Shadows and lights in enzyme kinetics: quasi-steady state approximation revisited

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Abstract

In this paper we re-examine the commonly accepted meaning of the two kinetic constants characterizing any enzymatic reaction, according to Michaelis-Menten kinetics. Introducing a new asymptotic expansion (in terms of exponentials) of the solutions of the ODEs governing the reaction, we determine a new constant, which corrects some misinterpretations of current biochemical literature and suggest a new method to estimate the characteristic parameters of the reaction.

Keywords: Michaelis-Menten kinetics, quasi-steady state approximations, asymptotic expansions
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1   Introduction

The Michaelis-Menten-Briggs-Haldane approximation, or standard quasi-steady state approximation (sQSSA) [20, 6, 32] represents a milestone in

the mathematical modeling of enzymatic reactions.

The hypothesis of quasi-steady state is crucial for the interpretation of the reaction and must be handled with much care. It is based on the assumption that the complex can be considered "substantially" constant, but this statement has led to many misinterpretations of the model.

In fact, as Heineken et al. showed in [13], the exact mathematical interpretation of the quasi-steady state assumption is that when we expand asymptotically the solutions of the ODEs governing the process with respect to an appropriate parameter, the sQSSA is the first order approximation of the solution.

As already observed by Michaelis and Menten by a chemical point of view, this approximation is valid only when the parameter of the expansion is sufficiently small. Heineken et al. used the parameter given by the ratio of the initial concentrations of enzyme E and substrate S, obtaining the well-known chemical requirement.

In 1988 Segel [31] and in 1989 Segel and Slemrod [32] obtained the Michaelis-Menten approximation expanding the solutions in terms of a new parameter, including the so-called Michaelis constant and showing that the sQSSA is valid in a wider range of parameters than the one supposed before.

However it is well known that while *in vitro* the condition on the concentrations can be easily fulfilled, *in vivo* it is not always respected. This means that, though very useful, this approximation cannot always be applied.

Michaelis-Menten kinetics has recently become very popular thanks to the recent explosion of Systems Biology and in particular of mathematical modeling of intracellular enzyme reactions, but in most literature any *apriori* analysis of the applicability of sQSSA is absent, even in very complex reaction networks. This fact has led to several problems concerning the study of particular phenomena, like oscillations [10, 25], bistability [8], ultrasensitivity [26] or Reverse Engineering [24].

Following [17], recent papers [5, 37, 38, 23, 8, 26, 39, 19, 9, 25, 3] have introduced and explored a new approximation, called total quasi-steady state approximation (tQSSA), which has been shown to be more precise than the sQSSA and to be roughly always valid.

Nevertheless, since it is in any case an approximation, also the tQSSA can dramatically fail, as shown in [25], but it is doubtless that it is much more affordable than the sQSSA.

One of the main problems of the mathematical treatment of the sQSSA is the misinterpretation of the hypothesis that the complex time concentration has zero derivative. Many papers and even monographies interpret the "substantial" equilibrium as a real equilibrium. This is obviously false. Nevertheless much literature uses equations that seem scandalous to any mathematicians ([13], p. 97), obtaining results which are absolutely inconsistent and false.

In this paper we want to re-examine some mathematical aspects of Michaelis-

Menten reaction and of the sQSSA, trying to clarify some aspects of the enzyme reactions.

The paper is organized in the following way: in Section 2 we recall the most important notions and results on Michaelis-Menten kinetics and sQSSA; in Section 3 we discuss the biochemical and mathematical meaning of the tQSSA, comparing it with the sQSSA; in Section 4 we analyse the consequences of the misuse of the sQSSA, reconsidering the meaning of the two kinetic constants V_{max} and K_M; in Section 5 we introduce a new expansion in terms of exponentials, which is valid for every choice of the parameters and enzyme initial concentrations; the new expansion is the most appropriate to approximate the asymptotic behavior of the solution for large values of t; in Section 6 we use the new expansion to solve a serious incoherence present in literature, related to the biochemical interpretation of the constant K_M; in Section 7, using the expansions introduced in Section 5, we suggest a new approach to the estimate of the kinetic constants, based on fitting by least squares the experimental data of the time courses of reactant concentrations.

Finally, let us remark that, due to the stiffness of the system (2), for its numerical integration we used a standard stiff MATLAB integrator, ode23s.

2   Notations, definitions and main known results

The model of biochemical reactions was set forth by Henri in 1901 [14, 15, 16] and Michaelis and Menten in 1913 [20] and further developed by Briggs and Haldane in 1925 [6]. This formulation considers a reaction where a substrate S binds an enzyme E reversibly to form a complex C. The complex can then decay irreversibly to a product P and the enzyme, which is then free to bind another molecule of the substrate.

This process is summarized in the scheme

$$E + S \; \underset{d}{\overset{a}{\rightleftarrows}} \; C \xrightarrow{k} E + P, \tag{1}$$
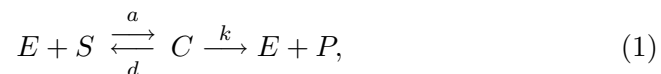
where a, d and k are kinetic parameters (supposed constant) associated with the reaction rates: a is the second order rate constant of enzyme-substrate association; d is the rate constant of dissociation of the complex; k is the catalysis rate constant.

Following the mass action principle, which states that the concentration rates are proportional to the reactant concentrations, the formulation leads to an ODE for each involved complex and substrate. We refer to this as the *full system*.

From now on we will indicate with the same symbols the names of the enzymes and their concentrations.

The ODEs describing (1) are

$$\frac{dS}{dt} = -a(E_T - C)S + d\,C,$$

$$\frac{dC}{dt} = a(E_T - C)S - (d + k)C, \qquad (2)$$

with initial conditions

$$S(0) = S_T, \quad C(0) = 0, \qquad (3)$$

and conservation laws

$$E + C = E_T, \quad S + C + P = S_T \ . \qquad (4)$$

Here E_T is the total enzyme concentration assumed to be free at time $t = 0$. Also the total substrate concentration, S_T, is free at $t = 0$. This is the so-called Michaelis-Menten (MM) kinetics [20, 4].

Let us observe that (2) - (4) admits trivially the asymptotic solution given by $C = S = 0$, $P = S_T$ and $E = E_T$. This means that all the substrate eventually becomes product due to the irreversibility, while the enzyme eventually is free and the complex concentration tends to zero.

Assuming that the complex concentration is approximately constant after a short transient phase leads to the usual Michaelis-Menten (MM) approximation, or *standard quasi-steady state approximation* (sQSSA): we have an ODE for the substrate while the complex is assumed to be in a quasi-steady state (i.e., $\frac{dC}{dt} \approx 0$):

$$C \cong \frac{E_T \cdot S}{K_M + S} \quad , \quad \frac{dS}{dt} \cong -kC \cong -\frac{V_{max}S}{K_M + S}, \quad S(0) = S_T, \qquad (5)$$

where

$$V_{max} = k\,E_T, \quad K_M = \frac{d + k}{a} \ . \qquad (6)$$

K_M is the so-called *Michaelis constant*.

The advantage of a quasi-steady state approximation is not only that it reduces the dimensionality of the system, passing from two equations (full system) to one (MM approximation or sQSSA) and thus speeds up numerical simulations greatly, especially for large networks as found *in vivo*, but also that it allows a theoretical investigation of the system which cannot be obtained with the numerical integration of the full system. Moreover, the kinetic constants in (1) are usually not known, whereas finding the kinetic parameters for the MM approximation is a standard *in vitro* procedure in biochemistry. See e.g. [4] for a general introduction to this approach. We stress here that this is an approximation to the full system, and that it is only

valid when the enzyme concentration is much lower than either the substrate concentration or the Michaelis constant K_M, i.e., (see, for example, [32])

$$\varepsilon_{MM} := \frac{E_T}{S_T + K_M} \ll 1 \tag{7}$$

This condition is usually fulfilled for *in vitro* experiments, but often breaks down *in vivo* [35, 34, 33, 1]. We refer to [29] for a nice, general review of the kinetics and approximations of (1).

3   The total quasi-steady state approximation (tQSSA)

As mentioned in the previous section, in general we cannot assume *in vivo* a low enzyme concentration, and hence the MM approximation can not be expected to hold. This fact is well known in literature. Apart from the above cited paper by Schnell and Maini [29] and references therein, it is useful to quote the recent papers [10, 41, 22, 8, 25] which discuss the applicability of the sQSSA.

In order to solve this problem, in 1955 Laidler [17], discussing the mathematical theory of the transient phase, found expressions for the behavior of P in the quasi-steady state and found several sufficient conditions for the applicability of the approximations. These conditions were much more general than $\dfrac{E_T}{S_T} \ll 1$. The importance of Laidler's results can be understood comparing his approach to a recent one, based on the so-called *total quasi-steady state approximation* (tQSSA). It was introduced by Borghans et al. [5] and refined by Tzafriri [37] for isolated reactions. It arises introducing the *total substrate*

$$\overline{S} = S + C, \tag{8}$$

and assuming that the complex is in a quasi-steady state as for the sQSSA. Reaction (1) then gives the tQSSA [5, 17]:

$$\frac{d\overline{S}}{dt} \cong -k\, C_-(\overline{S}), \quad \overline{S}(0) = S_T, \tag{9}$$

where

$$C_-(\overline{S}) = \frac{(E_T + K_M + \overline{S}) - \sqrt{(E_T + K_M + \overline{S})^2 - 4E_T\overline{S}}}{2}. \tag{10}$$

Numerical integration of (9) gives the time behavior of $\overline{S}$ and then (8) and (10) give the corresponding C and S.
Tzafriri [37] showed that the tQSSA (9) is valid whenever

$$\varepsilon_{tQSSA} := \frac{K}{2S_T}\left(\frac{E_T + K_M + S_T}{\sqrt{(E_T + K_M + S_T)^2 - 4E_T S_T}} - 1\right) \ll 1, \qquad (11)$$

(where $K = \frac{k}{a}$), and that this is at least roughly valid for any sets of parameters, in the sense that

$$\varepsilon_{tQSSA} \le \frac{K}{4K_M} \le \frac{1}{4}. \qquad (12)$$

This means that, for *any* combination of parameters and initial conditions, (9) is a good approximation to the full system (2). The parameter K is known as the Van Slyke-Cullen constant. The so-called *dissociation constant* $K_D = \frac{d}{a}$ [4] is related to the previous kinetic constants by the simple formula $K_D = K_M - K$.

Tzafriri [37] expanded equations (10) and (11) in terms of

$$\varepsilon_{Tz} := r(S_T) := \frac{4E_T \cdot S_T}{(E_T + K_M + S_T)^2} \qquad (13)$$

and assuming the validity of the tQSSA ($\epsilon_{tQSSA} \ll 1$) and $r \ll 1$, he found

$$\frac{d\bar{S}}{dt} \cong -kC_-(\bar{S}) \cong -\frac{V_{max}\bar{S}}{K_M + E_T + \bar{S}}, \quad \bar{S}(0) = S_T, \qquad (14)$$

as a first order approximation to (9). Expression (14) is identical to the formula obtained by [5] by means of a two point Padé approximant technique. This approximation is valid at low enzyme concentrations $E_T \ll S_T + K_M$, where it reduces to the MM expression (5), but holds moreover at low substrate concentrations $S_T \ll E_T + K_M$ [37]. Thus, with minimal effort performing the substitutions of S by $\bar{S}$ and of K_M by $K_M + E_T$, one obtains a significantly improved MM-like approximation, without any need of more advanced mathematics.

Moreover Tzafriri found that

$$\varepsilon_{tQSSA} = \frac{K \cdot E_T}{(E_T + K_M + S_T)^2} + o(r) . \qquad (15)$$

The expression $\frac{K \cdot E_T}{(E_T + K_M + S_T)^2}$ appears in the necessary condition, found by Borghans et al., for the validity of the tQSSA

$$\frac{K \cdot E_T}{(E_T + K_M + S_T)^2} \ll 1 \qquad (16)$$

which reduces to the condition given by Laidler for the validity of his approximations, setting $\bar{S} = S_T - P$.
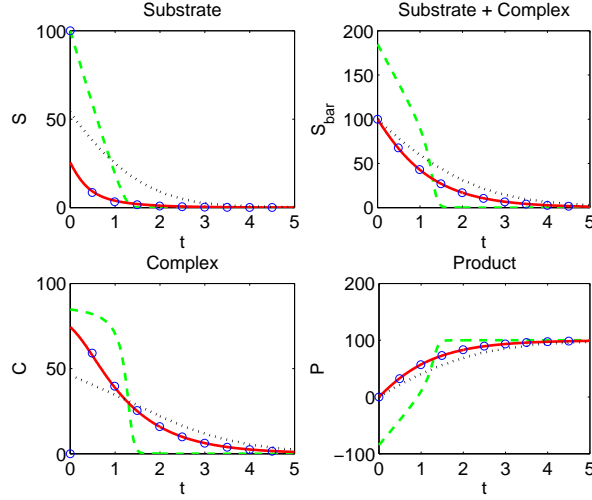
Figure 1: Dynamics of the model (1) for a=1, k=1, d=4, $S_T = 100$, $E_T = 89$. Plots show (top-left, bottom-right) S, $\bar{S}$, C, P. Circles: numerical solution of the full system; dashed line: sQSSA; solid: tQSSA; dotted: first order approximation of tQSSA.

It is important to remark, however, that the sufficient conditions found by Borghans et al. and by Tzafriri for the validity of (16) are much more general than those ones found by Laidler.

It is noteworthy the fact that the tQSSA uses the same parameters (V_{max}, K_M) of the sQSSA.

In [39] Tzafriri and Edelman gave the analytical solution of (9), in the reverse form $t = t(y)$, where y is a new variable depending on $\bar{S}$:

$$y = \bar{S} + \sqrt{(K_M + E_T + \bar{S})^2 - 4E_T \bar{S}} \ . \tag{17}$$

Very recently Dingee and Anton [9] reinterpreted the tQSSA as the first order term of a two-parameter asymptotic expansion where the perturbative parameters are

$$\varepsilon = \frac{K \cdot E_T}{(E_T + K_M + S_T)^2} \tag{18}$$

(that is (16), as one could naturally expect) and

$$\delta = \frac{S_T \cdot E_T}{(E_T + K_M + S_T)^2} \ . \tag{19}$$

With an accurate analysis of the time scales, they find the inner and the outer solutions. The leading order term coincides with the expression (14) obtained by Borghans et al. and Tzafriri.
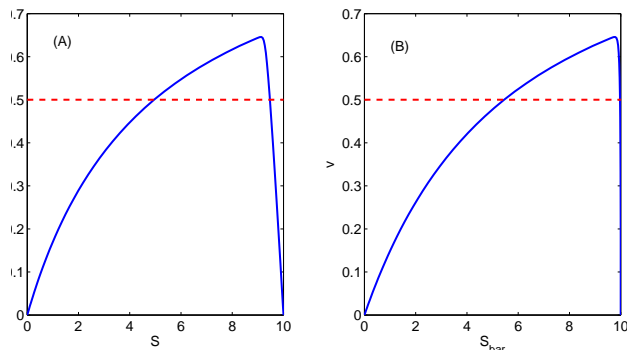
Figure 2: Plot of (A) $v = v(S)$ and (B) $v = v(\bar{S})$ for a=1, k=1, d=4, $S_T = 10$, $E_T = 1$. The dashed line represents the value of $\frac{V_{max}}{2}$.

4 Use and misuse of the Quasi-Steady State Approximation (sQSSA)

The roles of V_{max}, the maximal reaction velocity, and K_M, the Michaelis constant, become essential when characterizing biochemical reactions *in vitro* as well as *in vivo*. Moreover, the description of cooperative reactions, inhibition and many other biochemical processes have up to now exploited the fundamental ideas of the MM scheme, i.e., the sQSSA and the parameters V_{max} and K_M (see, e.g., [4]). However, these approximations cannot be expected to be valid *in vivo*.

Figure 1 shows that, for particular values of the parameters and the initial conditions, the sQSSA cannot be adequate to approximate the solutions of the full system at the beginning of the process, failing largely also the time in which the system reaches its equilibrium. This is due to the fact that (7) is not always fulfilled; nevertheless it is common in the biochemistry community not to consider this limitation and carry on calculations that can lead, therefore, to wrong estimations of the related kinetic parameters K_M and V_{max}.

As observed in [25], while every reaction is characterized by three constant rates (a, d, k), every QSSA works with only two parameters: V_{max} and K_M. Since $K_M = \frac{k+d}{a}$ and $V_{max} = kE_T$, we have

$$k = \frac{V_{max}}{E_T}, \qquad a = \frac{k+d}{K_M} = \frac{V_{max} + dE_T}{E_T K_M}. \qquad (20)$$

Setting $d = \gamma k$ we have

$$k = \frac{V_{max}}{E_T}, \qquad d = \frac{\gamma V_{max}}{E_T}, \qquad a = \frac{k+d}{K_M} = \frac{(1+\gamma)V_{max}}{E_T K_M}, \qquad (21)$$

8

	$S_{f.s.}$	$\bar{S}_{f.s.}$	S_{MM}	$\bar{S}_{tQSSA}$	$\bar{S}_{Tz}$
a)	4.995	5.045	5	5.05	5
			$\varepsilon_{MM} \cong 0.007$	$\varepsilon_{tQSSA} \cong 0.0004$	$\varepsilon_{Tz} \cong 0.0175$
			$\mathrm{err}_{MM} \cong 10^{-3}$	$\mathrm{err}_{tQSSA} \cong 10^{-3}$	$\mathrm{err}_{Tz} \cong 9 \cdot 10^{-3}$
b)	4.95	5.45	5	5.5	6
			$\varepsilon_{MM} \cong 0.067$	$\varepsilon_{tQSSA} \cong 0.004$	$\varepsilon_{Tz} \cong 0.156$
			$\mathrm{err}_{MM} \cong 10^{-2}$	$\mathrm{err}_{tQSSA} \cong 9 \cdot 10^{-3}$	$\mathrm{err}_{Tz} \cong 0.1$
c)	0.105	44.6	1	45.5	90
			$\varepsilon_{MM} \cong 0.88$	$\varepsilon_{tQSSA} \cong 0.034$	$\varepsilon_{Tz} \cong 0.99$
			$\mathrm{err}_{MM} \cong 8.52$	$\mathrm{err}_{tQSSA} \cong 2 \cdot 10^{-2}$	$\mathrm{err}_{Tz} \cong 1.018$

Table 1: Comparison of true and approximated values of S and $\bar{S}$ corresponding to $v = \frac{V_{max}}{2}$; $S_{f.s.}$ e $\bar{S}_{f.s.}$ are obtained integrating numerically the full system (2) - (4) with parameters given in Table 2; $S_{MM}, \bar{S}_{tQSSA}$ and $\bar{S}_{Tz}$ are respectively obtained from formulas (24), (25), (26); the values of their corresponding perturbative parameters and relative errors are reported, too.

with a degree of freedom, represented by the value of γ. Consequently, it is possible to vary the triplet (a, d, k) obtaining the same pair (V_{max}, K_M). However, the different choices of (a, d, k) could produce significantly different outputs, and thus predict different behaviors in the solutions of the full system and of its sQSSA. On the other hand, since tQSSA has shown to be always roughly valid, we can approximate the solution knowing only K_M and V_{max} because the presence of a degree of freedom in the choice of (a, d, k) does not substantially affect the validity of the approximation. Thus it is fundamental to understand how to appropriately estimate K_M and V_{max}, in order to approximate the solutions via the tQSSA.

The experimental estimates of V_{max} and K_M are usually performed repeat-

	E_T	S_T	a	k	d	K_M	V_{max}
a)	0.1	10	1	1	4	5	0.1
b)	1	10	1	1	4	5	1
c)	89	100	1	0.9	0.1	1	80.1

Table 2: Parameters used to obtain Table 1.

ing several experiments, with different values of S_T, in order to plot the dependence of the product velocity

$$v := \frac{dP}{dt} = kC \tag{22}$$

on the concentration of S and are based on the aprioristic (and not always true) assumption that the sQSSA is valid.

In this case

$$v = kC \cong \frac{V_{max} \cdot S}{K_M + S} \ . \tag{23}$$

Consequently V_{max} is usually intended as the limit of the "initial velocity" for the S concentration tending to infinity and K_M as the value of S such that

$$v(S = K_M) = \frac{V_{max}}{2} \ . \tag{24}$$

The values of V_{max} and K_M are obtained by means of the so-called Lineweaver-Burk plot [4].

The so-called "initial velocity" v must be intended as "initial velocity after the transient phase". This transient phase is mathematically characterized by a convex growth of P (during which C, and consequently $\frac{dP}{dt}$, grows) and ends when P graph changes its concavity, i.e., when C, at time t_{max}, reaches its maximal value, given by $C_{max} = C(t_{max}) = \frac{k \cdot E_T \cdot S(t_{max})}{K_M + S(t_{max})}$.

Obviously $\frac{dC}{dt} = 0$ iff $t = t_{max}$.

In the literature we always read that after the transient phase the growth of P is almost linear. But if we recall that the velocity of P is proportional to the concentration of C and if we look at Figure (1), we easily understand that C can hardly be considered as "almost constant"; thus by (22) P never grows in a linear way. This means that the experimental determination of the initial velocity will strongly depend on the time discretization used for the determination of the slope.

In other words the intrinsic bug of using v is strictly connected to the already discussed problem of stating that $\frac{dC}{dt}$ is really *equal* to zero, instead of being *almost* equal to zero.

Significantly, the original title of Schnell and Maini's paper [29], as shown on the web site http://www.cirs-tm.org/researchers/researchers.php?id=694, was "A century of enzyme kinetics. Should we believe in the K_m and v_{max} estimates?". In fact they and some of the authors cited by them criticize the way used to experimentally compute the parameters.

However, the problem of the above mentioned method is not only the low accuracy of any estimation of the initial velocity, obtained by means of the slope of a line passing through two different (possibly close each other) points of the time course of P just after the transient phase.

The problem we want to focus on is not HOW to estimate V_{max} or K_M, but IF it is really meaningful to determine the exact behavior of reactants by means of two parameters which can be determined only through some quasi-steady state approximation.

Moreover, we face with the problem that the biochemical interpretation of K_M fails not only when the sQSSA is not valid, but also in many other cases,

even when sQSSA holds: for example, when $K_M > S_T$. This means that, again, oppositely to what the great majority of biochemistry monographies reports, definition (24) is not true, in general.

Since the tQSSA is much more appropriate than the sQSSA, we can use formula (10) and very simple algebra to define in a more appropriate way K_M (if $S_T > K_M$):

i) when the value of the total substrate is equal to $\overline{S} = K_M + \dfrac{E_T}{2}$, then the rate of P is equal to $\dfrac{V_{max}}{2}$:

$$v\left(\overline{S} = K_M + \frac{E_T}{2}\right) = \frac{V_{max}}{2} \tag{25}$$

This result can also be found in [36].

Let us remark, by the way, that if we used Tzafriri approximating formula (14), we would obtain the following definition:

ii) when the value of the total substrate is equal to $\overline{S} = K_M + E_T$, then the velocity of P is equal to $\dfrac{V_{max}}{2}$:

$$v\left(\overline{S} = K_M + E_T\right) = \frac{V_{max}}{2} \tag{26}$$

Then the estimate given by (26) becomes largely incorrect for high values of E_T.

Figure (2) shows the behavior of v, as a function of S and $\overline{S}$. The graph has been obtained numerically integrating the full system (2) - (4) and computing v by means of formula (22). Let us observe that S is a decreasing function of t; thus the phase where C grows from 0 to C_{max} corresponds to the quasi-steady state phase, while the phase where C decreases from C_{max} to 0 corresponds to the transient phase.

We also plotted the horizontal line r of equation $v = \dfrac{V_{max}}{2}$ and determined the values of S and $\bar{S}$ corresponding to the intersection of the graph of v with r, respectively called $S_{f.s.}$ and $\bar{S}_{f.s.}$. Since the initial velocity is computed in the QSS phase, we are interested only in the first intersection.

In Tables 1 and 2 we give some examples, where we show the good accuracy of definition (i), even in extremal cases, in contrast with the usual definition (24) and definition (ii): in fact, differently from tQSSA, sQSSA can yield even very bad estimates of K_M. In Table 1 we report also the relative errors, given by

$$\text{err}_{MM} = \frac{|S_{MM} - S_{f.s.}|}{S_{f.s.}} \quad ; \quad \text{err}_{tQSSA} = \frac{|\overline{S}_{tQSSA} - \overline{S}_{f.s.}|}{\overline{S}_{f.s.}} \quad ; \quad \text{err}_{Tz} = \frac{|\overline{S}_{Tz} - \overline{S}_{f.s.}|}{\overline{S}_{f.s.}} \tag{27}$$

where $S_{MM}, \overline{S}_{tQSSA}$ and $\overline{S}_{Tz}$ are respectively given by formulas (24), (25), (26).

5   A new asymptotic expansion for large t

Though the sQSSA is based on the approximation $\dfrac{dC}{dt} \cong 0$, several biochemistry textbooks (see for example [18, 42, 27, 12]) misuse it, considering the approximation as a true equality.

As a consequence, the Michaelis constant is determined equating to zero the right hand side of equation (2) for C, obtaining

$$K_M = \frac{E \cdot S}{C} = \frac{(E_T - C) \cdot S}{C} \ . \tag{28}$$

Actually, as shown in Figure (1), the derivative of C is equal to zero only at time $t = t_{max}$. Consequently we cannot declare that the right hand side in (28) remains constant.

On the other hand, we could interpret K_M as the equilibrium value for $\dfrac{E \cdot S}{C}$, reached for t tending to infinity, in the same way as the dissociation constant K_D is interpreted in the original Michaelis-Menten reaction, where $k = 0$ [27].

Actually, while this last reaction, which is completely reversible, reaches a steady-state where both S and C are different from zero, in reaction (1), as above remarked, S and C tend to zero and consequently we cannot use (28), which gives an undefined ratio, for $t \to \infty$.

Thus the equality $K_M = \dfrac{E \cdot S}{C}$ is valid for every reaction only at $t = t_{max}$, when C reaches its maximum value.

We can however try to solve the indetermination of the ratio for $t \to \infty$ in the following way.

From Figure (1) we can observe that, after the transient phase, all the reactants seem to follow asymptotically an exponential behavior, with negative exponent. If we suppose that the asymptotic decay of C is proportional to $\mathrm{e}^{-\alpha t}$, for some α, formula (22) implies that also $S_T - P$ will be asymptotically proportional to $\mathrm{e}^{-\alpha t}$. By means of the conservation laws (4) we can conclude that also S and $E_T - E$ will follow the same asymptotic behavior as C.

Thus let us expand S and C in powers of $\mathrm{e}^{-\alpha t}$: we have

$$S(t) = S_0 + S_1 \, \mathrm{e}^{-\alpha t} + S_2 \, \mathrm{e}^{-2\alpha t} + o(\mathrm{e}^{-2\alpha t}) \tag{29}$$

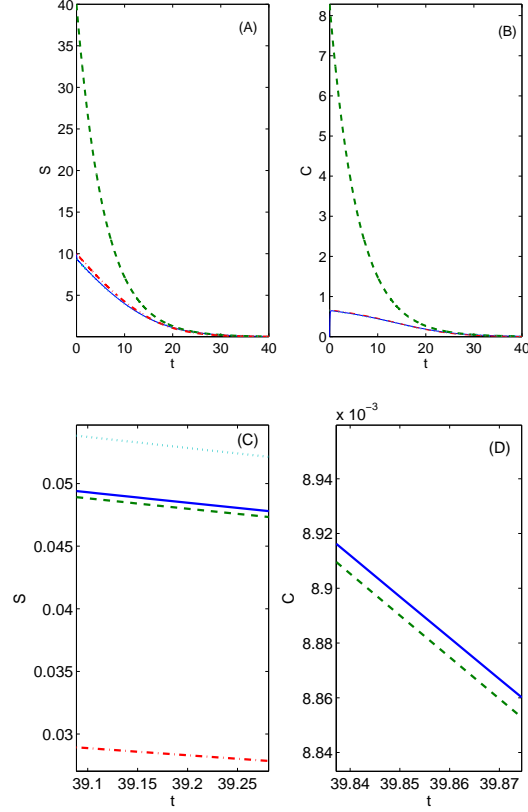$$C(t) = C_0 + C_1 \, \mathrm{e}^{-\alpha t} + C_2 \, \mathrm{e}^{-2\alpha t} + o(\mathrm{e}^{-2\alpha t}) \tag{30}$$

Figure 3: Dynamics of the model for a=1, k=1, d=4, $S_T = 10$, $E_T = 1$. Plots show: (A) S with (C) zoom and (B) C with (D) zoom. Solid line: numerical solution of the full system; dashed: asymptotic expansion, given by formulas (40), (41); dashed-dotted: sQSSA; dotted: tQSSA. $S_1 \sim 40$; $\alpha \sim 0.1716$; $\varepsilon_{MM} \sim 0.07$; $\varepsilon_{tQSSA} \sim 0.004$.

Substituting (29)–(30) in (2) we get

$$C_0 = S_0 = 0 \tag{31}$$
$$\alpha\, S_1 - aE_T S_1 + dC_1 = 0 \tag{32}$$
$$\alpha\, C_1 + aE_T S_1 - (d+k)\, C_1 = 0 \tag{33}$$
$$2\alpha\, S_2 - a[E_T S_2 - C_1 S_1] + dC_2 = 0 \tag{34}$$
$$2\alpha\, C_2 + a[E_T S_2 - C_1 S_1] - (d+k)C_2 = 0 \tag{35}$$

Formula (31) is biologically consistent with the asymptotic decay of S and C to 0. By Cramer's theorem, in order to have non-trivial solutions of (32)–(33), we must have

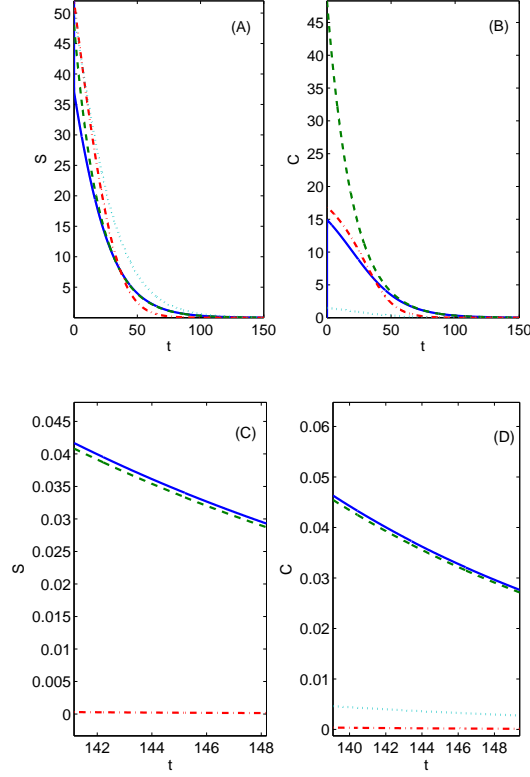$$\alpha^2 - [k + d + aE_T]\,\alpha + akE_T = 0$$

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Figure 4: Dynamics of the model for a=0.5, k=0.1, d=12.4, $S_T = 52$, $E_T = 25$. Plots show: (A) S with (C) zoom and (B) C with (D) zoom. Solid line: numerical solution of the full system; dashed: asymptotic expansion; dashed-dotted: sQSSA; dotted: tQSSA. $S_1 \sim 48.1$; $\alpha \sim 0.0782$; $\varepsilon_{MM} \sim 0.32$; $\varepsilon_{tQSSA} \sim 0.0008$

Then

$$\alpha_\pm = \frac{k + d + aE_T \pm \sqrt{(k + d + aE_T)^2 - 4akE_T}}{2} \qquad (36)$$

It is a matter of trivial algebra to prove that $\alpha_+ > k$, which is biologically unrealistic (see also (37)). So we can conclude that the only admissible solution is

$$\alpha_- = \frac{k + d + aE_T - \sqrt{(k + d \mp aE_T)^2 \pm 4akE_T}}{2} =$$

$$\frac{a}{2}\left[(K_M + E_T) - \sqrt{(K_M + E_T)^2 - \frac{4k}{a}E_T}\right] =$$

$$\frac{a}{2}(K_M + E_T)\left[1 - \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}}\right]$$

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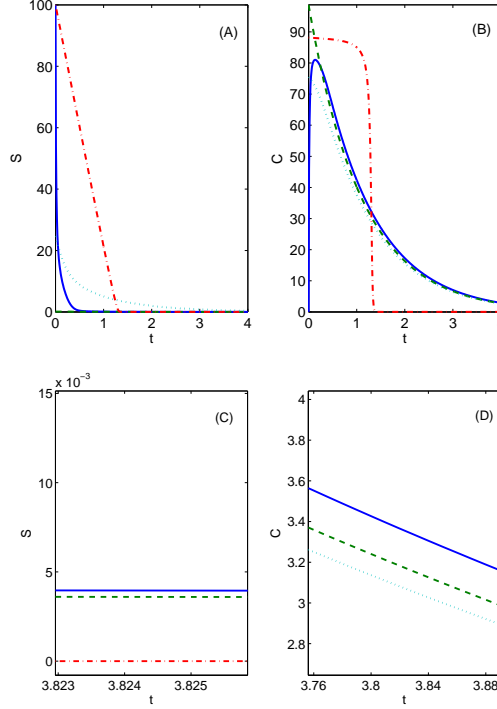
Figure 5: Dynamics of the model for a=1, k=0.9, d=0.1, $S_T = 100$, $E_T = 89$. Plots show: (A) S with (C) zoom and (B) C with (D) zoom. Solid line: numerical solution of the full system; dashed: asymptotic expansion; dashed-dotted: sQSSA; dotted: tQSSA. $S_1 \sim 0.112$; $\alpha \sim 0.899$; $\varepsilon_{MM} \sim 0.88$; $\varepsilon_{tQSSA} \sim 0.034$.

With this choice of α and dropping the minus for the sake of simplicity, we get

$$C_1 = \frac{\alpha}{k - \alpha} S_1 \qquad (37)$$

Now let us consider the terms of order 2. Inserting α in (34)–(35), by Cramer's theorem and (37) we find:

$$S_2 = \frac{-a\,\alpha\,(k - 2\alpha)\,S_1^2}{(k - \alpha)\,[(2\alpha - aE_T)(k - 2\alpha) + 2\alpha d]} \qquad (38)$$

$$C_2 = \frac{-2a\,\alpha^2\,S_1^2}{(k - \alpha)\,(2\alpha - aE_T)\,(k - 2\alpha) + 2\alpha d} \qquad (39)$$

So the required asymptotic expansions up to terms of the second order are:

$$S_{as}(t) = S_1\,e^{-\alpha t} - \frac{a\,\alpha\,(k - 2\alpha)\,S_1^2}{(k - \alpha)\,[(2\alpha - aE_T)(k - 2\alpha) + 2\alpha d]}e^{-2\alpha t} \cong S_1\,e^{-\alpha t} \qquad (40)$$

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$$C_{as}(t) = \frac{\alpha}{k-\alpha} S_1 \, \mathrm{e}^{-\alpha t} - \frac{2a \, \alpha^2 \, S_1^2}{(k-\alpha)(2\alpha - aE_T)(k-2\alpha) + 2\alpha d} \mathrm{e}^{-2\alpha t} \cong \frac{\alpha}{k-\alpha} S_1 \, \mathrm{e}^{-\alpha t}$$

$$(41)$$

There is still an unknown parameter, S_1, which could be estimated from experimental data via a least-squares procedure.

Figures (3) - (5) compare the time courses of the full system solutions, their sQSSA and tQSSA and the asymptotic expansions given by (40), (41).

In Figure (3) both sQSSA and tQSSA are good approximations, but, as shown in the zoom plots, the asymptotic expansions approximate the solutions much better for large t.

In Figure (4) the sQSSA begins to fail ($\varepsilon_{MM} \cong 0.32$), while the tQSSA follows in a very good way the solution. Nevertheless, again the asymptotic expansion approximates much better the solution for large t.

In Figure (5) the sQSSA cannot hold ($\varepsilon_{MM} \cong 0.88$), while the tQSSA still represents a good approximation ($\varepsilon_{tQSSA} \cong 0.034$). Though initially the asymptotic approximation considerably differs from the numerical solution, for large t it represents again the best approximation.

As shown in Figures (3) - (5), the estimate of S_1 is not related to S_T. In particular, in Figure (5) $S_T = 100$ and $S_1 \cong 0.112$. This fact is not in contrast with our results, because the intent of formulas (40), (41) is to approximate the solutions for large values of t, no matter what happens for small t.

6   The equilibrium constant revisited

Let us first state the main result of this section.

Theorem 6.1. *For* $t \to \infty$

$$\frac{E\,S}{C}(t) \cong \frac{E_{as}\,S_{as}}{C_{as}}(t) \to \left(\frac{k-\alpha}{\alpha}\right) E_T =: K_W \qquad (42)$$

Proof. From formulas (40), (41) we get

$$\frac{S_{as}}{C_{as}}(t) = \frac{1 - \frac{a\alpha(k-2\alpha)S_1}{(k-\alpha)[(2\alpha - aE_T)(k-2\alpha)+2\alpha d]}\mathrm{e}^{-\alpha t}}{\frac{\alpha}{k-\alpha}\left[1 - \frac{2a\,\alpha\,S_1}{(2\alpha - aE_T)(k-2\alpha)+2\alpha d}\mathrm{e}^{-\alpha t}\right]} \qquad (43)$$

When $t \to \infty$ we have $\frac{S_{as}}{C_{as}}(t) \to \frac{k-\alpha}{\alpha}$. Consequently, since $E_{as}(t) \to E_T$,

$$\frac{E\,S}{C}(t) \cong \frac{E_{as}\,S_{as}}{C_{as}}(t) \to \left(\frac{k-\alpha}{\alpha}\right) E_T =: K_W \qquad (44)$$
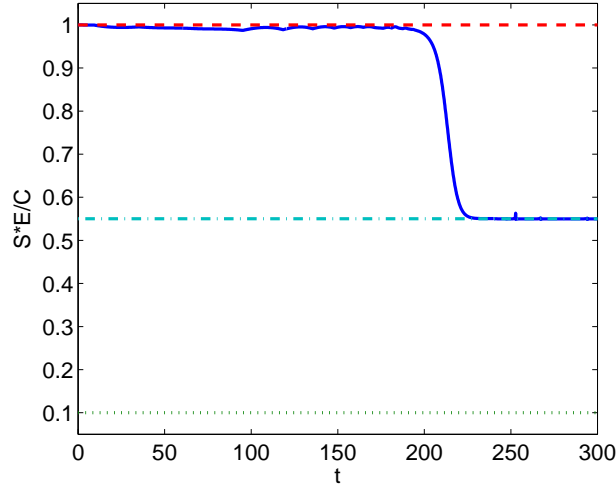
$\square$

Figure 6: Plot of $\frac{E\,S}{C}$ for a=1, k=0.9, d=0.1, $S_T = 100$, $E_T = 0.55$. Solid line: numerical solution of the full system; dashed: K_M; dashed-dotted: K_W; dotted: K_D. Parameters and initial conditions were chosen to give $K_W = \dfrac{K_M + K_D}{2}$.

The constant K_W, here introduced for the first time, gives the exact asymptotic value of the ratio $\dfrac{E\,S}{C}$ and, in contrast with biochemical literature [18, 42, 27, 12], in general is different from K_M. This result is clearly illustrated in Figures (6) - (8), where we have plotted the time course of the ratio $\dfrac{E\,S}{C}$, where the values E, S, C are obtained by the numerical integration of system (2) - (4).

Let us remark that, for $t \to 0$, $C(t) \to 0$; then $\dfrac{E\,S}{C}(t) \to \infty$. Consequently, Figures (6) - (8) start at an initial time $0 < t_0 \ll 1$. As expected, $\dfrac{E\,S}{C}$ initially decreases and assumes the value K_M only at time t_{max}, when $\dfrac{dC}{dt} = 0$. Then it tends to the value K_W.

When $K_M \gg E_T$ then $\alpha \cong \frac{kE_T}{K_M + E_T}$ and so, when $t \to \infty$,

$$\frac{E\,S}{C}(t) \to \left(\frac{k}{\alpha} - 1 \right) E_T \cong \left(\frac{K_M + E_T}{E_T} - 1 \right) E_T = K_M. \qquad (45)$$

Since, from (7), condition $K_M \gg E_T$ is sufficient to guarantee the validity of the sQSSA, formula (45) is consistent to what is generally asserted in literature, concerning the asymptotic value of $\dfrac{E\,S}{C}$ (see, for example, Figure
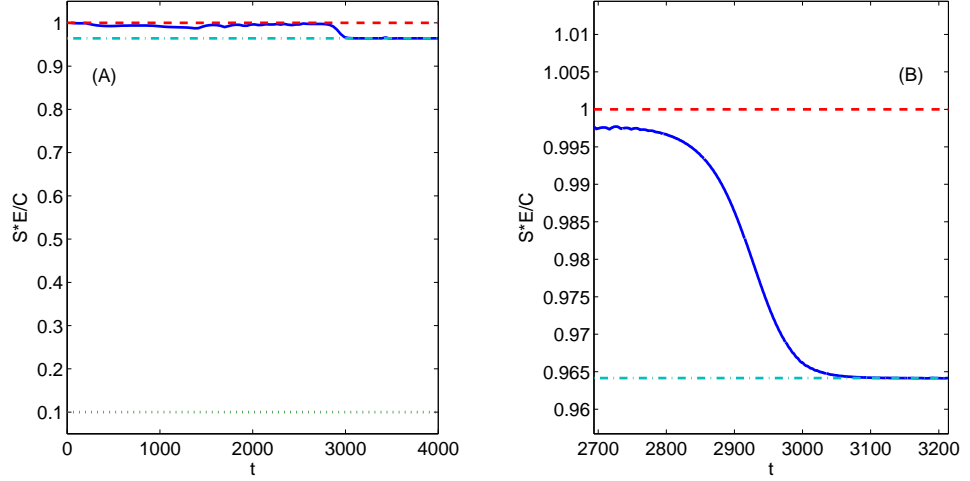
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(7)).

Figure 7: (A) Plot and (B) zoom of $\frac{E\,S}{C}$ for a=1, k=0.9, d=0.1, $S_T = 100$, $E_T = 0.04$. Solid line: numerical solution of the full system; dashed: K_M; dashed-dotted: K_W; dotted: K_D.

On the other hand, if $K_M \ll E_T$ then

$$\left(\frac{k-\alpha}{\alpha}\right) E_T = \frac{\left\{ k - \frac{a}{2}\,E_T \left(1 + \frac{K_M}{E_T}\right) \left[1 - \sqrt{1 - \frac{4kE_T}{a(K_M+E_T)^2}}\,\right] \right\} E_T}{\frac{a}{2}\,E_T \left(1 + \frac{K_M}{E_T}\right) \left[1 - \sqrt{1 - \frac{4kE_T}{a(K_M+E_T)^2}}\,\right] \right\} E_T} \tag{46}$$

$$\cong E_T \left(\frac{K_M}{E_T} + 1\right) \left[1 - \frac{1}{\frac{K_M}{E_T} + 1} - \frac{K}{E_T(\frac{K_M}{E_T} + 1)^3}\right] \tag{47}$$

$$= \frac{1}{(\frac{K_M}{E_T} + 1)^2} \left[K_M \left(\frac{K_M}{E_T} + 1\right)^2 - K\right] \cong K_M - K = K_D . \tag{48}$$

In this case the ratio $\dfrac{E\,S}{C}$ tends to a value which can be consistently different from K_M, as shown in Figure (8).

Let us now state some important properties of K_W.

Theorem 6.2. *For any admissible choice of the kinetic parameters and the initial conditions, the following inequalities hold:*
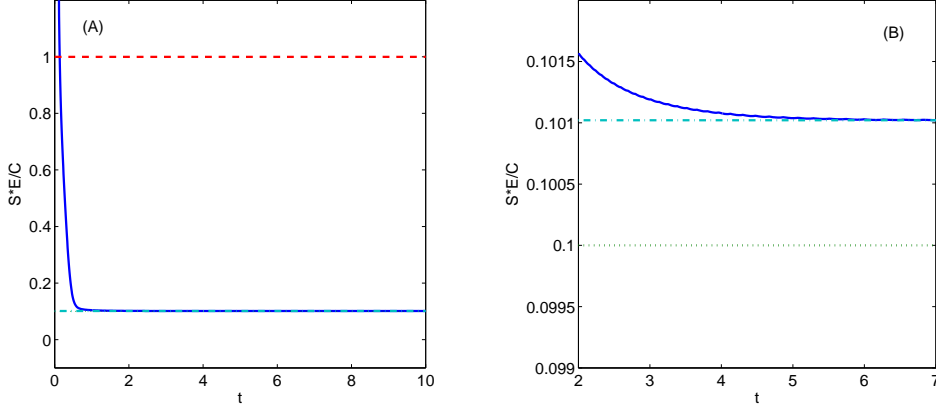
$$K_D \le K_W \le K_M . \tag{49}$$

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Figure 8: (A) Plot and (B) zoom of $\frac{ES}{C}$ for a=1, k=0.9, d=0.1, $S_T = 100$, $E_T = 89$. Solid line: numerical solution of the full system; dashed: K_M; dashed-dotted: K_W; dotted: K_D.

Proof. Let us begin with the right inequality. It holds iff

$$\left(\frac{k}{\alpha} - 1\right) E_T \leq K_M \tag{50}$$

$$\Leftrightarrow \frac{a}{2}(K_M + E_T)^2 \left[1 - \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}}\right] \geq kE_T \tag{51}$$

$$\Leftrightarrow \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}} \leq 1 - \frac{2kE_T}{a(K_M + E_T)^2} \tag{52}$$

Now, being

$$1 - \frac{2kE_T}{a(K_M + E_T)^2} > 1 - \frac{4kE_T}{a(K_M + E_T)^2} \geq 0$$

the right side of (49) follows squaring both sides of (52).
Let us now consider the left hand side of (49):

$$\left(\frac{k}{\alpha} - 1\right) E_T \geq K_D \tag{53}$$

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Being $K_D = K_M - \frac{d}{a}$, (53) holds iff

$$\alpha \left(K_M + E_T - \frac{k}{a} \right) \leq kE_T$$

$$\Leftrightarrow 1 - \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}} \leq \frac{2kE_T}{a(K_M + E_T)(K_M + E_T - \frac{k}{a})}$$

$$\Leftrightarrow \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}} \geq 1 - \frac{2kE_T}{a(K_M + E_T)(K_M + E_T - \frac{k}{a})}$$

If the right side of the last inequality is ≤ 0, the proof is over; if this is not the case, squaring both sides we get

$$1 - \frac{4kE_T}{a(K_M + E_T)^2} \geq 1 + \frac{4k^2 E_T^2}{a^2(K_M + E_T)^2(K_M + E_T - \frac{k}{a})^2}$$

$$- \frac{4kE_T}{a(K_M + E_T)(K_M + E_T - \frac{k}{a})};$$

$$\Leftrightarrow \left(K_M + E_T - \frac{k}{a} \right)^2 + \frac{k}{a}E_T - (K_M + E_T)\left(K_M + E_T - \frac{k}{a} \right) \leq 0$$

$$\Leftrightarrow -\frac{k}{a}\left(K_M - \frac{k}{a} \right) \leq 0 \Leftrightarrow \quad kd \geq 0$$

and the proof is over. $\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\qquad\square$

Actually, we can say something more: varying appropriately the parameter values, we can obtain for K_W every value between K_D and K_M. Let us show this fact varying only E_T.

Theorem 6.3. *For any admissible choice of the kinetic parameters and for any $\bar{K} \in (K_D, K_M)$, there exists $\bar{E}_T$ such that $\frac{ES}{C} \to \bar{K}$ when $t \to \infty$.*

Proof. Let us write

$$\bar{K} = \beta K_M + (1 - \beta)K_D = \frac{d + \beta k}{a} \tag{54}$$

for some $\beta \in [0, 1]$. Then

$$\left(\frac{k}{\alpha} - 1 \right) E_T = \frac{d + \beta k}{a} \Leftrightarrow \alpha = \frac{kE_T}{E_T + \frac{d + \beta k}{a}} \tag{55}$$

Comparing (55) with the expression of α, we have then

$$\frac{a}{2}(E_T + K_M)\left(E_T + \frac{d + \beta k}{a}\right)\left[1 - \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}}\right] = kE_T$$

$$\Leftrightarrow 1 - \sqrt{1 - \frac{4kE_T}{a(K_M + E_T)^2}} = \frac{2kE_T}{a(E_T + K_M)(E_T + \frac{d+\beta k}{a})}$$

$$\Leftrightarrow \frac{1}{K_M + E_T} + \frac{kE_T}{a(K_M + E_T)\left[K_M + E_T + \frac{k(\beta-1)}{a}\right]^2} - \frac{1}{K_M + E_T + \frac{k(\beta-1)}{a}} = 0$$

$$\Leftrightarrow a\left[K_M + E_T + \frac{k(\beta-1)}{a}\right]^2 + kE_T - a(E_T + K_M)\left[K_M + E_T + \frac{k(\beta-1)}{a}\right] = 0$$

$$\Leftrightarrow [a(K_M + E_T + k(\beta-1)](\beta-1) + aE_T = 0$$

and finally we have

$$\bar{E}_T = \frac{(k+d)(1-\beta) - k(1-\beta)^2}{\alpha\beta} = \frac{1-\beta}{\beta}\left[\frac{d + \beta k}{a}\right] = \frac{1-\beta}{\beta}\left[\beta K_M + (1-\beta)K_D\right] .$$
$$(56)$$
$$\square$$

Formula (54) justifies the symbol K_W: this constant can be seen as a convex combination of K_D and K_M, where β is the weight of the combination.

Substituting in (56) the expression of β given by (54), we find

$$\bar{E}_T = \bar{K}\frac{K_M - \bar{K}}{\bar{K} - K_D} \tag{57}$$

Moreover, we can express the corresponding α in a simpler way:

$$\alpha = k(1-\beta) = a(K_M - \bar{K}) \tag{58}$$

When $\beta = 0$, i.e. $\bar{K} = K_D$, formula (56) does not hold. In fact, the asymptotic value K_D is the equilibrium value of the original Michaelis-Menten reaction, obtained from (1) putting $k = 0$ [27]. Thus the case $\beta = 0$ is biologically unrealistic for reaction (1).

In any case, we can suppose $\beta \ll 1$ and consequently $\bar{E}_T \cong \frac{d}{a\beta} = \frac{K_D}{\beta}$. Therefore in this case we have $\frac{\bar{E}_T}{K_D} \gg 1$, that is, $\bar{E}_T \gg K_D$. In other words, as $E_T \to \infty$, K_W tends to K_D, as shown in Figure (8).

When $\beta = 1$, i.e. $\bar{K} = K_M$, formula (56) would give $E_T = 0$, which clearly is not admissible. Let us suppose therefore $\beta = 1 - \epsilon$ with $\epsilon \ll 1$. Then we get, at leading order,

$$\bar{E}_T \cong \frac{\epsilon}{1-\epsilon}\left[\frac{d + \beta k}{a}\right] \cong \epsilon(1 + \epsilon)\left[\frac{d + \beta k}{a}\right]$$

$$\cong \epsilon\left[\frac{d + k}{a}\right] = \epsilon K_M$$

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Then we have, in this case, $\frac{E_T}{K_M} = \epsilon \ll 1$, that is, $E_T \ll K_M$.
These last results are in good agreement with (45) and (48).

7 A new approach to the rate constants estimation

The analysis of the techniques for parameter estimation (inverse problem) is far beyond the scopes of this paper. We refer to the very interesting paper by Schnell and Maini [29] for an extended discussion of the problem and for a large bibliography. Moreover several authors have recently discussed new approaches for the experimental determination of the parameter values (see for example [7, 21, 36, 28, 40, 2] and references therein).
Schnell and Mendoza suggest to estimate the parameter values following the time course of reactants, fitting the experimental data with the formula obtained in [30] and based on the Lambert function W, defined by the implicit formula

$$W(t) exp\left[W(t)\right] = t \; , \tag{59}$$

which is the solution of the sQSSA equation (5), instead of repeating several times the same experiment, varying the initial reactant concentrations, in order to determine the values of the so-called "initial velocity" v.
Their theoretical considerations have been positively experimentally tested by Goudar et al. [11].
However the closed formula obtained by Schnell and Mendoza is valid only if the sQSSA holds. Otherwise we cannot use it.
Making use of the asymptotic approximations introduced in Section 5 (which are valid for every reaction and every parameter or initial concentration value) and by some simple considerations, we are able to suggest a new way of estimating the three constant rates a, d, k, starting from the experimental data of the time courses of S, E, C and P.
Let us first observe that in literature it is usually declared that at the beginning, mainly when the initial substrate concentration S_T is high, we can affirm that S remains constant.
Actually, when we determine from (2) the initial rates of the concentrations, we have

$$\frac{dS}{dt}(0) = \frac{dE}{dt}(0) = -\frac{dC}{dt}(0) = -aE_T S_T \quad ; \quad \frac{dP}{dt}(0) = 0 \; . \tag{60}$$

Then the mathematical model says that, for increasing values of S_T, $\left|\frac{dS}{dt}(0)\right|$ grows, while the ratio

$$\frac{\frac{dS}{dt}(0)}{S_T} = -aE_T \tag{61}$$

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does not depend on S_T.

On the other hand, when we use the tQSSA, we have $\dfrac{d\bar{S}}{dt} = -kC$ and consequently $\dfrac{d\bar{S}}{dt}(0) = 0$.

This means that during the transient phase we cannot suppose that S is approximately constant, but we can reasonably suppose $\bar{S} \cong S_T$, as correctly set for the inner solutions, for $t < t_{max}$, in the tQSSA asymptotic expansions [17, 5, 37, 9].

i) estimate of a

Equations (60) give a way to determine experimentally the parameter a, starting from the experimental data at the very beginning of the reaction: given t_1 and t_2, such that $t_1, t_2 \ll 1$,

$$a \cong \frac{1}{E_T S_T}\left[\frac{S(t_1) - S(t_2)}{t_2 - t_1}\right] \cong \frac{1}{E_T S_T}\left[\frac{E(t_1) - E(t_2)}{t_2 - t_1}\right] \cong \frac{1}{E_T S_T}\left[\frac{C(t_2) - C(t_1)}{t_2 - t_1}\right] \ . \tag{62}$$

The advantage of this estimate is given by the fact that, in the same experiment, the value of a can be determined in three different ways at the same two times t_1 and t_2.

The other parameters can be estimated by means of formula (42), using the experimental data of C, S and E for t sufficiently large.

ii) estimate of d

Performing an experiment with a very high initial concentration of E_T ($E_T \to \infty$) and for sufficiently large values of t,

$$K_D = \frac{d}{a} \cong \frac{S(t)E(t)}{C(t)} = \frac{S(t)\left[E_T - C(t)\right]}{C(t)} \tag{63}$$

thus, knowing a from (62), we have

$$d = aK_D \cong a \cdot \left[\frac{S(t)E(t)}{C(t)}\right] \ . \tag{64}$$

In this case we can test the validity of K_D (and a) estimates computing the ratio $\dfrac{S(t)E(t)}{C(t)}$ at different (sufficiently large) times.

iii) estimate of k

The case $E_T \gg 1$ ($\beta \ll 1$) can be used also for the estimate of k: in fact, in this case, from formula (58), $\alpha \cong k$.

Thus, fitting the experimental data of S with formula (40), we can estimate both S_1 and α and thus k, too.

On the other hand, since, for $E_T \to 0$, $\dfrac{SE}{C} \to K_W \cong K_M$ for $t \to \infty$, we can perform experiments with $E_T \ll 1$, estimating K_M (and thus $k = aK_M - d$) by means of the values of $\dfrac{SE}{C}(t)$ for t sufficiently large.

Though the approach here proposed needs the replication of experiments with different values of E_T (in particular $E_T \gg 1$ and $E_T \ll 1$), it has the advantage that it can directly estimate the three kinetic parameters a, d, k and that for every experiment the same estimate can be repeated for several values of t, using the experimental time course of the concentrations.

Acknowledgements

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