derivation of Borghans *et al.* (1996, *Bull. Math. Biol.*, **58**, 43–63). Moreover, the present rederivation yielded the noteworthy result that the tQSSA is at least roughly valid for any substrate and enzyme concentrations. This reinforces and extends the original assertion that the parameter domain for which the tQSSA is valid overlaps the domain of validity of the standard quasi-steady state approximation and includes the limit of high enzyme concentrations. The criteria for the uniform validity of the original (linearized) tQSSA are corrected, and are used to derive approximate solutions that are uniformly valid in time. These approximations overlap and extend the domains of validity of the standard and reverse quasi-steady state approximations.

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1. INTRODUCTION

The Michaelis–Menten scheme (Michaelis and Menten, 1913) is extensively used in biochemistry to describe enzymatic processes in solution. This scheme reads



where E and C denote the free and bound enzyme, respectively, S denotes the free substrate and P denotes the product, k_1 is the rate constant of formation of the enzyme substrate complex, k_{-1} the rate constant of dissociation of the enzyme substrate complex and k_2 is the catalysis rate constant. For enzymatic reactions in closed systems, kinetic scheme (1) implies the following conservation relations

$$E_T = E + C, \quad (2)$$

$$S_T = S + C + P, \quad (3)$$

where E_T and S_T are, respectively, the total concentrations of the enzyme and the substrate in the system. Applying the law of mass action to kinetic scheme (1), and using equation (2) to eliminate, yields the following set of differential equations

$$\frac{dS}{dt} = -k_1(E_T - C)S + k_{-1}C, \quad (4)$$

$$\frac{dC}{dt} = k_1((E_T - C)S - K_M C), \quad (5)$$

$$\frac{dP}{dt} = k_2 C, \quad (6)$$

where we employed the standard notation

$$K_M = \frac{k_{-1} + k_2}{k_1}. \quad (7)$$

Typically, enzymatic reactions are studied subject to the following uniform initial conditions

$$(E, S, C, P) = (E_T, S_T, 0, 0), \quad t = 0. \quad (8)$$

Namely, at the beginning of the experiment the enzyme is free and there are no products.

Equations (4)–(8) are only integrable in closed form for the trivial case of vanishing catalysis rate, $k_2 = 0$ ($P \equiv 0$), in which case the problem reduces to simple bimolecular binding. Nevertheless, starting with [Briggs and Haldane \(1925\)](#), the quasi-steady state approximation (QSSA), also referred to as the pseudo-steady-state hypothesis, has proven very fruitful in the analysis of equations (4)–(8), yielding approximate analytical solutions and simple parameter estimation schemes.

Several recent papers have reviewed and extended these results ([Segel and Slemrod, 1989](#); [Borghans *et al.*, 1996](#); [Schnell and Mendoza, 1997](#); [Schnell and Maini, 2000](#)). Adopting the nomenclature of [Borghans *et al.* \(1996\)](#) we distinguish between three different QSSA. The Briggs–Haldane approximation, which will henceforth be referred to as the standard QSSA (sQSSA), is derived by assuming that a short initial transient is closely followed by a QSS such that $dC/dt \approx 0$ ([Segel, 1988](#); [Segel and Slemrod, 1989](#)). The alternative of defining the QSS by $dS/dt \approx 0$ was first studied in [Segel and Slemrod \(1989\)](#), and is referred to as the reverse QSSA (rQSSA). Finally, [Borghans *et al.* \(1996\)](#) recently extended the validity of the sQSSA by employing a change of variable from S , to the total substrate concentration, $S + C = S_T - P$. In keeping with these authors, we shall refer to the latter QSSA as the total QSSA (tQSSA).

An important result of [Borghans *et al.* \(1996\)](#) was that the parameter domain for which the tQSSA is valid overlaps the domain of validity of the sQSSA and

considerably extends it. Thus, whereas $E_T \ll S_T + K_M$ guarantees the validity of the sQSSA (Segel, 1988), the tQSSA is also valid for cases such that $E_T \gg K_M$. Moreover, Borghans *et al.* (1996) argued that the tQSSA is valid in a strictly larger parameter domain than the rQSSA, and that when both approximations are valid they yield virtually identical results. Nevertheless, the validity of the tQSSA at high enzyme concentrations has recently been challenged by Schnell and Maini (2000), on the grounds that at high enzyme concentrations the correct physical assumption is that of the rQSSA, $dS/dt \approx 0$ and not $dC/dt \approx 0$.

The main goals of the present work are to reinforce and correct the results of Borghans *et al.* (1996) and to derive approximations of S , C and P that are uniformly valid in time. These goals are attained by rederiving the tQSSA subject to the principle of minimal simplification (Kruskal, 1963; Segel and Slemrod, 1989), which states that in the process of simplifying a system of equations no term should be neglected without good reason. In the present context this means that we retain quadratic terms in C . Such a procedure yields an integrable (Riccati) differential equation for the initial transient of C , that is analogous to the equations of bimolecular binding. This observation is a crucial feature in our derivation and allows us to show that the validity of the approximation $dC/dt \approx 0$ is much wider than subsequent results obtained by Borghans *et al.* (1996). Moreover, under certain well defined conditions, the results of Borghans *et al.* (1996) are recovered by a first order expansion of the rederived tQSSA.

2. THE TOTAL QUASI-STEADY STATE APPROXIMATION

Following Borghans *et al.* (1996) we define

$$\bar{S} = S + C \quad (9)$$

and add equations (4) and (5) to obtain

$$\frac{d\bar{S}}{dt} = -k_2 C. \quad (10)$$

Substituting equation (9) into (5) we obtain an equation for the concentration of bound enzyme

$$\frac{dC}{dt} = k_1(C - C_+(\bar{S}))(C - C_-(\bar{S})), \quad (11)$$

where

$$C_{\pm}(\bar{S}) = \frac{(E_T + K_M + \bar{S}) \pm \sqrt{(E_T + K_M + \bar{S})^2 - 4E_T\bar{S}}}{2}, \quad (12)$$

are the roots of the quadratic equation

$$C^2 - (E_T + K_M + \bar{S})C + E_T\bar{S} = 0. \quad (13)$$

Finally, P is found from the conservation equation

$$P = S_T - \bar{S}. \quad (14)$$

Initial conditions (8) imply that during the initial transient we can substitute $\bar{S} = S_T$ into equation (11) to obtain

$$\frac{dC}{dt} = k_1(C - C_+(S_T))(C - C_-(S_T)). \quad (15)$$

The solution of this Riccati equation is

$$C_i = C_-(S_T) \left(\frac{1 - e^{-t/t_C}}{1 - (C_-(S_T)/C_+(S_T))e^{-t/t_C}} \right), \quad (16)$$

where we defined

$$t_C \equiv \frac{1}{k_1(C_+(S_T) - C_-(S_T))} = \frac{1}{k_1 \sqrt{(E_T + K_M + S_T)^2 - 4E_T S_T}}. \quad (17)$$

The validity of the initial transient approximation requires its self-consistency (Lin and Segel, 1988). Namely, result (16) is valid for times t such that substitution of $C_i(t)$ into equation (10) yields $\bar{S}(t) \approx S_T$. This criterion can be made explicit by requiring that the fractional decrease of $\bar{S}(t)$ during the initial transient should be small (Segel, 1988)

$$\frac{S_T - \bar{S}(t)}{S_T} \leq \left(\frac{k_2 C_i(t)}{S_T} \right) t \ll 1. \quad (18)$$

Since the duration of the initial transient is on the order of t_C and moreover

$$C_-(S_T) = \lim_{t \rightarrow \infty} C_i(t), \quad (19)$$

equation (18) implies the following *sufficient* condition for the validity of the initial transient approximation (Borghans *et al.*, 1996)

$$\varepsilon \equiv \left(\frac{k_2 C_-(S_T)}{S_T} \right) t_C \ll 1. \quad (20)$$

Assuming that the latter condition is valid, we note that equation (16) implies that $C_i(t)$ grows and in a time of order t_C approaches the maximal asymptotic value implied by the initial conditions, $C_-(S_T)$, which in turn implies that the enzyme-substrate complex eventually enters a QSS such that

$$\frac{dC}{dt} \approx 0, \quad t > t_C \quad (21)$$

and

$$C \approx C_-(\bar{S}), \quad t > t_C. \quad (22)$$

Moreover, since the validity of equation (20) guarantees that the fractional decrease of \bar{S} is negligible during the initial transient, the tQSSA reduces the problem to a single nonlinear differential equation,

$$\frac{d\bar{S}}{dt} \approx -k_2 C_-(\bar{S}), \quad t > t_C, \quad (23)$$

subject to the initial condition

$$\bar{S} = S_T, \quad t = t_C. \quad (24)$$

In this context, the term *total* refers to the fact that the QSSA yields an equation for the total substrate (Borghans *et al.*, 1996). In order for the tQSSA to be uniformly valid for $t > 0$, the induction period prior to the QSS, t_C , has to be much shorter than, $t_{\bar{S}}$, the time scale for the depletion of \bar{S} during the *beginning* of the QSS phase,

$$\frac{t_C}{t_{\bar{S}}} \ll 1. \quad (25)$$

Equations (23) and (24) imply that

$$t_{\bar{S}} = \frac{S_T}{k_2 C_-(S_T)}. \quad (26)$$

Comparing equation (26) to the definition of ε in equation (20), we find

$$\frac{t_C}{t_{\bar{S}}} = \varepsilon, \quad (27)$$

which entails that self consistency of the initial transient approximation [e.g., equation (20)] is a sufficient condition for the tQSSA to be uniformly valid in time. Namely, $\varepsilon \ll 1$ guarantees that we can substitute $t_C = 0$ into equations (23) and (24), and moreover, that

$$C \approx C_-(\bar{S}) \left(\frac{1 - e^{-t/t_C}}{1 - (C_-(S_T)C_+(S_T))e^{-t/t_C}} \right), \quad 0 < t < \infty. \quad (28)$$

2.1. Sufficient conditions for the uniform validity of the tQSSA. An explicit form of the sufficient condition for the uniform validity of the tQSSA is obtained by substituting the definitions of $C_-(S_T)$ and t_C , equations (12) and (17), into (20). In order to simplify the algebra we introduce the notation

$$r(\bar{S}) \equiv \frac{4E_T\bar{S}}{(E_T + K_M + \bar{S})^2}. \quad (29)$$

Since $r < 1$, we can rewrite equation (12) in the form

$$C_{\pm}(\bar{S}) = \frac{(E_T + K_M + \bar{S})(1 \pm \sqrt{1 - r(\bar{S})})}{2}. \quad (30)$$

Incorporating the latter results, into equations (17) and (20), yields, respectively

$$t_C \equiv \frac{1}{k_1(E_T + K_M + S_T)\sqrt{1 - r(S_T)}} \quad (31)$$

and

$$\varepsilon \equiv \left(\frac{K}{2S_T}\right) f(r(S_T)) \ll 1, \quad (32)$$

where

$$K \equiv k_2/k_1 \quad (33)$$

is the so-called *Van Slyke–Cullen* constant (van Slyke and Cullen, 1914), and

$$f(r) \equiv (1 - r)^{-1/2} - 1. \quad (34)$$

Given the values of E_T , S_T , k_1 , k_2 and K_M , equation (32) can be used to verify the validity of the tQSSA. Although equation (32) seems rather cumbersome, it is easy to verify that $\varepsilon(S_T, E_T)$ has a global maximum at $S_T = 0$, $E_T = K_M$, so that (see Appendix B for details)

$$\varepsilon(S_T, E_T) \leq \varepsilon(0, K_M) = \frac{K}{4K_M} \leq \frac{1}{4}. \quad (35)$$

This entails that the tQSSA is always at least roughly valid, and that the smaller the ratio K/K_M , the better the approximation. Figure 1 shows a contour plot of ε for the case when the prefactor K/K_M is equal to unity. The white regions in Fig. 1 guarantee that $\varepsilon \leq 0.1$, which implies that the tQSSA is a good approximation there. This is also guaranteed whenever $S_T > 15K_M$ or $E_T > 16K_M$.

Since the tQSSA problem, equations (23) and (24), cannot be solved in closed form, these results are more of a qualitative nature. In the next section we approximate $C_-(\bar{S})$ by a more manageable form, which can be integrated in closed form.

2.2. The first order tQSSA. As is demonstrated in Appendix A

$$r(S_T, E_T) < 1. \quad (36)$$

Thus, whenever the tQSSA is valid ($\varepsilon \ll 1$) it is useful to expand equations (30)–(32) to first order in r , to obtain, respectively

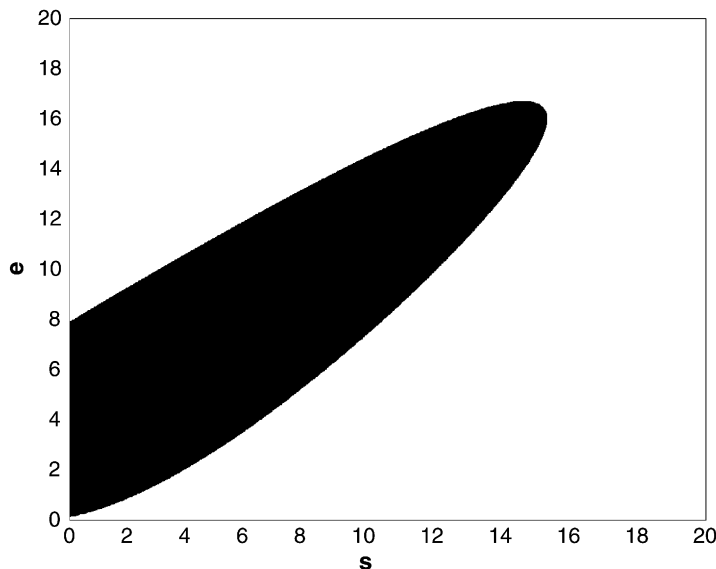


Figure 1. Validity of the tQSSA for the case $K = K_M$. Notations: $s = S_T/K_M$ and $e = E_T/K_M$. White domain: tQSSA valid ($\varepsilon(s, e) \leq 0.10$); Black domain: tQSSA roughly valid ($0.10 < \varepsilon(s, e) \leq 0.25$).

$$C_+(\bar{S}) = (E_T + K_M + \bar{S})(1 - r/4) + O(r^2), \tag{37}$$

$$C_-(\bar{S}) = \frac{E_T \bar{S}}{E_T + K_M + \bar{S}} + O(r^2), \tag{38}$$

$$t_C = \frac{1 + r(S_T)/2}{k_1(E_T + K_M + S_T)} + O(r^2) \tag{39}$$

and

$$\varepsilon = \left(\frac{K}{S_T}\right) \frac{r(S_T)}{4} + O(r^2) = \frac{K E_T}{(E_T + K_M + S_T)^2} + O(r^2). \tag{40}$$

Moreover, equations (37) and (38) imply that

$$\frac{C_-(S_T)}{C_+(S_T)} = \frac{E_T S_T}{(E_T + K_M + S_T)^2} + O(r^2) = \frac{r(S_T)}{4} + O(r^2). \tag{41}$$

The relative errors incurred by the linearizations of equations (38)–(41) are less than 10%, provided that $r \leq 0.6$, $r \leq 0.45$, $r \leq 0.10$, $r \leq 0.15$, respectively.

Substituting equation (38) into (23) and (28), and neglecting terms of order $O(r^2)$ we obtain the *first order* tQSSA

$$\frac{d\bar{S}}{dt} \approx -\frac{k_2 E_T \bar{S}}{E_T + K_M + \bar{S}}, \tag{42}$$

$$\bar{S} = S_T, \quad t = 0, \tag{43}$$

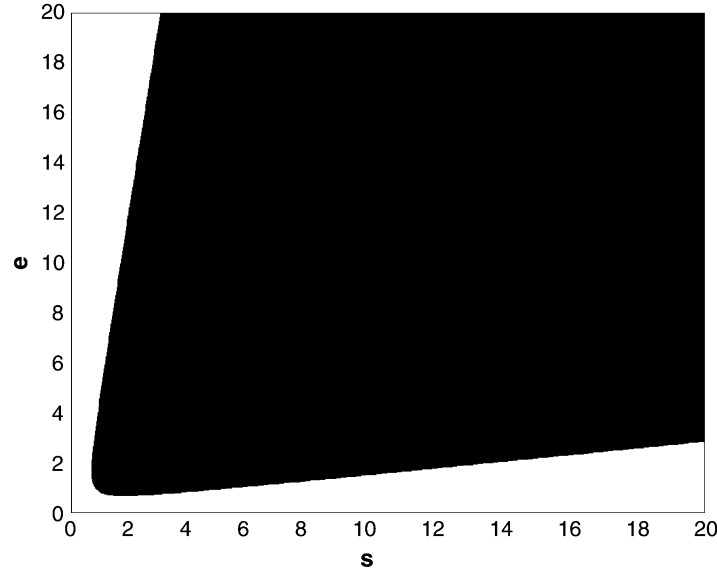


Figure 2. Validity of the first order tQSSA for the case $K \ll K_M$. Notations: $s = S_T/K_M$ and $e = E_T/K_M$. White domain: first order tQSSA valid ($r(s, e) \leq 0.40$); Black domain: first order tQSSA invalid ($0.40 < r(s, e) < 1.0$).

$$C \approx C_-(\bar{S}) \left(\frac{1 - e^{-t/tc}}{1 - (r(S_T)/4)e^{-t/tc}} \right) \approx \frac{E_T \bar{S}}{E_T + K_M + \bar{S}} (1 - e^{-t/tc}),$$

$$0 < t < \infty. \tag{44}$$

Equations (38)–(44) are closely related to the results of Borghans *et al.* (1996).

2.2.1. *Sufficient conditions for the validity of the first order tQSSA.* Although $\varepsilon \ll 1$ guarantees the uniform validity of the tQSSA, the resulting equations do not have a closed form solution unless r is sufficiently small to justify the validity of the first order tQSSA [equations (42)–(44)]. As is shown in Appendix A, each of the following conditions guarantees that $r(\bar{S}) \ll 1$

$$S_T + K_M \gg E_T, \tag{45}$$

$$E_T + K_M \gg S_T. \tag{46}$$

The contour plot shown in Fig. 2 validates these criteria and lends them a more quantitative meaning. White regions in this plot correspond to $r \leq 0.40$. Equation (45) is a well-known necessary condition for the validity of the sQSSA (Segel, 1988; Segel and Slemrod, 1989). As it also guarantees that $\varepsilon \ll 1$, equation (45) is also a sufficient condition for the sQSSA. In contrast, inspection of Fig. 1 reveals that equation (46) does not guarantee that $\varepsilon \ll 1$, when $K \approx K_M$. Thus, equation (46) guarantees the validity of the first order tQSSA only if $K \ll K_M$, in which

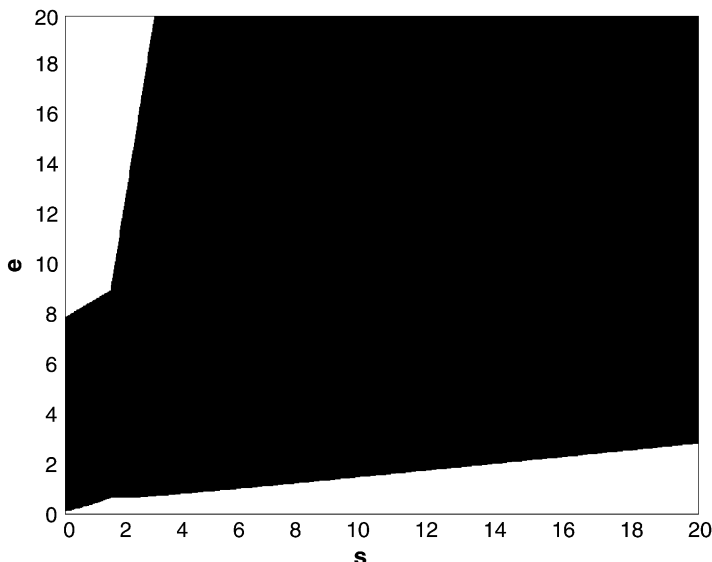


Figure 3. Validity of the first order tQSSA for the case $K = K_M$. Notations: $s = S_T/K_M$ and $e = E_T/K_M$. White domain: first order tQSSA valid ($\varepsilon(s, e) \leq 0.10$ and $r(s, e) \leq 0.40$); Black domain: first order tQSSA roughly valid ($0.10 < \varepsilon(s, e) \leq 0.25$ or $0.40 < r(s, e) < 1.0$).

case equation (35) implies that $\varepsilon \leq 0.25(K/K_M) \ll 0.25$. Stated differently, Fig. 2 delineates the validity domain of the first order tQSSA *only* if $K \ll K_M$.

As noted in Section 2.1, $\varepsilon \leq 0.1(K/K_M)$ is guaranteed whenever $E_T > 16K_M$. Hence, whenever $K \approx K_M$, condition (46) must be augmented by the additional requirement that

$$E_T \gg K_M, \tag{47}$$

which guarantees that $\varepsilon \ll 1$. Taken together, inequalities (46) and (47) imply that $E_T \gg S_T$. The validity of these considerations is illustrated by the contour plot shown in Fig. 3. White regions in this plot guarantee the validity of the first order tQSSA for the case $K \approx K_M$, here defined as $\varepsilon \leq 0.10$ and $r \leq 0.40$.

In summary, each of the following criteria guarantees the validity of the first order tQSSA: equation (45),

$$E_T + K_M \gg S_T \quad \text{and} \quad K \ll K_M, \tag{48}$$

or

$$E_T \gg S_T \quad \text{and} \quad E_T \gg K_M \approx K. \tag{49}$$

These results are different than the criteria in the original derivation of Borghans *et al.* (1996). Basing their analysis on a linearized form of equation (32) [e.g., equation (40)], these authors claimed that the validity of the first order tQSSA is

guaranteed by any of the following conditions: equation (45),

$$k_{-1} \gg k_2, \quad (50)$$

or

$$E_T + S_T \gg K. \quad (51)$$

Equation (50) is equivalent to the requirement that $K \ll K_M$, which as we have shown guarantees that $\varepsilon \ll 1$, but does not guarantee that $r \ll 1$ (see also Figs 6 and 7 in the discussion). Similarly, equation (51) wrongly implies that the first order tQSSA is valid for $E_T \approx S_T \gg K$, even though the latter implies that $r \approx 1$ (see Appendix A and Fig. 2).

Schnell and Maini (2000) derived the following necessary condition for the validity of the rQSSA

$$E_T \gg S_T \quad \text{and} \quad E_T \gg K. \quad (52)$$

Whenever $K \approx K_M$, equation (52) is equivalent to the criterion for the validity of the first order tQSSA, equation (49). In contrast, whenever $K \ll K_M$, the validity of the rQSSA [equation (52)] guarantees the validity of the first order tQSSA [equation (48)], but the validity of equation (48) does *not* imply the validity of equation (52). We therefore find that as originally asserted by Borghans *et al.* (1996), the parameter domain of the validity of the first order tQSSA overlaps and extends the domain of validity of the rQSSA.

2.3. Uniformly valid approximations. Assuming that the first order tQSSA is valid and integrating equation (42) subject to initial condition (43), we obtain

$$\bar{S} + (E_T + K_M)\ln\bar{S} \approx -k_2 E_T t + S_T + (E_T + K_M)\ln S_T. \quad (53)$$

Rewriting the latter as

$$\frac{\bar{S}}{E_T + K_M} \exp\left(\frac{\bar{S}}{E_T + K_M}\right) \approx \frac{S_T}{E_T + K_M} \exp\left(\frac{S_T - k_2 E_T t}{E_T + K_M}\right), \quad (54)$$

yields

$$\frac{\bar{S}}{E_T + K_M} \approx W\left(\frac{S_T}{E_T + K_M} \exp\left(\frac{S_T - k_2 E_T t}{E_T + K_M}\right)\right), \quad 0 < t < \infty. \quad (55)$$

Here W is the Lambert W function, defined as the real valued solution of the following equation (Corless *et al.*, 1996)

$$W(x)e^{W(x)} = x. \quad (56)$$

Moreover, substitution of equation (55) into (44) yields a uniformly valid approximation of C . The corresponding approximations of S and P are obtained by recalling the identities $S \equiv \bar{S} - C$ and $P \equiv S_T - \bar{S}$.

Results (55) and (44) are a natural extension of the closed form solution of the sQSSA derived by Schnell and Mendoza (1997). The validity of the latter approximation is guaranteed by equation (45). We shall now pause to derive the uniformly valid approximations implied by equations (48) and (49).

2.3.1. *High enzyme concentrations.* At high enzyme concentrations such that equation (46) is valid

$$x \equiv S_T/(E_T + K_M) \ll 1, \quad (57)$$

the following approximation of the Lambert W function holds (Corless *et al.*, 1996)

$$W(x) \approx x. \quad (58)$$

The validity of equation (46) therefore implies the following approximations

$$\bar{S} \approx S_T \exp\left(-\frac{k_2 E_T t}{E_T + K_M}\right), \quad (59)$$

$$C \approx \frac{E_T S_T}{E_T + K_M} \exp\left(-\frac{k_2 E_T t}{E_T + K_M}\right) (1 - e^{-k_1 (E_T + K_M)t}), \quad (60)$$

$$S \approx S_T \exp\left(-\frac{k_2 E_T t}{E_T + K_M}\right) \left(1 - \frac{E_T}{E_T + K_M} (1 - e^{-k_1 (E_T + K_M)t})\right). \quad (61)$$

These approximations are valid whenever $K \ll K_M$ or $E_T \gg K_M$. In the latter case equations (59)–(61) reduce to

$$\bar{S} \approx S_T e^{-k_2 t}, \quad (62)$$

$$C \approx S_T (e^{-k_2 t} - e^{-k_1 E_T t}), \quad (63)$$

$$S \approx S_T e^{-k_1 E_T t}. \quad (64)$$

Schnell and Maini (2000) used the rQSSA formalism to obtain the following approximations [subject to the validity of equation (52)]

$$C \approx S_T (e^{-k_2 t} - e^{-k_1 E_T t}), \quad (65)$$

$$S \approx S_T e^{-k_1 E_T t} + S_T (K_D/E_T) (e^{-k_2 t} - e^{-k_1 E_T t}), \quad K_D \equiv k_{-1}/k_1. \quad (66)$$

It is noteworthy that the lowest order rQSSA of C is the same as equation (63), the lowest order tQSSA of C subject to the validity of equation (49). Actually, the

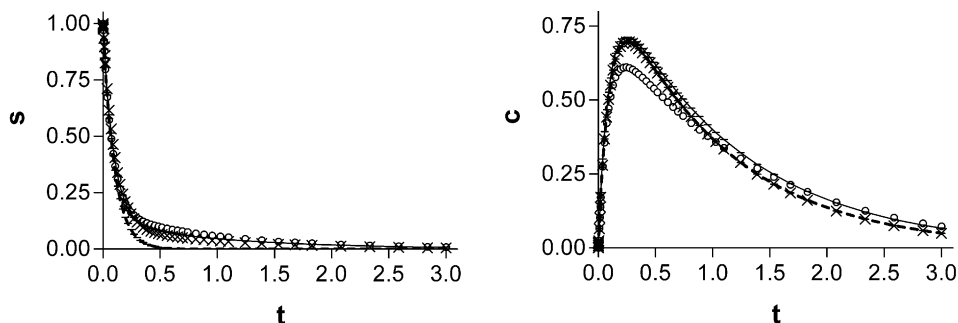


Figure 4. rQSSA vs. tQSSA for a case that satisfies equation (52). Notations: $s = S/S_T$ and $c = C/S_T$. (Solid line) Numerical solution of equations (4) and (5); (crosses) the rQSSA, equations (65) and (66); (dashes) the high enzyme concentration limit of the tQSSA, equations (63) and (64); (circles) the first order tQSSA, equations (44) and (55). The parameter values used in these simulations are from Fig. 1 in Schnell and Maini (2000): $k_1 = 1, k_{-1} = 1, k_2 = 1, S_T = 1$ and $E_T = 10 (K_M = 2, K = 1)$. For these values $r(S_T) = 0.24$ and $\varepsilon(S_T, E_T) = 0.072$.

same is true for S , since as noted in Schnell and Maini (2000), equation (64) is the lowest order rQSSA of S . Figure 4 compares the rQSSA predictions to the tQSSA predictions, for the parameter values $k_1 = 1, k_{-1} = 1, k_2 = 1, S_T = 1$ and $E_T = 10$ ($K_M = 2, K = 1$). These parameter values guarantee the validity of the rQSSA (Schnell and Maini, 2000) and imply that $r(S_T) = 0.24$, $\varepsilon(S_T, E_T) = 0.072$. As is readily seen, the fit of the rQSSA to the numerical solution of equations (4) and (5) is very good. Moreover, although the rQSSA and the high enzyme concentration limit of the tQSSA are indistinguishable for C , Fig. 4 illustrates a case where equation (66) can significantly improve upon equation (64). The fit of the first order tQSSA of S [equation (55)] to the numerical solution of equation (4) is also very good. In contrast, the first order tQSSA of C [equation (44)] correctly captures the overall qualitative behavior of the numerical solution of equation (5), but is quantitative only during the early and late phases. During the late phase, equation (44) is a better estimate than equation (65).

As indicated in Section 2.2.1, there are situations where the first order tQSSA is valid, but the rQSSA is not. For example, Fig. 5 illustrates that the condition $K_M \gg E_T \approx K \gg S_T$ guarantees the validity of (48) and the invalidity of (52).

3. DISCUSSION

The basic contribution of this work is the rederivation of the tQSSA of equations (4)–(8) in a consistent manner which allowed us to correct and elucidate the criteria for its validity, previously obtained in Borghans *et al.* (1996), and moreover, to obtain approximate solutions that are uniformly valid in time when these criteria are met. In contrast with Borghans *et al.* (1996), the present paper explored the

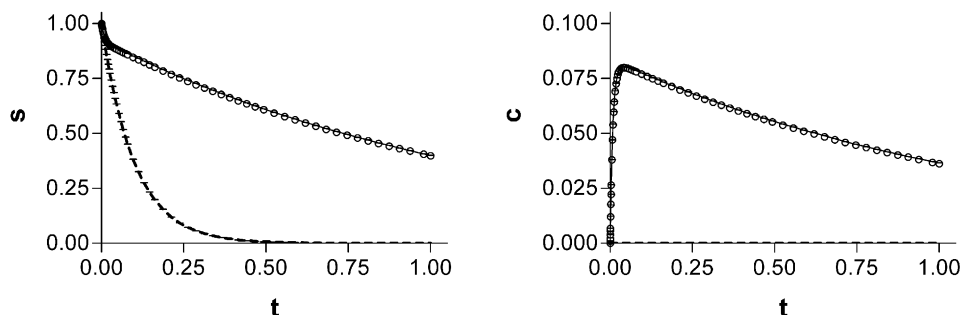


Figure 5. rQSSA vs. tQSSA for a case such that $K_M \gg E_T \approx K \gg S_T$. Notations: $s = S/S_T$ and $c = C/S_T$. (Solid line) Numerical solution of equations (4) and (5); (dashes) the rQSSA, equations (65) and (66); (circles) first order tQSSA, equations (60) and (61). The parameter values used in these simulations are: $k_1 = 1$, $k_{-1} = 100$, $k_2 = 10$, $S_T = 1$ and $E_T = 10(K_M = 110, K = 10)$. For these values $r(S_T) = 0.003$ and $\varepsilon(S_T, E_T) = 0.006$.

consequences of retaining terms proportional to C^2 , in keeping with the principle of minimal simplification (Kruskal, 1963; Segel and Slemrod, 1989). This procedure yielded an improved approximation of the initial transient.

An important result of the present paper is that self consistency of the initial transient is a sufficient condition for the validity of the QSS assumption $dC/dt \approx 0$, and moreover guarantees that the duration of the initial transient is short compared to the QSS phase, $t_C/t_{\bar{S}} \ll 1$. Namely, the sufficient condition for self consistency of the initial transient, $\varepsilon \ll 1$ [equation (20)], was found to guarantee the uniform validity of the tQSSA. The finding that $\varepsilon \leq 0.25K/K_M < 0.25$ therefore implies that the QSS assumption $dC/dt \approx 0$ is roughly valid for any combination of initial substrate and enzyme concentrations, and is an excellent approximation whenever $K \ll K_M$. It is noteworthy that the latter criterion is satisfied for most metabolic enzymes [see Chapter 5 of Atkinson (1977)].

However, it is important to note that $\varepsilon = t_C/t_{\bar{S}} \ll 1$ does not guarantee the validity of the first order tQSSA as previously asserted by Borghans *et al.* (1996). More specifically, $k_{-1} \gg k_2(K \ll K_M)$ guarantees the validity of the tQSSA, but not of the first order tQSSA. These considerations are illustrated in Figs 6 and 7 using the kinetic parameters corresponding to the hydrolysis of acetyl-L-phenylalanine ether by chymotrypsin (Gutfreund and Hammond, 1959). Lim (1973) demonstrated that the sQSSA is invalid for this case and noted that $C_-(\bar{S})$ from equation (12) is equivalent to the sQSSA result $C(S) \approx E_T S/(K_M + S)$. However, he did not compare the tQSSA to the numerical solution. As is evident in Figs 6 and 7, the tQSSA is in fact a very good approximation for this problem, and even the first order tQSSA yields adequate results for the case considered in Fig. 6. Moreover, it is noteworthy that the first order tQSSA yields significantly better estimates of the lumped variable \bar{S} , than of C and S . This stems from the fact that the linearization leading to equation (42) is of wider validity than the

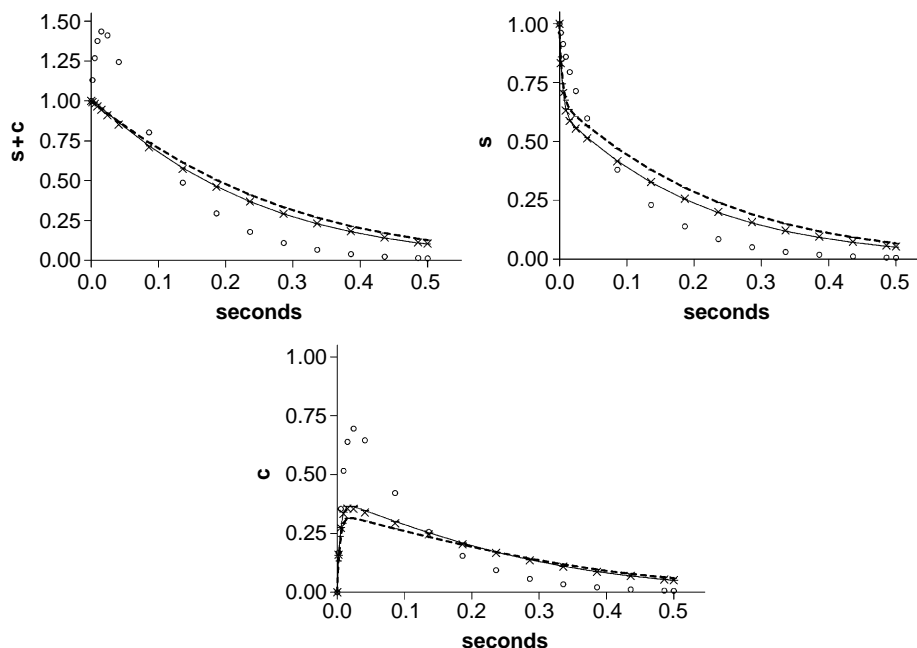


Figure 6. Comparison of the rQSSA to the tQSSA and the first order tQSSA for a case of intermediate enzyme concentrations. Notations: $s = S/S_T$ and $c = C/S_T$. (Solid line) Numerical solution of equations (4) and (5); (crosses) tQSSA; (dashes) first order tQSSA; (circles) rQSSA. The parameter values used in these simulations were: $k_1 = 1 \mu\text{M}^{-1} \text{s}^{-1}$, $k_{-1} = 90 \text{s}^{-1}$, $k_2 = 10 \text{s}^{-1}$, $S_T = K_M$ and $E_T = K_M$ ($K_M = 100 \mu\text{M}$), and are taken from Fig. 1 of Lim (1973). For these values $r(S_T) = 0.44$, $K/K_M = 0.1$ and $\varepsilon = 0.017$, which implies that the tQSSA is a good approximation, but that the first order tQSSA is only marginally valid.

linearizations leading to equation (44). The rQSSA is a poor approximation for both the cases considered in Figs 6 and 7. This is not surprising since although both examples satisfy the first necessary condition for the validity of the rQSSA, $E_T \gg K$, they do not satisfy the second necessary condition, $E_T \gg S_T$. The observation that the rQSSA can predict that $\bar{S} > S_T$ stems from the fact that the rQSSA approximation of S , equation (66), is of higher order than the corresponding approximation of C , equation (65).

Whenever $\varepsilon \gg 1$ and $r \ll 1$, the tQSSA derived by Borghans *et al.* (1996) emerges as a uniformly valid approximation. The criteria for the uniform validity of the first order tQSSA are given by equations (45), (48) and (49). These observations enabled us to derive uniformly valid approximations, subject to the validity of equations (48) and (49). When equation (45) is valid, the sQSSA is valid and equations (44) and (55) reduce to the uniformly valid approximations derived by Schnell and Mendoza (1997). In the opposite extreme, when equation (48) [equation (49)] is valid, the uniformly valid approximations of C and S reduce to an extremely simple form, equations (60) and (61) [equations (63) and (64)].

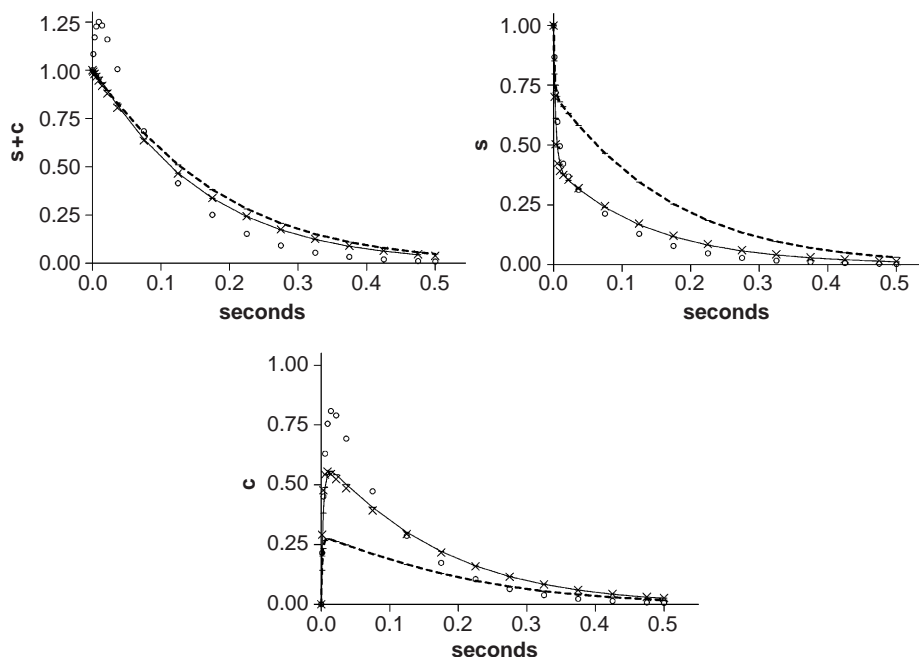


Figure 7. Comparison of the rQSSA to the tQSSA and the first order tQSSA for a case of intermediate enzyme concentrations. Notations: $s = S/S_T$ and $c = C/S_T$. (Solid line) Numerical solution of equations (4) and (5); (crosses) tQSSA; (dashes) first order tQSSA; (circles) rQSSA. The parameter values used in these simulations were: $k_1 = 1 \mu\text{M}^{-1} \text{s}^{-1}$, $k_{-1} = 90 \text{s}^{-1}$, $k_2 = 10 \text{s}^{-1}$, $S_T = K_M$ and $E_T = 2K_M$ ($K_M = 100 \mu\text{M}$), and are taken from Fig. 1 of Lim (1973). For these values $r(S_T) = 0.50$, $K/K_M = 0.1$ and $\varepsilon = 0.021$, which implies that the tQSSA is a good approximation, but not the first order tQSSA.

Moreover, the criteria for the validity of the first order tQSSA at high enzyme concentrations, equations (48) and (49), were shown to overlap and extend the necessary condition for the validity of the rQSSA, equation (52). In contrast to equation (45), which is mostly valid under *in vitro* conditions, equations (48) and (49) may also be valid under *in vivo* conditions. Indeed, whereas *in vivo* substrate concentrations are normally close to or below their K_M value [Chapter 2 of Atkinson (1977) and Sols and Marco (1970)], *in vivo* enzyme concentrations can significantly exceed K_M (Sols and Marco, 1970).

Although we have shown that the tQSSA is virtually a global approximation, and that the linearized version of Borghans *et al.* (1996) overlaps and extends the validity domains of the sQSSA and the rQSSA, this does not mean that the tQSSA is a better approximation when the latter approximations are valid. Indeed, Fig. 4 illustrates a case where the rQSSA results of Schnell and Maini (2000) are a better approximation than the first order tQSSA. Moreover, Schnell and Maini (2002) recently applied the nonlinear lumping technique of Li *et al.* (1993) to equations (4) and (5), which seems to be more accurate than the tQSSA and of wider validity than the first order tQSSA.

However, in contrast to the first order tQSSA, which yields closed form approximations for C and S , the simplified equations that are obtained by nonlinear lumping can only be solved numerically (Li *et al.*, 1993; Schnell and Maini, 2002). This balance between validity and simplicity makes the uniformly valid approximations derived in this work a good choice for fitting experimental progress curves using closed form solutions, when no prior knowledge on the kinetic parameters is available, especially if k_2 and K_M are of prime concern. This has recently been demonstrated by Tzafiriri *et al.* (2002) in their analysis of a novel model of the enzymatic degradation of insoluble fibrillar matrices. Additional examples of biological importance wherein the tQSSA may be useful are discussed in Borghans *et al.* (1996).

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APPENDIX A: ANALYSIS OF r

Introducing the dimensionless variables $e = E_T/K_M$ and $s = \bar{S}/K_M$ in equation (29) yields

$$r = \frac{4es}{(1 + e + s)^2}. \quad (\text{A.1})$$

Direct inspection of the latter yields the following simple results

$$1 + s \gg e \Rightarrow r \ll 1, \quad (\text{A.2})$$

$$1 + e \gg s \Rightarrow r \ll 1. \quad (\text{A.3})$$

As long as r is positive we can rewrite equation (A.1) as

$$r = \frac{4es}{(e + s)^2} \times \frac{1}{\left(1 + \frac{1}{e+s}\right)^2}. \quad (\text{A.4})$$

Thus,

$$r \leq \frac{1}{\left(1 + \frac{1}{e+s}\right)^2} < 1, \quad (\text{A.5})$$

$$r < \frac{4es}{(e + s)^2} = \frac{4\theta}{(1 + \theta)^2} \leq 1, \quad \theta \equiv s/e. \quad (\text{A.6})$$

Moreover, equations (A.5) and (A.6) imply, respectively

$$r \leq 1 - \frac{2}{e+s} \approx 1, \quad e+s \gg 1, \quad (\text{A.7})$$

$$r \leq (e+s)^2 \ll 1, \quad e+s \ll 1 \quad (\text{A.8})$$

and

$$r \leq 4\theta \ll 1, \quad \theta \equiv s/e \ll 1, \quad (\text{A.9})$$

$$r \leq \frac{4}{\theta} \ll 1, \quad \theta \equiv s/e \gg 1. \quad (\text{A.10})$$

Finally, result (A.7) along with the fact that $4\theta/(1+\theta)^2$ attains its maximum value (unity) at $\theta = 1$ implies that

$$r \approx 1 \Leftrightarrow e \approx s \gg 1. \quad (\text{A.11})$$

APPENDIX B: ANALYSIS OF ε

Introducing the dimensionless variables $e = E_T/K_M$ and $s = S_T/K_M$ into equation (32) we obtain

$$\varepsilon = \frac{K}{K_M} \frac{f(r(s, e))}{2s}. \quad (\text{B.1})$$

As a first step, we shall show that ε is bounded from above and below. The lower bound is obviously $\varepsilon = 0$ which is attained for $e = 0, s > 0$. In proving that ε is bounded from above, two regions in parameter space are of prime importance: $s \approx e \gg 1$ and $s \rightarrow 0$. The former, because it is a necessary and sufficient condition for $f(r)$ to diverge, and the latter because it implies that $1/s$ diverges. If $s \rightarrow 0$ then $r \rightarrow 0$, so that $f(r) \approx r/2$ and $\varepsilon \approx (K/K_M)e/(1+e)^2$, which is bounded by $0.25(K/K_M)$. Conversely, if $e \approx s \gg 1$, then introducing the dimensionless variable $\theta \equiv e/s \approx 1$ we have [see equation (A.7)]

$$r(\theta, e) \approx 1 - \frac{2}{e(1+\theta)}, \quad (\text{B.2})$$

$$f(r(\theta, e)) \approx \sqrt{e \left(\frac{1+\theta}{2} \right)}, \quad (\text{B.3})$$

$$\varepsilon(\theta, e) \approx \left(\frac{K}{K_M} \right) \left(\sqrt{\frac{1+\theta}{8\theta s}} - \frac{1}{2s} \right) \ll 1. \quad (\text{B.4})$$

Since ε is a continuous function in the first quadrant of the Cartesian plane, it has a maximum and a minimum in any closed set $[0, R]^2$. A necessary condition for a maximum in $(0, R)^2$ is that $\partial\varepsilon/\partial e = \partial\varepsilon/\partial s = 0$. Using the simple results

$$\frac{\partial f}{\partial r} = \frac{1}{2(1-r)^{3/2}}, \tag{B.5}$$

$$\frac{\partial r}{\partial s} = \frac{4e(1+e-s)}{(1+e+s)^3}, \tag{B.6}$$

$$\frac{\partial r}{\partial e} = \frac{4s(1+e-s)}{(1+e+s)^3}, \tag{B.7}$$

we find

$$\frac{\partial\varepsilon}{\partial e} = \left(\frac{K}{K_M}\right) \frac{1}{2s} \frac{\partial r}{\partial e} \frac{df}{dr} = \left(\frac{K}{K_M}\right) \frac{1+s-e}{(1+e+s)^3(1-r)^{3/2}} \tag{B.8}$$

and

$$\frac{\partial\varepsilon}{\partial s} = \frac{K}{K_M} \left(\frac{1}{4s(1-r)^{3/2}} \frac{\partial r}{\partial s} - \frac{f}{2s^2} \right). \tag{B.9}$$

Thus, $\partial\varepsilon/\partial e = 0$ implies that

$$1+s-e=0 \tag{B.10}$$

and

$$r = \frac{s}{s+1}, \tag{B.11}$$

$$f(r) = \sqrt{1+s} - 1, \tag{B.12}$$

$$\frac{\partial r}{\partial s} = \frac{1}{(1+s)^2}, \tag{B.13}$$

$$\begin{aligned} \frac{\partial\varepsilon}{\partial s} &= \frac{K}{K_M} \left(\frac{(1+s)^{3/2}}{4s} \frac{1}{(1+s)^2} - \frac{\sqrt{1+s}-1}{2s^2} \right) \\ &= \frac{K}{K_M} \left(\frac{1}{4s\sqrt{1+s}} - \frac{\sqrt{1+s}-1}{2s^2} \right) < 0. \end{aligned} \tag{B.14}$$

In turn, this implies that ε attains its maximum and minimum on the boundary of the set $[0, R]^2$. Since $\varepsilon \rightarrow 0$ for $e \rightarrow 0$, we conclude that the maximum is attained for $s \rightarrow 0$, and is therefore $K/(4K_M)$, regardless of how large R is.

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