A New Perturbation Solution to the Michaelis-Menten Problem

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We develop a new dimensionless representation of the time-dependent mass balances for the Michaelis–Menten (MM) reaction mechanism; we identify several dimensionless parameters that control the fundamental nature of the solution; and we solve the scaled equations with a combined regular and singular perturbation expansion. Unlike several approximate solutions to the MM problem offered previously in the literature, each of which is valid only for some limited range of conditions, the new solution converges accurately for any combination of initial substrate concentration, initial enzyme concentration, and kinetic rate constants. We discuss the physical significance and interdependence of the dimensionless parameters that emerge from our scaling analysis; we use these parameters to categorize previous approximations for the MM problem and to delimit their accuracy; and we verify the accuracy of our solution via comparisons to an exact numerical solution and various approximations offered previously by others. © 2008 American Institute of Chemical Engineers *AIChE J,* 54: 1344–1357, 2008
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Introduction

Homogeneous, enzyme-catalyzed reactions nearly always occur via a two-step process known as the "Michaelis–Menten" (MM) mechanism.[1–3] In the first elementary reaction, the enzyme E attaches reversibly to the substrate molecule S to form a complex C, i.e.

$$E + S \underset{k_{-1}}{\overset{k_1}{\leftrightarrow}} C; \tag{1}$$

and in the second elementary reaction, the bound enzyme converts the substrate irreversibly to product P and releases it, which returns the enzyme to solution, i.e.,

$$C \xrightarrow{k_2} P + E. \tag{2}$$

The parameters k_1 [units mol/vol-time], k_{-1} [time^{-1}], and k_2 [time^{-1}] are the kinetic rate constants for each elementary reaction as shown.[4] One frequently seeks to measure these rate constants (or combinations thereof) as accurately as possible for various enzyme/substrate combinations to compare their performance or for use in simulations of complex, multi-step, multi-component biochemical processes.[5,6]

Four ordinary differential equations are required to describe the time-dependent concentrations $E(t)$, $S(t)$, $C(t)$, and $P(t)$ in a reactor where an MM reaction is occurring:

$$\frac{dE}{dt} = -k_1(ES - K_{\mathrm{M}}C); \tag{3a}$$

$$\frac{dS}{dt} = -k_1(ES - K_{\mathrm{D}}C); \tag{3b}$$

$$\frac{dC}{dt} = k_1(ES - K_{\mathrm{M}}C); \tag{3c}$$

$$\frac{dP}{dt} = k_1KC. \tag{3d}$$

From this point forward we use combined kinetics parameters that are preferred by the biochemistry community:

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$K = k_2/k_1$ is the Van Slyke-Cullen constant[7]; $K_D = k_{-1}/k_1$ is the dissociation equilibrium constant of the enzyme-substrate complex; and $K_M = K + K_D = (k_{-1} + k_2)/k_1$ is the MM constant.[4] Each of these has units of concentration [mol/vol] and can range between zero and infinity for different enzyme-substrate combinations. They quantify in some sense the relative tendency of enzyme and substrate to distribute among initial, intermediate, and final states during the reaction. Typically, the reaction is carried out in a closed batch reactor charged with an initial concentration of enzyme (E_T) and substrate (S_T), in which case there are two algebraic mass constraints:

$$E_T = E + C; \tag{4a}$$

$$S_T = S + P + C. \tag{4b}$$

Equations 3 and 4 can be combined into two independent ordinary differential equations, which, when solved with the initial conditions $E(t = 0) = E_T$, $S(t = 0) = S_T$, $C(t = 0) = 0$, and $P(t = 0) = 0$, give the time-dependent concentrations of all species in the reactor. The solution of these equations is a function of five parameters: the rate constants k_1, k_{-1}, and k_2 (or the combinations K, K_D, and K_M), which one is typically trying to measure in MM experiments, and the initial concentrations E_T and S_T, which one can vary in experiments to ensure good data are collected.

Unfortunately, the governing Eq. 3 are nonlinear and coupled, so no exact, closed-form solution is available, which makes it difficult to extract estimates of the kinetic parameters from curve-fits to concentration-vs.-time data. Various simplifications and approximations have been proposed to circumvent this difficulty, each of which is only accurate for some limited range of conditions. We will discuss and compare them in some detail later in this article after we present and justify a new, more accurate perturbation solution to the MM equations.

Scaling and Solving the Governing Eqs. 3 and 4

We begin by combining (3) and (4) to recast the problem in terms of $C(t)$ and $P(t)$, which we find to be the most expedient pair of concentrations to use for the ensuing analysis:

$$\frac{dC}{dt} = k_1[(E_T - C)(S_T - P - C) - K_M C] \quad \text{with } C(0) = 0; \tag{5a}$$

$$\frac{dP}{dt} = k_1 K C \quad \text{with } P(0) = 0. \tag{5b}$$

Since the intermediate complex must form according to reaction (1) before the substrate can be converted to product according to reaction (2), there are two natural time-scales in this coupled, nonlinear pair of equations. If the two time-scales are widely disparate, i.e., complex forms quickly and product forms slowly, which we will show to be unavoidably the case here, and if the nonlinearities are relatively weak, the MM equations can be solved to arbitrary accuracy with a rigorous approximation method known as "perturbation theory."[8–10]

To apply perturbation theory, the first and most crucial move is to "scale"[8] the dependent variables, in this case C and P, with appropriate combinations of the constant parameters in the equations to generate new, dimensionless variables that are certain to be $O(1)$ [11] over the full range of the independent variable, in this case $0 \leq t < \infty$. When the scaling causes a small dimensionless parameter, which we will call δ, to appear as a coefficient of nonlinear terms in the equation that are otherwise $O(1)$ functions of the dependent variables, a regular perturbation is indicated. In this situation the equations can be solved by orders in δ with a perturbation series. When the scaling procedure reveals two different time-scales, which can be used to scale the independent variable t, a singular perturbation is indicated. The ratio of the short time-scale to the long time-scale appears as a small dimensionless parameter, which we will call ε. One casts the equations in terms of the short-time variable and solves them by orders in ε with a perturbation series, and then recasts them in terms of the long-time variable and solves them again by orders in ε with a perturbation series. The "inner" solution for short time and the "outer" solution for long time are then matched asymptotically in the intermediate region where the two time-scales overlap. Our analysis of the MM problem is unusual in that perturbations of both types appear together and must be solved simultaneously. For a more detailed discussion of these methods and their applicability to problems of this type, we refer the reader to any of many fine textbooks on the subject.[8–10]

Perturbation methods offer several advantages over numerical techniques for problems of this type. First and foremost, the leading-order, δ, $\varepsilon \to 0$ portion of the solution, which is often easy to find, describes the exact solution to sufficient accuracy for many uses and reveals its dominant behavior. Furthermore, a properly scaled perturbation solution is uniformly valid over the entire range of the independent variable and not subject to the "stacking up" of numerical integration errors. Because of the ordering scheme, the equations for higher-order corrections are certain to be linear and soluble, and because the scaling ensures the dimensionless variables are $O(1)$, the difference between the perturbation and exact solutions at every point is certain to be at most $O(\delta^n$ or $\varepsilon^n)$, where n is the order of the first neglected term in the perturbation expansion. One retrieves an orderly prescription for finding an analytical solution with arbitrary and quantifiable accuracy.

We begin our analysis by transforming the Eq. 5 to scaled, dimensionless forms and identifying the characteristic short and long time-scales. Note first that Eq. 5a has a constant "source" (+) term $k_1 E_T S_T$ on the right hand side. The correct choice of scales must drive this term to one for $O(1)$ balancing against the remaining terms. Note also that the form of (5) ensures that $C(t)$ rises from zero at $t = 0$, passes through a maximum, and then decreases to zero as $t \to \infty$, whereas $P(t)$ increases monotonically from zero at $t = 0$ to S_T as $t \to \infty$. Let the constant parameter β be a leading-order estimate of the maximum in $C(t)$, and define dimensionless, $O(1)$ dependent variables $x = C/\beta$ and $y = P/S_T$. Substituting x and y as described earlier and dividing by $k_1 E_T S_T$ to convert the source term to one converts (5a) to the following dimensionless form:

$$\left(\frac{\beta}{k_1 E_T S_T}\right)\frac{dx}{dt} = 1 - \left(\frac{E_T + S_T + K_M}{E_T S_T}\right)\beta x + \left(\frac{\beta^2}{E_T S_T}\right)x^2 - \left[1 - \left(\frac{\beta}{E_T}\right)x\right]y. \tag{6}$$

Since we anticipate that complex forms rapidly relative to product, we solve for β by setting $y \cong 0$ in this equation and considering the $t \to \infty$ limit $\left(\text{i.e., } dx/d\tau \to 0\right)$ of the remaining terms. Pair-wise balancing of the remaining terms on the right-hand side confirms that the constant and the linear term are dominant, and the term quadratic in x is subdominant,[12] from which we find

$$\beta = \frac{E_T S_T}{E_T + S_T + K_M}. \tag{7}$$

Note that this choice of β sets the coefficient of the term linear in x equal to one, ensuring an $O(1)$ balance of the dominant source and sink terms.

Since the scaled variable x accounts for complex formation, the dominant process on the short time-scale, and since Eq. 6 is dimensionless as shown, one can immediately identify the coefficient of the derivative as the scaling factor for t on the short time-scale. Consequently, we define a dimensionless independent variable $\tau = t/\theta_C$, where

$$\theta_C = \frac{\beta}{k_1 E_T S_T} = \frac{1}{k_1(E_T + S_T + K_M)} \tag{8}$$

is the characteristic (short) time for complex formation. Note the rational dependence on kinetics parameters evident in θ_C: it is the ratio of the amount of complex to be produced on the short time-scale (β) to a characteristic rate of complex formation ($k_1 E_T S_T$).

Now one is left with two groups of constants on the right-hand side of Eq. 6. For convenience we define two additional dimensionless parameters:

$$\gamma = \frac{\beta}{E_T} = \frac{S_T}{E_T + S_T + K_M}; \tag{9}$$

$$\delta = \frac{\beta^2}{E_T S_T} = \frac{E_T S_T}{(E_T + S_T + K_M)^2}. \tag{10}$$

These dimensionless parameters have useful physical meanings. Since β is a leading-order estimate of the maximum complex concentration, γ is clearly a leading-order estimate of the fraction of enzyme initially charged to the reactor that forms complex on the time-scale θ_C. Note that $0 < \gamma < 1$ for all possible choices of the parameters E_T, S_T, and K_M ($0 \leftrightarrow \infty$). The meaning of the parameter δ is less obvious but can be gleaned from the following consideration. If the MM reaction is conducted in a flow reactor with continuous replenishment of enzyme at a fixed concentration E_T, so that the mass constraint for total enzyme, Eq. 4a, does not enter the problem, the term δx^2 does not appear in Eq. 6, or alternatively, $\delta = 0$. Thus δ quantifies the extent to which equilibrium of the complex-formation reaction (1) is perturbed by the fact that the reaction is conducted in a closed vessel with a finite supply of enzyme. Note by inspection of (10) that $0 < \delta < ¼$ for any possible combination of E_T, S_T, and K_M, so the nonlinear term $\delta x^2 \ll 1$ always, which will allow us to treat it as a regular perturbation.

When one propagates the same scaling to Eq. 5b, one finds

$$\frac{dy}{d\tau} = \left(\frac{\theta_C k_1 K \beta}{S_T}\right)x. \tag{11}$$

This equation must reveal the long time-scale over which product formation is the dominant process. Recall we set $y \cong 0$ when analyzing Eq. 6 to identify the dominant behavior on the short time-scale. This condition is met if the dimensionless coefficient on the right hand side of Eq. 11 is small and represents the ratio of the short and long time-scales. Consequently, we identify

$$\varepsilon = \frac{\theta_C k_1 K \beta}{S_T} = \frac{\theta_C}{\theta_P} = \frac{K E_T}{(E_T + S_T + K_M)^2} \tag{12}$$

as the singular perturbation parameter for this problem. The form of (12) ensures that $0 < \varepsilon < ¼$ for any possible combination of E_T, S_T, K, and K_M ($=K + K_D$), and combining (10) and (12) reveals that $\delta + \varepsilon < ¼$, which is indeed a fortunate circumstance, as we will show in the Appendix. It follows immediately that

$$\theta_P = \frac{S_T}{k_1 K \beta} = \frac{E_T + S_T + K_M}{k_1 K E_T} \tag{13}$$

is the characteristic (long) time-scale for product formation. Note that θ_P is the ratio of the amount of product to be formed on the long time-scale (S_T) to a characteristic rate of product formation ($k_1 K \beta$). Finally, one can identify a "stretched" form of the independent variable $T = t/\theta_P = \varepsilon\tau$ that is appropriate for the long time-scale, where product formation is the dominant process.

Incorporating everything to this point, the short-time ($\tau = T/\varepsilon$) or "inner" representation of Eqs. 5a–b becomes

$$\frac{dx}{d\tau} = 1 - x + \delta x^2 - (1 - \gamma x)y \quad \text{with } x(0) = 0; \tag{14a}$$

$$\frac{dy}{d\tau} = \varepsilon x \quad \text{with } y(0) = 0. \tag{14b}$$

Note that x is constrained to be $O(1)$ and y is constrained to be $O(\varepsilon)$ on the short time-scale where τ is $O(1)$, since $\delta < ¼$ and $\gamma < 1$ always. The long-time ($T = \varepsilon\tau$) or "outer" representation of the problem, distinguished here by use of capital letters for the dependent variables, becomes

$$\varepsilon\left(\frac{dX}{dT}\right) = 1 - X + \delta X^2 - (1 - \gamma X)Y; \tag{15a}$$

$$\frac{dY}{dT} = X. \tag{15b}$$

Now one observes for the same reasons as before that X and Y are both constrained to be $O(1)$ on the long time-scale, where T is $O(1)$. The outer equations have no initial conditions; rather, they must asymptotically match the inner solutions in the region where the inner and outer solutions overlap.

The form of Eq. 15a reveals a special physical significance of the singular perturbation parameter ε. Since X, Y, and T are $O(1)$ on the outer time-scale, dX/dT is $O(1)$ also, and this derivative vanishes from Eq. 15a when $\varepsilon \rightarrow 0$, which makes (15a) an algebraic rather than a differential equation. This is the familiar "quasi-steady-state approximation" (QSSA) of chemical kinetics.[13–15] One concludes that the magnitude of ε quantifies the extent to which the QSSA is valid for the complex concentration in the MM problem, since $X(T)$ is the dimensionless representation of $C(t)$. Recall we found earlier that $0 < \varepsilon < 1/4$, which confirms that the QSSA $dC/dt \cong 0$ is always at least approximately valid for the MM problem.[16] Stated another way, the scaling and ordering scheme reveals that no matter what values of E_T and S_T are chosen for experiments and what values of the elementary rate constants k_1, k_{-1}, and k_2 obtain for the enzyme/substrate system under investigation, the relative errors in quantities extracted from MM data analyses that use the QSSA will be less than ~25%. A more thorough discussion of the QSSA for MM kinetics will be presented later in this article.

Our scaling has successfully constrained the dependent variables x and y to be $O(1)$ in both the inner and outer representations, and this ensures that their perturbation representations will be orderly convergent for all possible values of the parameters δ, γ, and ε. The equations are now properly transformed for solution with a combined regular (parameter δ) and singular (parameter ε) perturbation series. We refer the reader now to the Appendix, where the solution is described in detail.

Limiting Forms of the Perturbation Solution

Once x and y have been determined by solving Eqs. 14 and 15, the algebraic mass constraints (4) can be used to find $E(t)$ or $S(t)$. It is useful to recast this part of the problem in dimensionless form, too. The natural scales for $E(t)$ and $S(t)$ are E_T and S_T, of course, so we define additional dimensionless variables $w = E/E_T$ and $z = S/S_T$. In terms of these, the mass constraints (4) can be rearranged to read

$$w = 1 - \gamma x; \tag{16a}$$

$$z = 1 - \mu x - y. \tag{16b}$$

A new dimensionless parameter appears,

$$\mu = \frac{\beta}{S_T} = \frac{E_T}{E_T + S_T + K_M}, \tag{17}$$

which quantifies the fraction of substrate initially present that is converted into complex on the short time-scale θ_C. Two ancillary dimensionless ratios can also be identified,

$$\eta = \frac{K\beta}{E_T S_T} = \frac{K}{E_T + S_T + K_M}; \tag{18}$$

$$\phi = \frac{K_D \beta}{E_T S_T} = \frac{K_D}{E_T + S_T + K_M}. \tag{19}$$

These can be thought of as kinetic branching ratios. When $\eta \rightarrow 0$, complex is converted slowly to product, and when $\phi \rightarrow 0$, complex is returned slowly to enzyme and substrate. When both are small, $K_M \ll E_T + S_T$, thus complex accumulates to its maximum possible concentration [cf. Eq. 7 for β], and when both approach one, $K_M \gg E_T + S_T$, thus complex does not accumulate relative to E_T or S_T. Clearly, $\gamma + \eta + \mu + \phi = 1$, but more importantly, the magnitudes of these serve to distinguish among several approximate solutions for the MM problem that have appeared previously in the literature. We will return to this in the next section "The Original Michaelis-Menten Equation and Related Quasi-Steady-State Approximations."

Since all six dimensionless parameters we have identified—the ratios γ, μ, η, and ϕ, and the perturbation parameters δ and ε—come from only four physical quantities in the original MM problem—E_T, S_T, K, and K_D—there are additional relationships among them, for example,

$$\delta = \gamma\mu, \quad \delta \leq \gamma(1-\gamma), \quad \delta \leq \mu(1-\mu), \text{ and } \delta \leq \frac{1}{4}(1-\eta)^2; \tag{20}$$

$$\varepsilon = \eta\mu, \quad \varepsilon \leq \frac{1}{4}(1-\gamma)^2, \quad \varepsilon \leq \mu(1-\mu), \text{ and } \varepsilon \leq \eta(1-\eta). \tag{21}$$

We present these because they show that some limiting values ($\rightarrow 0$ or $\rightarrow 1$) of γ, μ, η, or ϕ translate directly into limiting forms of the perturbation solution.

There are 14 possible inequality relationships among the MM parameters E_T, S_T, K, and K_D, which we list in Table 1 with corresponding consequences for the dimensionless ratios and perturbation parameters. The limiting cases for γ, δ, and ε are clearly most important, because they appear in the original scaled Eqs. 14 and 15, hence control the fundamental nature of the solution. The implications of limiting values for the other parameters η, μ, and ϕ are more subtle, but we will demonstrate briefly here and in more detail in the next section how they serve to differentiate various physical scenarios for the MM mechanism.

A key concern in previous analyses of the MM mechanism has been determining when can one assume the complex concentration remains relatively low, so that either the free enzyme concentration remains nearly constant, or the substrate initially charged to the system exists thereafter only as product or as substrate remaining to be converted. Each of these limiting circumstances simplifies the mass balances (3). Under what circumstances can one expect them to occur?

Consider first the case where the free enzyme concentration remains effectively constant, or $E(t) = E_T - C(t) \cong E_T$. One intuitively expects this to occur when enzyme is present in large excess, i.e. $E_T \gg S_T$, however the dimensionless form of the enzyme mass constraint, Eq. 16a, shows that this is actually achieved, i.e. $w \cong 1$, when $\gamma = (\beta/E_T) \rightarrow 0$. Rows (a) through (g) of Table 1 list six different limiting relationships among the MM parameters that cause $\gamma \rightarrow 0$. Only row (a) accounts for the intuitively obvious excess-enzyme scenario, because $\gamma \rightarrow (S_T/E_T) \rightarrow 0$ when $E_T \gg S_T + K_M$, but rows (b–g) describe five other scenarios where $\gamma \rightarrow 0$ can be realized without firm constraints on the relative magnitude of E_T and S_T.

Consider instead the case where the initial substrate is present only as remaining substrate or as product, or $S(t) =$

Table 1. Limiting Values of the Dimensionless MM Parameters

Case	Description	γ	η	μ	ϕ	δ	ε	QSSA	UVA
(a)	$E_T \gg S_T + K_M$	$\to 0$	$\to 0$	$\to 1$	$\to 0$	$\to 0$	$\to 0$	22,26,28	31
(b)	$E_T + K_M \gg S_T$	$\to 0$	<1	<1	<1	$\to 0$	$<1/4$		31
(c)	$E_T + K_D \gg S_T + K$	$\to 0$	$\to 0$	<1	<1	$\to 0$	$\to 0$	22,26,28	31
(d)	$E_T + K \gg S_T + K_D$	$\to 0$	<1	<1	$\to 0$	$\to 0$	$<1/4$		31
(e)	$K \gg E_T + S_T + K_D$	$\to 0$	$\to 1$	$\to 0$	$\to 0$	$\to 0$	$\to 0$	19,20,26	31
(f)	$K_D \gg E_T + S_T + K$	$\to 0$	$\to 0$	$\to 0$	$\to 1$	$\to 0$	$\to 0$	19,20,26,28	31
(g)	$K_M \gg E_T + S_T$	$\to 0$	<1	$\to 0$	<1	$\to 0$	$\to 0$	19,20,26	31
(h)	$S_T + K_M \gg E_T$	<1	<1	$\to 0$	<1	$\to 0$	$\to 0$	19,20,26	21–23
(i)	$S_T \gg E_T + K_M$	$\to 1$	$\to 0$	$\to 0$	$\to 0$	$\to 0$	$\to 0$	19,20,26,28	21–23
(j)	$S_T + K_D \gg E_T + K$	<1	$\to 0$	$\to 0$	<1	$\to 0$	$\to 0$	19,20,26,28	21–23
(k)	$S_T + K \gg E_T + K_D$	<1	<1	$\to 0$	$\to 0$	$\to 0$	$\to 0$	19,20	21–23
(l)	$E_T + S_T + K_D \gg K$	<1	$\to 0$	<1	<1	$<1/4$	$\to 0$	26,28	
(m)	$E_T + S_T \gg K_M$	<1	$\to 0$	<1	$\to 0$	$<1/4$	$\to 0$	26,28	
(n)	$E_T + S_T + K \gg K_D$	<1	<1	<1	$\to 0$	$<1/4$	$<1/4$		

The limiting values are consequences of inequality relationships among the four independent MM parameters E_T, S_T, K and K_D (Recall: $K_M = K + K_D$.) The dimensionless ratios γ, η, μ, and ϕ are defined in terms of the MM parameters by Eqs. 9, 18, 17, and 19, and the perturbation parameters δ and ε are defined by Eqs. 10 and 12, respectively. The columns labeled "QSSA" and "UVA" list references to quasi-steady-state and uniformly-valid approximations, respectively, that are accurate for each case. Tzafriri and Edleman's uniformly-valid solution[17] is accurate in all situations to within errors of $O(\varepsilon)$, and the perturbation solution we present here is accurate in all situations to within errors of $O(\delta + \varepsilon)^2$.

$S_T - C(t) - P(t) \cong S_T - P(t)$. One intuitively expects this to occur when substrate is present in large excess, i.e. $S_T \gg E_T$, however the dimensionless form of the substrate mass constraint, Eq. 16b, shows that this is achieved, i.e. $z \cong 1 - y$, when $\mu = (\beta/S_T) \to 0$. Only row (i) accounts for the intuitively obvious excess-substrate scenario, because $\mu \to (E_T/S_T) \to 0$ when $S_T \gg E_T + K_M$, but rows (e–h) and (j–k) describe five other scenarios where $\mu \to 0$ can be realized without firm constraints on the relative magnitude of E_T and S_T.

The Original Michaelis-Menten Equation and Related Quasi-Steady-State Approximations

The QSSA is a clever "trick" for simplifying the complicated sets of differential equations that are ubiquitous in chemical kinetics analyses.[18,19] The premise of the QSSA is that some intermediate chemical species are consumed so rapidly after they form in a reaction mechanism, they can not accumulate to an appreciable extent; consequently, time-derivatives of their concentrations are nearly zero. This simplification converts differential equations into algebraic equations and reduces the order and complexity of the mathematical problem at hand, but at the expense of completeness, because removing time-derivatives from the problem ensures that the solution cannot meet the initial conditions for all species. One typically uses chemical intuition and insight to decide when the QSSA might be valid, but the best decider is a rigorous scaling analysis like we have presented here, as it reveals what relationships among parameters and initial conditions actually justify a QSSA.

The "MM approximation," which is included in the discussion of enzyme kinetics in any biochemistry textbook,[1,3] is often cited as a prototypical example of the utility of the QSSA.[14] One begins by requiring that all MM experiments are conducted with excess substrate, i.e. $E_T/S_T \to 0$, so $C(t) \ll S(t) + P(t)$, and $S(t) + P(t) \cong S_T$ are assured, and one may safely assume $dC/dt \cong 0$. This particular set of conditions and assumptions is also referred to in the literature as the "standard QSSA" or sQSSA of MM kinetics.[20] Substituting (4a) into (3c) and simplifying via the QSSA gives

$$C \cong \frac{E_T S}{S + K_M}. \tag{22}$$

Combining this with (3d) and (4b) generates the so-called "MM equation:"

$$\frac{dP}{dt} = -\frac{dS}{dt} \cong \frac{V_{\max} S}{S + K_M}; \tag{23}$$

where $V_{\max} = k_1 K E_T$ is the maximum attainable rate for a given enzyme concentration.

The sQSSA can be better understood if reexamined in the context of our scaled representation of equation set (3). The assumption that an initial complex-forming transient has passed implies that a time $t \gg \theta_C$ passes before data are collected, so that the outer Eq. 15 apply. The assumption $dC/dt \cong 0$ is equivalent to $\varepsilon \to 0$ in (15a), and the experimental constraint $E_T/S_T \to 0$ is equivalent to $\mu \to 0$ in (17), which ensures $C(t) \ll S(t) + P(t)$ according to (16b). But, because $\varepsilon = \eta\mu$ according to (21), the single criterion $\mu \to 0$ is sufficient to ensure that the sQSSA is valid. The sQSSA criteria $E_T/S_T \to 0$ first proposed by Laidler,[21] $E_T/K_M \to 0$ proposed more recently by Seshadri and Fritch,[22] and $E_T/(S_T + K_M) \to 0$ advocated by Segel and Slemrod[23] are all special cases of the more general criterion $\mu \to 0$. All of the limiting scenarios that cause $\mu \to 0$ and thereby substantiate the sQSSA are enumerated in rows (e) through (k) of Table 1.

The first integral solution to the MM kinetic problem was derived by the solving the MM Eq. 23 with the initial condition $S(t = 0) = S_T$, which gives[2]

$$\frac{S}{S_T} = 1 - \left(\frac{V_{\max} t}{S_T}\right) - \left(\frac{K_M}{S_T}\right) \ln\left(\frac{S}{S_T}\right). \tag{24}$$

The criterion $\mu \to 0$ that justifies this approach also ensures that the $O(\delta)$ and $O(\varepsilon)$ terms in our perturbation solution become vanishingly small (cf. Table 1); consequently, the integrated MM equation is identical to our leading-order solution $Y_0(T)$, Eq. A14a, when $\mu \to 0$.

Experimentalists often collect $P(t)$ vs. t data of for a relatively short time-period and extract values of MM parameters by comparing the their data to the initial-rate form of (23), which is

$$V_0 = \left.\frac{dP}{dt}\right|_{t=0} = -\left.\frac{dS}{dt}\right|_{t=0} = \frac{V_{\max} S_T}{S_T + K_M}. \tag{25}$$

The additional assumption $S \cong S_T$ in this treatment is equivalent to $Y(T) \to 0$ in our dimensionless representation, which can occur only if insufficient time has passed to penetrate significantly into the outer time-scale, or alternatively $t \ll \theta_P$. Thus one requires both $t \gg \theta_C$ and $t \ll \theta_P$ to use Eq. 24, which can only be accomplished when the short and long time-scales are well separated, or $\varepsilon \to 0$. Once again we see that $\mu \to 0$ is what substantiates even this more restrictive assumption.

Several more sophisticated approximate solutions of equation set (3) have appeared in the literature, including singular perturbations similar to ours but with E_T/S_T,[8,24–26] E_T/K_M,[22] and $E_T/(S_T + K_M)$[23] as the perturbation parameters. These are not QSSAs, but rather are "uniformly-valid" approximations that meet the initial conditions for all species. Note that $\mu \to 0$ when any of these ratios is small, thus $\delta, \varepsilon \to 0$ in our representation; consequently, they duplicate our leading-order solution and the integrated MM Eq. 24 for $S(T)$ when $\mu \to 0$. However, they differ significantly from our solution and from the exact solution when μ is not small. We will return to this in the next section, where we compare phase plots for various approximate solutions of the MM problem.

Recall that the sQSSA uses $E_T/S_T \to 0$ to ensure $C(t)$ is small, so one may safely assume $dC/dt \cong 0$. Borghans et al. realized the QSSA arises fundamentally from a mismatch of time-scales and should apply regardless of the magnitude of $C(t)$.[27] Substituting (4b) into (3c) to eliminate $S(t)$ and setting $dC/dt \cong 0$ gives $P(t)$ as a quadratic function of $C(t)$, which can in principle be inverted and used to integrate (3d) for $P(t)$. They effectively linearized the C vs. P relation with a Pade' approximation, and they used their simplified equations to identify correctly the same short and long time-scales we identify by scaling Eq. 5 directly. They recognized that the ratio of these time-scales, which is exactly the same as our Eq. 12 for ε, is less than unity for all possible circumstances, but they did not exploit this to develop a uniformly valid solution for the full problem. They named their approach the "total QSSA" (tQSSA), which is an improvement over the sQSSA because it adds at least some effect of the terms in the mass balances (3) that are quadratic in $C(t)$. Because these are the same terms that appear as δx^2 in our scaled Eqs. 14a and 15a, respectively, and because the tQSSA is valid for $\varepsilon \to 0$, we conclude that the tQSSA extends the sQSSA to include entries (a), (c), (l) and (m) in Table 1.

A recent uniformly valid solution from Tzafriri and Edelman[17] is worthy of particular attention here, as it is similar to ours in some ways, yet has noteworthy differences. To facilitate a lucid comparison, we will recast their analysis in terms of our own dimensionless parameters.

Tzafriri[16] used intuition and physical arguments to correctly identify the same disparate time-scales—θ_C (8) and θ_P (13)—which we identified with a routine scaling analysis, but he included the small quadratic term δx^2 in the leading-order scaling, whereas we used a dominant-balance argument to exclude it. Consequently, he obtained the following scaling constants and singular perturbation parameter (denoted by *), which, when expanded for small δ, are identical to our equations (7), (8), (13), and (12) in leading order:

$$\beta^* = \beta \frac{1 - \sqrt{1 - 4\delta}}{2\delta} \approx \beta\left[1 + \delta + O\left(\delta^2\right)\right]; \tag{26a}$$

$$\theta_C^* = \frac{\beta^*}{k_1 E_T S_T} = \frac{\theta_C}{\sqrt{1 - 4\delta}} \approx \theta_C\left[1 + 2\delta + O\left(\delta^2\right)\right]; \tag{26b}$$

$$\theta_P^* = \frac{S_T}{k_1 K \beta^*} = \frac{2\delta}{1 - \sqrt{1 - 4\delta}} \theta_P \approx \theta_P\left[1 - \delta + O\left(\delta^2\right)\right]; \tag{26c}$$

$$\varepsilon^* = \frac{\theta_C^*}{\theta_P^*} = \frac{1 - \sqrt{1 - 4\delta}}{2\delta\sqrt{1 - 4\delta}} \varepsilon \approx \varepsilon\left[1 + 3\delta + O\left(\delta^2\right)\right]. \tag{26d}$$

Tzafriri and Edelman[17] solved the $O(1)$ inner equations, their equivalent of (14) with $\varepsilon = 0$, without resorting to a regular perturbation in δ, hence they obtained the Ricatti solution we give as Eq. A4. They solved the nonlinear $O(1)$ outer equations, their equivalent of (15) with $\varepsilon = 0$, with an ingenious variable transformation and then constructed a uniformly valid solution by multiplying the inner and outer solutions together rather than matching them asymptotically. Their ad hoc solution accounts accurately for leading and higher-order effects of the quadratic nonlinearity quantified by δ, but only the $O(1)$ effect of the two time-scales quantified by ε^* in (26d), hence its error, as quantified by the first neglected term, is $O(\varepsilon^*) \cong O(\varepsilon)$. Nonetheless, Tzafriri and Edelman's treatment is a dramatic improvement over all that preceded it, because it is the first uniformly valid solution to the full MM problem that is based on correct choices of the inner and outer time-scales, which ensures it is approximately valid for any possible combination of the MM parameters.

Finally, a separate QSSA treatment has been proposed for Eq. 3b that is referred to in the literature as the "reverse QSSA" (rQSSA).[23] Setting $dS/dt \cong 0$ in (3b) yields

$$C = \frac{E_T S}{S + K_D}. \tag{27}$$

The implications of this approximation become clearer if one inspects the corresponding outer equations in dimensionless form. Scaling Eqs. 3b and 3c appropriately and using (16a) to eliminate $W = E/E_T$ yields the following:

$$\eta\left(\frac{dZ}{dT}\right) = -[(1 - \gamma X)Z - \phi X]; \tag{28a}$$

$$\varepsilon\left(\frac{dX}{dT}\right) = (1 - \gamma X)Z - (\phi + \eta)X. \tag{28b}$$

Since $Z(T)$ is the dimensionless representation of $S(t)$, Eq. 28a reveals that the criterion for validity of the rQSSA is $\eta \to 0$, but Eq. 21 and Table 1 verify that this also ensures $\varepsilon \to 0$ in (28b). We conclude in agreement with others that

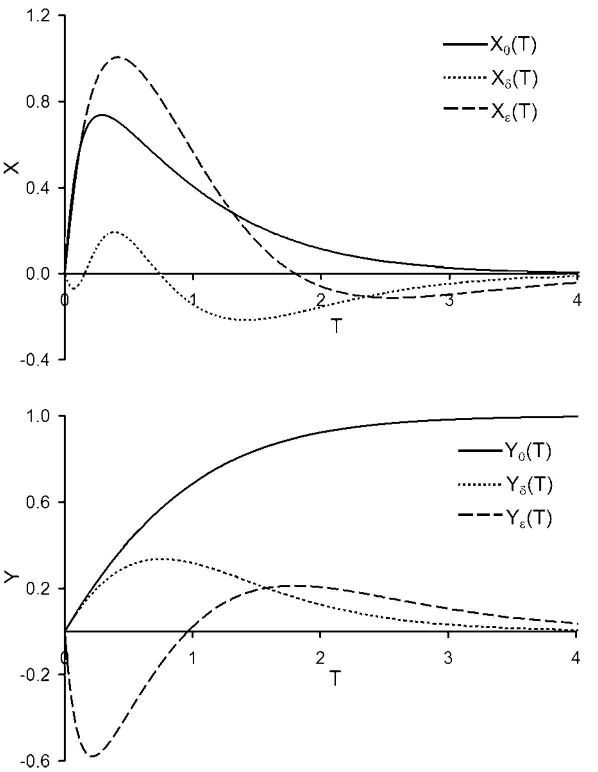

Figure 1. Constituent parts of the perturbation solution.

The curves in the top panel compare $X_0(T)$, $X_\delta(T)$, and $X_\varepsilon(T)$ from Eqs. A14a and A14b, and the curves in the bottom panel compares $Y_0(T)$, $Y_\delta(T)$, and $Y_\varepsilon(T)$ from Eqs. A14a and A14c, all for $\gamma = 0.333$ and $\varepsilon = 0.110$.

the rQSSA is more restrictive than the tQSSA, or stated another way, the assumption $dS/dt \cong 0$ must have associated with it the assumption $dC/dt \cong 0$. Segel and Slemrod proposed the criterion $E_T \gg K_M$ to justify the rQSSA,[23] whereas Schnell and Maini argued that assuming $dC/dt \cong 0$ was not necessary, but $E_T \gg K$ and $E_T \gg S_T$ were.[28] The former corresponds to $\eta,\phi,\varepsilon \to 0$ in our dimensionless representation [Table 1, row (m)], and the latter corresponds to $\gamma,\eta,\delta,\varepsilon \to 0$ [Table 1, rows (a), (c), and (f)]. Rows (i), (j), and (l) in Table 1 identify three other, previously unidentified relationships among the MM parameters that also cause $\eta \to 0$ and therefore render the rQSSA valid.

Quantitative Comparison of Solutions

First we present plots of $X(T)$ and $Y(T)$ that show the constituent parts of the perturbation solution and compare the assembled solutions to numerical solutions of the scaled outer Eq. (15).

Recall that the scaled perturbation solutions are assembled from three functions, e.g., $X(T) = X_0(T) + \delta X_\delta(T) + \varepsilon X_\varepsilon(T)$. The top panel of Figure 1 compares $X_0(T)$, $X_\delta(T)$, and $X_\varepsilon(T)$ from Eqs. (A14a) and (A14b), and the bottom panel compares $Y_0(T)$, $Y_\delta(T)$, and $Y_\varepsilon(T)$ from Eqs. (A14a) and (A14c) for representative values of the parameters $\gamma = 0.333$ and $\varepsilon = 0.110$. These functions are independent of δ, but depend parametrically on γ because it enters the governing Eqs. (A6) and (A8) at every order, and they also depend parametrically on ε because each perturbation function includes asymptotically matched contributions from both the short ($\tau = T/\varepsilon$) on long ($T = \varepsilon\tau$) time-scales [cf. Appendix]. We present them in terms of the outer time-variable T because it is the $O(1)$ time-scale for product formation.

The general shapes of the $O(\varepsilon)$ functions in Figure 1 are easy to interpret in the context of the MM reaction mechanism. Recall that ε is the ratio of the inner to outer time-scales according to Eq. (12). As ε grows from zero, the short-time process, i.e. formation of complex, accelerates relative to the long-time process, i.e. formation of product. Consequently, one expects $\varepsilon > 0$ to be evidenced as an increase in the amplitude of the complex curve $X(T)$ and a decrease in the amplitude of the product curve $Y(T)$ for short times, to be made up by opposite trends at long times. Notice how $X_\varepsilon(T)$ first goes positive and then negative, and $Y_\varepsilon(T)$ does just the opposite, so that the $O(\varepsilon)$ corrections $\varepsilon X_\varepsilon(T)$ and $\varepsilon Y_\varepsilon(T)$ contribute to $X(T)$ and $Y(T)$ in exactly the manner just described. Unfortunately, the general shapes of the $O(\delta)$ functions in Figure 1 are not amenable to such simple interpretation. One must analyze carefully the Eqs. A6c,d and A8c,d that generated them.

Figure 2 compares the assembled perturbation solutions to "exact" numerical solutions[29,30] of the outer Eq. 15 for $\gamma = 0.333$, $\delta = 0.111$, and $\varepsilon = 0.110$. Recall that the definitions of δ and ε, Eqs. 19 and 12, ensure that $\delta + \varepsilon < ¼$ for any combination of the MM parameters; consequently, the choice of $\delta + \varepsilon = 0.221$ for this calculation represents a near-worst-case scenario for our perturbation solution. Nonetheless, the agreement between the perturbation and exact solution is excellent, but this should be expected, since the scaling and ordering scheme (A5) ensures that relative errors are within $O(\delta + \varepsilon)^2 \cong 0.05$ in this case.

Next we compare five different solutions of the MM equations—an exact numerical solution, our perturbation approximation, and three uniformly-valid approximations offered previously by others—for four different combinations of the

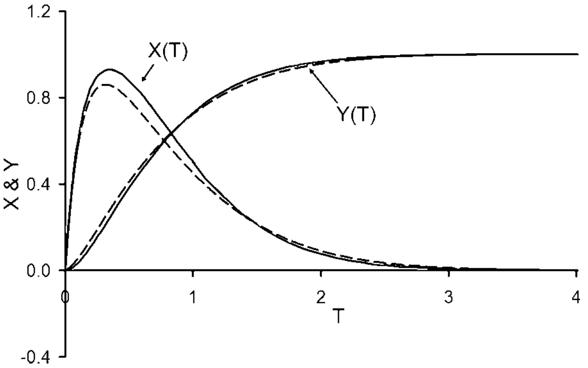

Figure 2. Comparison of solutions perturbation and exact.

These curves compare the composite perturbation solutions (dashed line) and exact numerical solutions (solid line) for $\gamma = 0.333$, $\delta = 0.111$, and $\varepsilon = 0.110$.

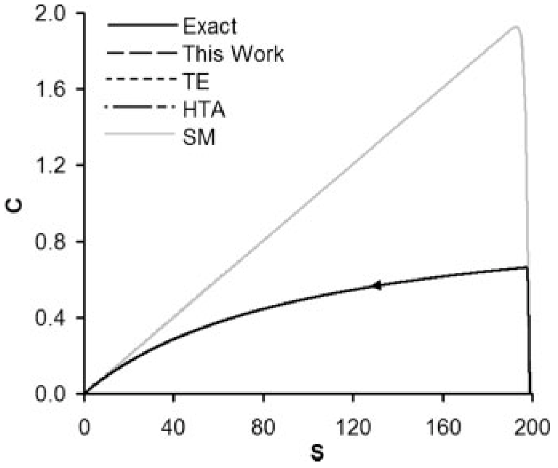

Figure 3. Case I.

These phase plots compare the HTA[24], the SM[31], the TE[17], the perturbation solution described in this work, and an exact numerical solution for the set of MM parameters listed as Case I in Table 2. Only the SM solution fails to track the exact solution in this circumstance. The arrow points in the direction of increasing time.

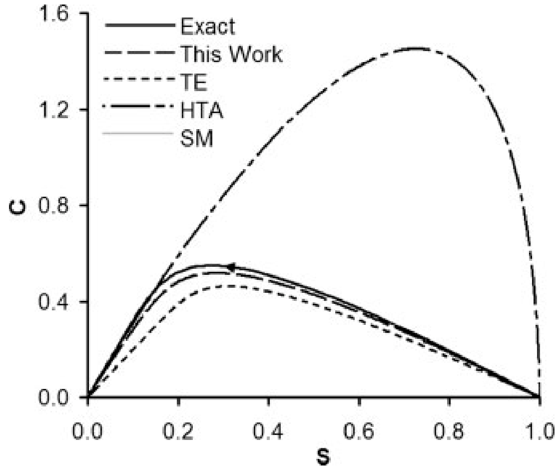

Figure 4. Case II.

These phase plots compare the HTA[24], the SM[31], the TE[17], the perturbation solution described in this work, and an exact numerical solution for the set of MM parameters listed as Case II in Table 2. The SM solution is virtually identical to the exact solution in this case. The arrow points in the direction of increasing time.

MM parameters, chosen to explore regions of parameter space where different approximations are likely to be valid or to fail. We make these comparisons with phase plots of $C(t)$ vs. $S(t)$, since the phase-plot construction adequately reveals the quantitative differences while reducing the density of visual data by a factor of two.

We chose three other uniformly-valid approximate solutions for this comparison to represent the diversity of thought and effort that MM problem has inspired. Before presenting the plots we will review each approximation briefly and use the inequalities in Table 1 to delimit their strengths and weaknesses. We do not offer any of the more rudimentary QSSA solutions for comparison here, because they cannot meet one of the initial conditions for the MM problem, hence they generate irrational phase plots.

The first approximate perturbation solution of the MM equations we use for our comparison was presented by Heineken, Tsuchiya, and Aris (HTA) in 1967.[24] It was the first treatment that went beyond a QSSA and included the full time-dependence of all the concentrations. They scaled the MM equations and solved them with the same singular perturbation method we have used, but, taking inspiration from the original sQSSA approximation,[15] they began with the ad hoc assumption that $\varepsilon = E_T/S_T$ was the correct singular perturbation parameter and adjusted the scaling of other terms in the equations to accommodate this choice. Their scaled equations include two dimensionless ratios – $\kappa = K_M/S_T$ and $\lambda = K/S_T$ – as coefficients of ostensibly $O(1)$ functions of the dimensionless concentration variables, and these ratios appear as coefficients in the equations at all orders of the perturbation hierarchy. Consequently, HTA's perturbation solution should converge in an orderly way only when the perturbation parameter $\varepsilon \to 0$, of course, and when κ and λ are $O(1)$ or smaller. The criterion $\varepsilon \to 0$, or equivalently $E_T \ll S_T$, is met when $\mu \to 0$ according to (17), and the criteria $\kappa,\lambda \leq O(1)$ are met for all circumstances except $K_M \gg S_T$, or alternatively $\gamma \to 0$ according to (9). On the basis of these arguments, one anticipates that the HTA solution we present in Figures 3–6 will converge to the exact solution when μ is small but γ is not, a circumstance which is accounted for by rows (h–k) of Table 1.

The authors of two subsequent papers criticized HTA's scaling arguments and presented alternative perturbation solutions. First Seshadri and Fritzsch argued that $\varepsilon = E_T/K_M$ is the correct singular perturbation parameter,[22] and later

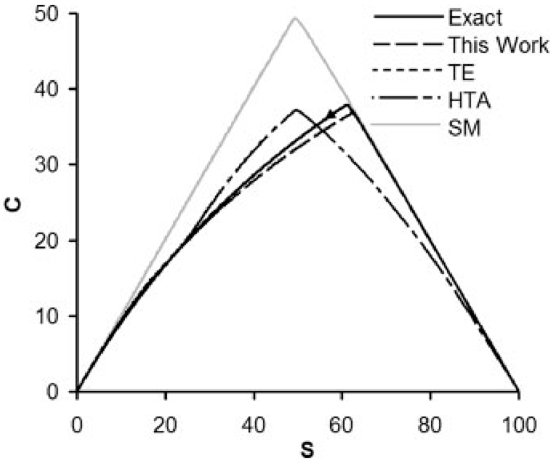

Figure 5. Case III.

These phase plots compare the HTA[24], the SM[31], the TE[17], the perturbation solution described in this work, and an exact numerical solution for the set of MM parameters listed as Case III in Table 2. The TE solution is virtually identical to the exact solution in this case. The arrow points in the direction of increasing time.

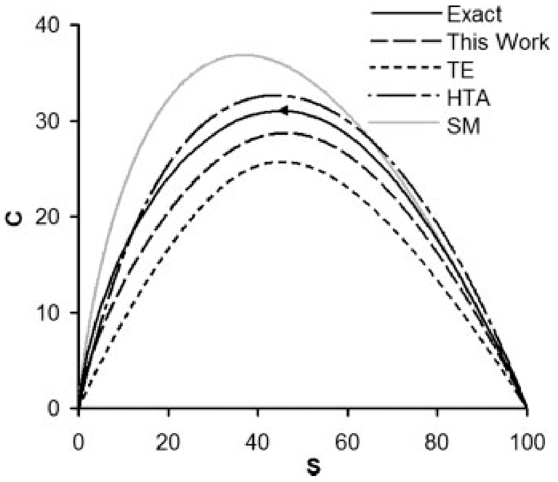

Figure 6. Case IV.

These phase plots compare the HTA[24], the SM[31], the TE[17], the perturbation solution described in this work, and an exact numerical solution for the set of MM parameters listed as Case IV in Table 2. The arrow points in the direction of increasing time.

Segel and Slemrod argued instead that $\varepsilon = E_T/(S_T + K_M)$ is correct.[23] These treatments evidence the same problem as HTA's, however, as they predict the perturbation functions will be $O(1)$ only if certain dimensionless coefficients that appear in the scaled equations are $O(1)$ or smaller. We have analyzed these solutions in thorough detail, and we were surprised to discover that both are in fact identical to the original HTA solution we use here for comparison. To verify this redundancy, one needs only to return all three analytical solutions from dimensionless to dimensioned, physical variables and compare them. Although each group of authors used different physical arguments to justify their scaling choices, in the end they achieve dominant balances among the same terms in the governing equations, which ensures that equivalent parts of the solutions appear at the same orders in their perturbation hierarchies. This unfortunate confusion and duplication of effort reveals how subtle the scaling and ordering exercise can be.

The second solution we use for comparison was derived by Schnell and Mendoza (SM),[31] who recognized that the governing equations are fortuitously linear and admit an exact solution whenever $K_M \gg S_T$, regardless of whether $E_T \ll S_T$ or vice versa. The ratio of short and long time-scales in this limit is $\varepsilon = KE_T/(E_T + K_M)^2$. Since the only restriction for validity of their approach is $K_M \gg S_T$, or $\gamma \to 0$ according to (9), the SM solution we present in Figures 3–6 is accurate for a wider range of circumstances than its authors claim, i.e. rows (a) through (g) of Table 1. We present the SM solution in the context of a different discussion as Eq. A16 of the Appendix.

The third solution we use for comparison was derived by Tzafriri and Edelman (TE)[17] and was discussed in detail in a previous section. These authors correctly identified the short and long time-scales; hence their solution is the first to give a reasonably accurate solution for all possible combinations of the MM parameters. Since the TE solution includes contributions at all orders from the equivalent of our regular perturbation parameter δ, but only the $O(1)$ contribution from the equivalent of our singular perturbation parameter ε, its estimated error relative to the exact solution is $O(\varepsilon)$, which is to be compared in Figures 3–6 with a relative error of $O(\delta + \varepsilon)^2$ for our perturbation solution.

Table 2 lists the MM parameters and associated dimensionless groups that were used to calculate the comparative phase plots of $C(t)$ vs. $S(t)$ in Figures 3 through 6.

Figure 3 compares the exact solution and four approximations for Case I of Table 2, which involves excess substrate $S_T \gg E_T$ and the limit $\mu \to 0$ (=0.003), so traditional simplifications like the sQSSA and tQSSA are approximately valid and can be used to calculate $S(t)$ or $P(t)$ [but not $E(t)$ or $C(t)$]. This criterion also ensures $\delta,\varepsilon \to 0$ in our representation (δ = 0.002, ε = 0.002), but the other parameters γ = 0.663 and $\eta = \phi$ = 0.167 are not at limiting values, so row (h) of Table 1 applies. As expected, the HTA, TE, and our solution all agree with each other and the exact solution in this case; indeed, they are indistinguishable within the resolution of the lines on the figure. The SM solution departs significantly, however, because γ = 0.663 is well outside its range of validity ($\gamma \to 0$).

Figure 4 compares them again for Case II of Table 2, which involves excess enzyme $E_T \gg S_T$ and the limits $\gamma \to 0$ (=0.003) and $\delta \to 0$ (=0.002). The other parameters $\eta = \phi$ = 0.167, μ = 0.663, and ε = 0.110 are not at limiting values, so row (b) of Table 1 applies. This case demonstrates the strength of the SM solution, which has moved into its range of validity and nearly duplicates the exact solution, and the weakness of the HTA solution, which now fails quite obviously because its singular perturbation parameter is

Table 2. Representative Combinations of MM Parameters

Quantity	Case I	Case II	Case III	Case IV
E_T (mol/vol)	1	199	100	100
S_T (mol/vol)	199	1	100	100
k_1 (vol/mol time)	1	1	1	1
k_{-1} (time^{-1})	50	50	99	1
k_2 (time^{-1})	50	50	1	99
K (mol/vol)	50	50	1	99
K_D (mol/vol)	50	50	99	1
$K_M = K + K_D$ (mol/vol)	100	100	100	100
β (mol/vol), Eq. 7	0.663	0.663	33.3	33.3
θ_C (time), Eq. 8	0.003	0.003	0.003	0.003
θ_P (time), Eq. 13	6	0.030	3	0.030
γ, Eq. 9	0.663	0.003	0.333	0.333
η, Eq. 18a	0.167	0.167	0.003	0.330
μ, Eq. 17	0.003	0.663	0.333	0.333
ϕ, Eq. 18b	0.167	0.167	0.330	0.003
δ, Eq. 10	0.002	0.002	0.111	0.111
ε, Eq. 12	0.001	0.110	0.001	0.110

These are the combinations of MM parameters (with units) and associated dimensionless groups that are used to calculate $C(t)$ vs. $S(t)$ in Figures 3–6. Case I involves excess substrate and δ, ε, $\mu \to 0$ [*cf.* equation (17), row (h) of Table 1], so the sQSSA and tQSSA are approximately valid. Case II involves excess enzyme and δ, $\gamma \to 0$ [*cf.* equation (9), row (b) of Table 1]. Case III involves ε, $\eta \to 0$ [*cf.* Eq. (18), row (l) of Table 1], so the rQSSA is approximately valid. Case IV is an extreme test of the accuracy of the perturbation solution (A14), since only $\phi \to 0$ [*cf.* equation (19), row (n) of Table 1], and the perturbation parameters are very near their limiting values $\delta + \varepsilon <$ ¼.

$\varepsilon = E_T/S_T = 199$. The TE solution does well with an estimated relative error of $O(\varepsilon) \cong 0.1$, but our solution does better, since the estimated error is only $O(\delta + \varepsilon)^2 \cong 0.01$ in this case.

Figure 5 compares them again for Case III of Table 2, where $\eta \to 0$ (=0.003), and the limits $\varepsilon \to 0$ (=0.003) and $\varepsilon \to 0$ (=0.001) are realized. The other parameters $\gamma = \mu \cong \phi = 0.33$ and $\delta = 0.111$ are not at limiting values, so row (l) of Table 1 applies. These are exactly the criteria that justify the rQSSA. Neither the HTA nor the SM solution was derived for this particular circumstance, so each of these deviates significantly, whereas the TE solution is best of all and virtually indistinguishable from the exact solution. This is because the combination $\delta = 0.111$ and $\varepsilon = 0.001$ exposes its particular strength: the error estimate $O(\varepsilon) \cong 0.001$ in this favorable circumstance is an order of magnitude lower than the estimate $O(\delta + \varepsilon)^2 \cong 0.01$ for our solution.

Finally, Figure 6 compares them for Case IV of Table 2, where $\gamma \cong \eta \cong \mu \cong 0.33$, $\phi = 0.003$, and $\delta \cong \varepsilon = 0.11$, so no dimensionless parameter is at a favorable limiting value, and no mathematical simplification like a QSSA applies. The SM solution fails as it did in Case I, because $\gamma = 0.333$ is outside its range of validity ($\gamma \to 0$), and the TE solution agrees with the exact solution to within its anticipated error of $O(\varepsilon) \cong 0.1$. Since the combination $\delta + \varepsilon = 0.221$ is near its worst-case limit of ¼ for our solution, Case IV a stringent test, but the accuracy of our perturbation solution is still good, because the error is limited to $O(\delta + \varepsilon)^2 \cong 0.05$. Recall that good agreement was also evident in Figure 2, above, which compared scaled versions of the $C(t)$ and $P(t)$ curves to an exact solution for the same set of dimensionless parameters.

The HTA solution shows remarkable accuracy in Figure 6, even though its perturbation parameter is $\varepsilon = E_T/S_T = 1$ in this case. This success is fortuitous, however, and should not be mistaken as evidence that this solution is generally superior. All one needs to do is begin with the MM parameters for Case IV in Table 2 and recalculate the phase curves for decreasing values of k_2, which decreases K and K_M concomitantly. One finds that our solution, TE's, and SM's behave rationally and maintain their relative accuracy as k_2 decreases, whereas the HTA solution becomes progressively worse and diverges to nonsensical behavior when k_2 is less than about 30.

On the basis of these comparisons and others for a broad range of MM parameters that we do not present here, we conclude that the HTA solution[24] (along with the redundant solutions from Seshadri and Fritzsch[22] and Segel and Slemrod[23]) is the most restrictive to use. One can easily choose MM parameters, particularly combinations like $E_T \gg S_T + K_M$ (or alternatively $\mu \to 1$ in our representation) that put it far outside of its intended range of validity and cause its perturbation series to diverge nonsensically. This is not the case for the others. The SM solution[31] gives rational if not accurate estimates of the $C(t)$- and $S(t)$-curves in any circumstance, whereas the TE solution[17] and ours always give quantitatively accurate curves with errors limited only by the asymptotic errors of their missing, higher-order terms—$O(\varepsilon)$ for the former and $O(\delta + \varepsilon)^2$ for the latter. In most circumstances our solution is more accurate than the TE solution, because it includes an important $O(\varepsilon)$ contribution that the TE solution does not.

Conclusions

We have developed a new dimensionless representation of the MM equations, and we have found an approximate solution with a combined regular and singular perturbation scheme. The new scaling we introduce is the first that constrains all dimensionless concentration variables to be $O(1)$ for all time, which ensures that our solution converges uniformly and accurately for any physically realizable combination of MM parameters. As a by-product of our scaling analysis we have identified four dimensionless ratios—γ, η, μ, and ϕ (cf. Table 1)—that serve to categorize all the previous approximations for the MM problem (sQSSA, integrated MM equation, initial-rate method, tQSSA, rQSSA, singular perturbations, and others), to delimit their accuracy, and to reveal some new regions of parameter space where traditional QSSAs for the MM problem are valid. Our perturbation solution properly shows the leading-order effects of an unavoidable quadratic nonlinearity in the MM equations [cf. δ, Eq. 10], and it includes important leading-order corrections for the effects of two widely disparate timescales in the MM equations [cf. ε, Eq. 12]. The cumulative effect of our analysis is a comprehensive, unprecedented description of the mechanistic subtleties of this important problem.

The only obvious avenue for progress is to improve accuracy by finding the neglected, higher-order terms our perturbation solution has omitted. One can envisage two ways to proceed. The first (and easiest) method would be to extend the perturbation expansion, Eq. A5, to include terms of $O(\delta^2)$, $O(\varepsilon^2)$, and $O(\delta\varepsilon)$, and to solve the higher-order equations, which would reduce the maximum relative error in the composite solution to $O(\delta + \varepsilon)^3 = (1/4)^3 \cong 0.02$. The higher-order equations are certain to be linear, hence soluble by the same methods we used to find the $O(\delta)$ and $O(\varepsilon)$ terms, but the effort would be formidable, and the reward would be small. A second (and more difficult) way to proceed would be to use the TE solution,[17] which already has higher-order effects of δ in it, as the $O(1)$ starting point for a singular perturbation in ε alone. However, the effort here would be even more intimidating, since the TE solution is the product of two solutions of nonlinear equations and is significantly more complicated than ours. Perhaps the only reasonable thing to consider is to "patch" the gap in the TE solution by adding our $O(\varepsilon)$ correction to it, but even then, the remaining errors would still be $O(\varepsilon)^2 = 1/16$. We conclude there is little incentive to proceed.

Finally, we offer a few comments on the pedagogical value of this work. The scaling and perturbation methods we have used here are standard tools of engineering analysis. Many of us are charged with the duty of teaching others how to use these tools properly, and we seek example problems that combine practical relevance with the potential for "clean" solutions that our students can easily derive and understand. Generations of chemical engineering graduate students have "cut their teeth" on singular perturbation theory by repeating Heineken, Tsuchiya, and Aris's classic analysis of this important MM problem[24]; indeed, it has been reproduced in graduate-level textbooks as a prototype for the singular perturbation method.[8,25,26] We believe our new analysis of the MM problem is actually a better example to learn from, because our scaling more transparently and effectively accomplishes the general goals of scaling analysis; our scaled perturbation Eqs. A6 and A8 are relatively easy to solve; and our method introduces regular and singular perturbations together in the context of solving a single problem, which helps beginners recognize and appreciate their differences.

Acknowledgments

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Appendix: Perturbation Solution of Eqs. 14–15

We begin by solving the inner Eq. 14. Our first move is to postulate a solution in the form of a perturbation expansion in ε as follows:

$$x(\tau) = x_0(\tau) + \varepsilon x_\varepsilon(\tau) + O(\varepsilon^2); \tag{A1a}$$

$$y(\tau) = y_0(\tau) + \varepsilon y_\varepsilon(\tau) + O(\varepsilon^2); \tag{A1b}$$

where, x_0, x_ε, y_0 and y_ε are as-yet unknown functions of τ. These functions must meet the initial conditions of the original problem for arbitrary values of ε, in which case $x_0(0) = y_0(0) = x_\varepsilon(0) = y_\varepsilon(0) = 0$. Substituting the (A1) into[14] and collecting terms by orders in ε, one finds at $O(1)$:

$$\frac{dx_0}{d\tau} = 1 - x_0 + \delta x_0^2 - (1 - \gamma x_0)y_0; \tag{A2a}$$

$$\frac{dy_0}{d\tau} = 0\,; \tag{A2b}$$

and at $O(\varepsilon)$:

$$\frac{dx_\varepsilon}{d\tau} = (-1 + 2\delta x_0 - y_0)x_\varepsilon - (1 - \gamma x_0)y_\varepsilon\,; \tag{A3a}$$

$$\frac{dy_\varepsilon}{d\tau} = x_0(\tau). \tag{A3b}$$

Neglecting terms of $O(\varepsilon^2)$ and higher introduces errors $O(1/16)$ or smaller, since $0 < \varepsilon < 1/4$ according to Eq. 12.

From (A2b) one obtains the trivial solution $y_0 = 0$, and from (A2a), which is a nonlinear but soluble Riccati equation, one obtains

$$x_0(\tau) = \frac{1 - \exp\left[-\left(\sqrt{1-4\delta}\right)\tau\right]}{1 - \frac{1}{2}\left(1 - \sqrt{1-4\delta}\right)\left\{1 + \exp\left[-\left(\sqrt{1-4\delta}\right)\tau\right]\right\}}. \tag{A4}$$

One finds quickly that the Riccati function makes Eqs. A3 for the $O(\varepsilon)$ corrections very tedious to solve. Recognizing this difficulty, and also recalling that $0 < \delta < 1/4$ according to Eq. 10, we linearize Eq. A2 by introducing regular perturbation expansions in the parameter δ for the functions $x(\tau)$ and $y(\tau)$. Now these functions become two-parameter expansions of the form

$$x(\tau) = x_0(\tau) + \delta x_\delta(\tau; \varepsilon = 0) + \varepsilon x_\varepsilon(\tau; \delta = 0) + O(\delta + \varepsilon)^2; \tag{A5a}$$

$$y(\tau) = y_0(\tau) + \delta y_\delta(\tau; \varepsilon = 0) + \varepsilon y_\varepsilon(\tau; \delta = 0) + O(\delta + \varepsilon)^2; \tag{A5b}$$

Since $\delta + \varepsilon < ¼$, including only the first term in δ and ε is sufficient to ensure that errors in $x(\tau)$ and $y(\tau)$, as quantified by the first neglected terms in the perturbation expansions, are $O(\delta + \varepsilon)^2$, which is once again $(1/16)$ or smaller.

One might wonder at this point why it is necessary to solve the equations with a two-parameter perturbation expansion. Since $\delta < ¼$, $\varepsilon < ¼$, and $\delta + \varepsilon < ¼$, why not recast the original Eqs. 14 and 15 in terms of ε only and solve them with a one-parameter expansion, as others have in the past?[22–24] The reason is subtle and worthy of explanation. Note that the ratio $\delta/\varepsilon = S_T/K = \gamma/\eta$ [cf. Eqs. 9, 10, 12, and 18] can range anywhere between zero and infinity, so δ and ε are in general of different order, even though they are independently bounded. If one solves the problem with a one-term singular perturbation expansion in ε, the unbounded ratio γ/η appears unavoidably as a coefficient in the $O(\varepsilon)$ equations; consequently, the $O(\varepsilon)$ functions in the perturbation hierarchy are $O(\gamma/\eta)$ rather than $O(1)$ and can diverge when this ratio is large. Using a two-term perturbation expansion in δ and ε circumvents this difficulty by ensuring all terms are properly bounded.

Inserting (A5) into (14) and collecting terms by orders in δ and ε, one finds the following six equations to solve on the inner time-scale τ:

$$\frac{dx_0}{d\tau} = 1 - x_0 - y_0 + \gamma x_0 y_0 \quad \text{with } x_0(0) = 0\,; \tag{A6a}$$

$$\frac{dy_0}{d\tau} = 0 \quad \text{with } y_0(0) = 0\,; \tag{A6b}$$

$$\frac{dx_\delta}{d\tau} = x_0^2 - x_\delta - y_\delta + \gamma x_\delta y_0 + \gamma x_0 y_\delta \quad \text{with } x_\delta(0) = 0\,; \tag{A6c}$$

$$\frac{dy_\delta}{d\tau} = 0 \quad \text{with } y_\delta(0) = 0\,; \tag{A6d}$$

$$\frac{dx_\varepsilon}{d\tau} = -x_\varepsilon - y_\varepsilon + \gamma x_\varepsilon y_0 + \gamma x_0 y_\varepsilon \quad \text{with } x_\varepsilon(0) = 0\,; \tag{A6e}$$

$$\frac{dy_\varepsilon}{d\tau} = x_0 \quad \text{with } x_\varepsilon(0) = 0. \tag{A6f}$$

The solution of these is straightforward, as they are all linear and coupled only in a cascading way. After solving at each order and substituting into (A5), one obtains the inner solutions:

$$\begin{aligned} x(\tau) &= x_0 + \delta x_\delta + \varepsilon x_\varepsilon = 1 - e^{-\tau} + \delta\left[1 - \left(2\tau + e^{-\tau}\right)e^{-\tau}\right] \\ &+ \varepsilon\left\{\left(\gamma - 1\right)\left(\tau - 2\right) + \left[\left(2\gamma - 1\right)\tau - \frac{1}{2}\gamma\tau^2 - 2 + \gamma\right]e^{-\tau} + \gamma e^{-2\tau}\right\}; \end{aligned} \tag{A7a}$$

$$y(\tau) = y_0 + \delta y_\delta + \varepsilon y_\varepsilon = \varepsilon\left(e^{-\tau} + \tau - 1\right). \tag{A7b}$$

Note that if one expands the exact Riccati solution (A4) for small δ, one obtains exactly the $O(1)$ and $O(\delta)$ portions of (A7a), above, which confirms that the perturbation in δ linearizes Eq. A2a. (If one tries to solve the outer equations without using a regular perturbation in δ to linearize the corresponding δX^2 term, the problem is even worse, as a closed-form solution for the leading-order contribution $Y_0(T)$ can only be obtained with a complicated variable transformation. See [27]).

We solve the outer Eqs. 15 by inserting perturbation series for $X(T)$ and $Y(T)$ analogous to Eqs. A1, above, and collecting terms by order in δ and ε, which generates the following set of equations to solve on the outer time-scale T:

$$0 = 1 - X_\mathrm{o} - Y_\mathrm{o} + \gamma X_\mathrm{o} Y_\mathrm{o}; \tag{A8a}$$

$$\frac{dY_\mathrm{o}}{dT} = X_\mathrm{o}\,; \tag{A8b}$$

$$0 = X_\mathrm{o}^2 - X_\delta - Y_\delta + \gamma Y_\mathrm{o} X_\delta + \gamma X_\mathrm{o} Y_\delta\,; \tag{A8c}$$

$$\frac{dY_\delta}{dT} = X_\delta\,; \tag{A8d}$$

$$\frac{dX_\varepsilon}{dT} = -X_\varepsilon - Y_\varepsilon + \gamma Y_\mathrm{o} X_\varepsilon + \gamma X_\mathrm{o} Y_\varepsilon\,; \tag{A8e}$$

$$\frac{dY_\varepsilon}{dT} = X_\varepsilon. \tag{A8f}$$

Since two derivatives have been lost in the ordering scheme (cf., A8a and c), the solutions of these equations cannot meet all the initial conditions of the overall problem, so one must solve them in terms of integration constants that will be determined by matching the outer solutions asymptotically to the inner solutions. Rearranging (A8a), one finds

$$X_\mathrm{o}\left(Y_0\right) = \frac{1 - Y_\mathrm{o}}{1 - \gamma Y_\mathrm{o}}\,; \tag{A9a}$$

which, when substituted into (A8b) and integrated gives

$$T\left(Y_\mathrm{o}\right) = \gamma Y_\mathrm{o} + \left(\gamma - 1\right)\ln\left(1 - Y_\mathrm{o}\right) + A; \tag{A9b}$$

where A is an integration constant. Equation A9b gives $Y_0(T)$ implicitly rather than explicitly, which proves inconvenient when solving the remaining outer equations, as they depend explicitly on $Y_0(T)$. To circumvent this difficulty, we use (A8b) to transform the independent variable from T to Y_0 in the remaining equations, i.e., $\frac{d}{dT} = \left(\frac{dY_0}{dT}\right)\frac{d}{dY_0} = X_0(Y_0)\frac{d}{dY_0}$. The final outer solutions in terms of Y_0 are

$$\begin{aligned} X\left(Y_\mathrm{o}\right) &= X_0 + \delta X_\delta + \varepsilon X_\varepsilon = \frac{1 - Y_\mathrm{o}}{1 - \gamma Y_\mathrm{o}} + \delta\left\{\frac{(1 - Y_\mathrm{o})^2}{(1 - \gamma Y_\mathrm{o})^3}\right. \\ &\left.\times\left[1 - \left(\frac{1 - \gamma}{1 - Y_\mathrm{o}}\right)\left(B - \frac{1}{\gamma}\ln(1 - \gamma Y_\mathrm{o})\right)\right]\right\} + \varepsilon\left\{\left(\frac{1 - Y_\mathrm{o}}{(1 - \gamma Y_\mathrm{o})^3}\right)\right. \\ &\left.\times\left[(1 - \gamma)\left(\frac{2}{1 - \gamma Y_\mathrm{o}} - C\right) + \ln\left(\frac{1 - Y_\mathrm{o}}{1 - \gamma Y_\mathrm{o}}\right)\right]\right\}; \end{aligned} \tag{A10a}$$

$$Y(Y_o) = Y_0 + \delta Y_\delta + \varepsilon Y_\varepsilon = Y_o + \delta\left[\frac{(1-Y_o)}{(1-\gamma Y_o)} \times\left(B - \frac{1}{\gamma}\ln(1-\gamma Y_o)\right)\right] + \varepsilon\left\{\left(\frac{1-Y_o}{1-\gamma Y_o}\right) \times\left[C + \frac{1}{\gamma Y_o - 1} + \frac{1}{\gamma - 1}\ln\left(\frac{1-Y_o}{1-\gamma Y_o}\right)\right]\right\}; \qquad \text{(A10b)}$$

where B and C are integration constants, and $Y_0(T)$ is given implicitly by Eq. A9b.

We must determine the integration constants A, B, and C by asymptotically matching the inner and outer solutions in the overlap region, where $\tau \gg 1$ and $T \ll 1$. The so-called "matching solutions" can be identified by recasting the inner solutions in terms of the outer variable T and finding their asymptotic behavior in the limit $\varepsilon \to 0$ with $T =$ finite. Once these are available, the integration constants in the outer solutions can be identified by recasting the outer solutions in terms of the inner variable τ, finding their asymptotic behavior in the limit $\varepsilon \to 0$ with $\tau =$ finite, and requiring them to reproduce the matching solutions. Composite solutions that are uniformly valid for the entire range $0 < t < \infty$ are constructed by summing the inner and outer solutions and subtracting away the redundant matching solution for the overlap region. This procedure is outlined in any of the numerous textbooks on singular perturbation theory.[8–10] One has in our case the added complication that the inner and outer solutions must match for arbitrary values of the regular perturbation parameter δ, but this introduces little difficulty.

The matching solutions derived from the inner solutions are

$$x_{\text{match}}(T) = 1 + (\gamma - 1)T + \delta - 2\varepsilon(\gamma - 1); \qquad \text{(A11a)}$$

$$y_{\text{match}}(T) = T - \varepsilon. \qquad \text{(A11b)}$$

Finding the outer matching solutions is complicated by the fact that the outer solutions are given explicitly in terms of Y_0 rather than T. Since $Y_0 \to 0$ when $T \to 0$, we expand (A9b) for small Y_0, which gives

$$T \approx A + Y_o + \frac{1}{2}(1-\gamma)Y_o^2 + O(Y_o^3); \qquad \text{(A12a)}$$

and we invert this expansion to obtain

$$Y_0 \approx (T - A) + \frac{1}{2}(\gamma - 1)(T - A)^2 + O(T - A)^3. \qquad \text{(A12b)}$$

Comparing to (A11a) requires $A = 0$ for matching at $O(1)$. Substituting the resulting expansion into the other outer solutions and expanding them for small T, one finds

$$X(T) \approx 1 + (\gamma - 1)T + O(T^2) + \delta[1 + B(\gamma - 1) + O(T)] + \varepsilon[(C - 2)(\gamma - 1) + O(T)]; \qquad \text{(A13a)}$$

$$Y(T) \approx T + O(T^2) + \delta[B + O(T)] + \varepsilon[C - 1 + O(T)]. \qquad \text{(A13b)}$$

Comparing to Eqs. (A11) reveals $B = C = 0$.

One can now combine all of the parts, i.e. (inner, A7) + (outer, A10) − (matching, A11), to formulate the uniformly valid composite solutions. We present them here in terms of the outer independent variable T ($=\varepsilon\tau$), since it is the relevant time-scale for product formation.

$$T = \gamma Y_o - (1-\gamma)\ln(1 - Y_o); \qquad \text{(A14a)}$$

$$\begin{aligned} X(T) &= X_0 + \delta X_\delta + \varepsilon X_\varepsilon + O(\delta+\varepsilon)^2 = \frac{1-Y_o}{1-\gamma Y_o} - e^{-T/\varepsilon} \\ &+ \delta\left[\frac{(1-Y_o)^2}{(1-\gamma Y_o)^3} + \frac{(1-\gamma)(1-Y_o)}{\gamma(1-\gamma Y_o)^3}\ln(1-\gamma Y_o)\right] \\ &- \delta e^{-T/\varepsilon}\left[2\left(\frac{T}{\varepsilon}\right) + e^{-T/\varepsilon}\right] + \varepsilon\frac{1-Y_o}{(1-\gamma Y_o)^3}\left[\frac{2(1-\gamma)}{1-\gamma Y_o} + \ln\left(\frac{1-Y_o}{1-\gamma Y_o}\right)\right] \\ &- \varepsilon e^{-T/\varepsilon}\left[\frac{\gamma}{2}(T/\varepsilon)^2 + (1-2\gamma)\left(\frac{T}{\varepsilon}\right) + 2 - \gamma\left(1 + e^{-T/\varepsilon}\right)\right] \\ &+ O(\delta+\varepsilon)^2; \end{aligned} \qquad \text{(A14b)}$$

$$\begin{aligned} Y(T) &= Y_0 + \delta Y_\delta + \varepsilon Y_\varepsilon + O(\delta+\varepsilon)^2 \\ &= Y_o - \delta\frac{1-Y_o}{\gamma(1-\gamma Y_o)}\ln(1-\gamma Y_o) - \varepsilon\left\{\left(\frac{1-Y_o}{1-\gamma Y_o}\right)\left[\frac{1}{1-\gamma Y_o} + \frac{1}{1-\gamma}\ln\left(\frac{1-Y_o}{1-\gamma Y_o}\right)\right] - e^{-T/\varepsilon}\right\} + O(\delta+\varepsilon)^2. \end{aligned} \qquad \text{(A14c)}$$

One can invert Eq. A14a by choosing values of Y_0 ranging from 0 to 1 and calculating corresponding values of T, which are then used to solve Eqs. A14b–c for X and Y.

Given values of the initial concentrations E_T and S_T and the kinetics parameters k_1, k_{-1}, and k_2 (or alternatively K, K_D, and K_M), one can calculate values of the various dimensionless groups and scaling parameters in Eqs. 7–13, and then use Eqs. A14 to find $C = \beta X$ and $P = S_T Y$. Finally, $E(t)$ and $S(t)$ can be obtained if necessary from the mass constraints (4). This procedure was used to construct Figures 3–6.

The effects of the perturbation parameters δ and ε in (A14) are obvious, but the effects of γ are not. Recall that $\gamma = \beta/E_T$ is the fraction of enzyme initially charged to the reactor that forms complex on the short time-scale, so $0 < \gamma < 1$, and that $\gamma \to 0$ ensures the free enzyme concentration remains nearly constant [cf. Eq. 9, and Table 1, rows (a)–(g)]. If one is interested specifically in the limiting circumstance $\gamma \to 0$, it makes sense to reexamine the original problem, Eqs. 14–15, treating γ as the regular perturbation parameter instead of δ, because $\delta = \gamma\mu$ according to Eq. 20, and $0 < \mu < 1$ according to (17). The procedure in this case parallels exactly what we outlined in (A5) through (A14). One finds:

$$\begin{aligned} X(T) &= e^{-T} - e^{-T/\varepsilon} + \gamma\Big\{(1-T)e^{-T} + (\mu - 1)e^{-2T} \\ &- \mu\left[2\left(\frac{T}{\varepsilon}\right) + e^{-T/\varepsilon}\right]e^{-T/\varepsilon}\Big\} + \varepsilon\left[(2-T)e^{-T} - \left(2 + \frac{T}{\varepsilon}\right)e^{-T/\varepsilon}\right] \\ &+ O(\varepsilon+\gamma)^2; \end{aligned} \qquad \text{(A15a)}$$

$$Y(T) = 1 - e^{-T} + \gamma T e^{-T} + \varepsilon\left[e^{-T/\varepsilon} - (1-T)e^{-T}\right] + O(\varepsilon+\gamma)^2. \quad \text{(A15b)}$$

This is effectively the small-γ limit of our more complete perturbation solution (A14), above, which is difficult to obtain by manipulating (A14) directly. When $\gamma = 0$ exactly, $\delta = 0$ also, and the governing Eqs. 14 or 15 are fortuitously linear, thus they can be solved exactly for arbitrary ε. This circumstance was first described and an analytical solution given by Schnell and Mendoza,[31] and we include it in our comparison of solutions, Figures 3–6. In terms of our scaled variables, one finds

$$X(T) = \frac{1}{\sqrt{1-4\varepsilon}}\left\{\exp\left[-\left(\frac{1-\sqrt{1-4\varepsilon}}{2\varepsilon}\right)T\right] - \exp\left[-\left(\frac{1+\sqrt{1-4\varepsilon}}{2\varepsilon}\right)T\right]\right\}; \quad \text{(A16a)}$$

$$Y(T) = 1 + \left(\frac{1-\sqrt{1-4\varepsilon}}{2\sqrt{1-4\varepsilon}}\right)\exp\left[-\left(\frac{1+\sqrt{1-4\varepsilon}}{2\varepsilon}\right)T\right] - \left(\frac{1+\sqrt{1-4\varepsilon}}{2\sqrt{1-4\varepsilon}}\right)\exp\left[-\left(\frac{1-\sqrt{1-4\varepsilon}}{2\varepsilon}\right)T\right]. \quad \text{(A16b)}$$

Since this solution includes contributions from all orders of ε, it is more accurate than our perturbation solution for this one restrictive case, $\gamma = \delta = 0$. One recovers the $\gamma = \delta = 0$ version of our perturbation solution by expanding (A16) to first order in ε.

There is only one circumstance for which $\gamma \to 1$, and that is $S_T \gg E_T + K_M$ [cf. Table 1, row (i)]. In this unusual situation all enzyme binds quickly to a large excess of substrate, remaining bound and making product at a constant rate until the substrate is nearly consumed. The scaled complex curve $X(T)$ rises almost instantly from zero to one, stays there until $T \cong 1$, and then falls almost instantly back to zero. The scaled product curve $Y(T)$ rises linearly from zero to one at $T \cong 1$, turns sharply, and remains at one thereafter. This odd behavior for $\gamma \to 1$ is evidenced in the governing equations as an intriguing boundary singularity near $T = 1$, and it requires that all terms in (A14a) for $Y_0(T)$ be retained in the limit $(1 - \gamma) \to 0$. We attempted to solve the original Eqs. 14 and 15 with a singular perturbation in the small parameter $(1 - \gamma)$, which would serve to identify the leading-order effect of $\gamma \to 1$, but we were unsuccessful. This is of no practical consequence, however, because the full solutions (A14) converge uniformly for any physically realizable circumstance, i.e. $0 < \gamma < 1$.

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