MATCH
Communications in Mathematical and in Computer Chemistry

MATCH Commun. Math. Comput. Chem. **70** (2013) 759-784

ISSN 0340 - 6253

Enzyme Kinetics: A Critique of the Quasi–Steady–State Approximation

Kamal Bhattacharyya[*], **Sharmistha Dhatt**

Department of Chemistry, University of Calcutta, Kolkata 700 009, India

(Received May 7, 2013)

Abstract

The standard two-step model of homogeneous-catalyzed reactions had been theoretically analyzed at various levels of approximations from time to time. The primary aim was to check the validity of the quasi-steady-state approximation, and hence emergence of the Michaelis-Menten kinetics, with various substrate-enzyme ratios. But, conclusions vary. We solve here the desired set of coupled nonlinear differential equations by invoking a new set of dimensionless variables. Approximate solutions are obtained via the power-series method aided by Padè approximants. The scheme works very successfully in furnishing the initial dynamics at least up to the region where existence of any steady state can be checked. A few conditions for its validity are put forward and tested against the findings. Temporal profiles of the substrate and the product are analyzed in addition to that of the complex to gain further insights into legitimacy of the above approximation. Some recent observations like the 'reactant stationary approximation' and the notions of different timescales are revisited. Signatures of the quasi-steady-state approximation are also nicely detected by following the various reduced concentration profiles in triangular plots. Conditions for the emergence of Michaelis-Menten kinetics are scrutinized and it is stressed how one can get the reaction constants even in the absence of any steady state.

[*] Corresponding author (pchemkb@gmail.com)

1. INTRODUCTION

The standard two-step model of homogeneous-catalyzed reactions leads to a set of coupled differential equations. Several interesting features [1–5] of nonlinearity in such reactions involve biochemical systems, either in isolation or as part of complex reaction networks. Therefore, simplifying assumptions are often made for the solutions. The most popular and useful result of these endeavors is the Michaelis–Menten (MM) form [6–8], particularly relevant to enzyme kinetics. One assumes here that the concentration of enzyme-substrate complex remains approximately constant over a considerable time span after a short transient. This is commonly known as the quasi-steady-state approximation (QSSA). It has been customary to test QSSA by choosing large substrate-enzyme ratios in keeping with *in vivo* studies. Therefore, one is led to believe, along with many authors [9–17] that the standard QSSA (s–QSSA) is valid only when the enzyme concentration is small enough, though the range of validity of the MM region is widened [13]. On the other hand, a number of studies [2,18–25] considered moderate-to-large enzyme-substrate ratios and found QSSA regions there too, under specific circumstances. These are relevant to interesting *in vitro* studies. Such endeavors, without any restriction on substrate-enzyme ratios, look for the applicability of total QSSA (t–QSSA). Experimental relevance of the MM kinetics in these situations is also available [4].

The two-step model corresponds to the reaction scheme

$$S+E \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} ES \xrightarrow{k_2} P+E. \qquad (1)$$

Several features of QSSA have been noted on its basis. For example, Laidler [26] put forward certain conditions for the applicability of QSSA. Borghans *et* al [19] distinguished s–QSSA from t–QSSA, and also remarked on reverse QSSA (r–QSSA) when the enzyme–substrate ratio is large. The idea was extended by Tzafriri [22]; subsequent extensions [23,24] followed. Various perturbation methods [10,13,18] have appeared with different scaled variables to understand QSSA. A nice summary of such works with further developments are available [27,28]. A variation-iteration method due to He [29–31] has recently [32] been found effective over a range of certain parameter values. Legitimacy of the MM approximation via a stochastic algorithm has also been forwarded [15].

From an experimental point of view, however, one can follow not only the rate of product formation, but the temporal profiles of substrate and product also. Theoretical studies, on the other hand, are centered chiefly on the profile of the complex. Conditions for the validity of QSSA also vary. So, while it is tempting to explore better and precise conditions of the

applicability of QSSA (or t–QSSA) from a different theoretical approach to the problem, one may also legitimately inquire whether its signatures exist in substrate–time and product–time plots. We also indicate the usefulness of triangular plots in deciphering the applicability of QSSA. A few other relevant queries include (i) relations among the maximum complex concentration, transient time and QSSA, if any, (ii) dependence of the steady–state region on the starting enzyme-substrate ratio [17], (iii) adequacy of the 'reactant stationary approximation' (RSA) [21,33], (iv) relevance of two time–scales and their relations in s–QSSA [18–20,22–24] and r–QSSA [19,21], etc.

Another important area concerns the validity of the MM kinetics. It is generally believed that the same rests on the assumption of the QSSA; thus, when QSSA fails to be obeyed, the MM kinetics loses its footing. However, we like to scrutinize the role of MM kinetics separately, irrespective of whether QSSA becomes valid or not. That this is possible will become clear in due course. One can also get the reaction constants from appropriate plots. Indeed, this turns out to be the most important part of the present work. Such a study has a good bearing on an early work [34] that claimed unacceptability of MM kinetics for about 800 enzymes!

To inspect the questions posed above, we choose a considerably different route. Casting the relevant equations in terms of a new set of dimensionless variables, we employ the standard power-series method of solution, supplemented by the construction of suitable Padè approximants (PA). Indeed, this is one of the most straightforward schemes to handle the problem. The success, however, depends on the choice of variables. In this respect, a preliminary investigation revealed that our scaling scheme performs nicely [35]. It extends the region of validity of the initial dynamics to intermediate times in a very successful manner. Our endeavor does not depend on the magnitude of the enzyme-substrate ratio [36] either. Hence, all the three types of QSSA can be dealt with on equal footing. However, here we choose to explore a number of areas that have not been covered earlier.

2. THE METHOD

2.1. Scaling

Denoting the concentrations of complex ES, enzyme E, substrate S and product P respectively by c, e, s and p, and their initial values by a subscript zero, we obtain from (1) the following differential equations:

$$ds / dt = -k_1 es + k_{-1} c, \tag{2}$$

$$dc / dt = k_1 es - (k_{-1} + k_2) c, \tag{3}$$

$$de/dt = -k_1 es + (k_{-1} + k_2)c, \tag{4}$$

$$dp/dt = k_2 c. \tag{5}$$

In addition, we have two mass conservation equations

$$e_0 = e + c, \tag{6a}$$

$$s_0 = s + c + p. \tag{6b}$$

To solve the above equations, various sets of scaled variables have been employed (see, e.g., Fraser [27], Murray [37], and works quoted therein). Here, we employ the following dimensionless variables:

$$\alpha = e/e_0, \beta = s/e_0, \gamma = c/e_0, \delta = p/e_0, \tau = k_2 t. \tag{7}$$

The conservation equations (6) now read as

$$\alpha + \gamma = 1 \tag{8a}$$

$$\beta + \gamma + \delta = \beta_0 \tag{8b}$$

The primary kinetic equations, out of (2) – (5), then follow as

$$-d\gamma/d\tau = d\beta/d\tau + \gamma, \tag{9}$$

$$d\beta/d\tau = -K_1\beta + (K_1\beta + K_2)\gamma, \tag{10}$$

with the initial conditions

$$\alpha_0 = 1, \beta_0 = s_0/e_0, \gamma_0 = 0 = \delta_0. \tag{11}$$

The constants K_1 and K_2 in (10) are given by

$$K_1 = k_1 e_0/k_2, K_2 = k_{-1}/k_2. \tag{12}$$

Thus, we could reduce the actual problem by choosing three variables and two constants. The usual strategy [37] has been to employ three variables and three constants.

2.2. Series expansions

Note that a large K_2 implies the equilibrium approximation in MM kinetics that had been extended [7] to QSSA in the same context long back. The above system of non-linear equations (9) – (10), with the aid of (11), can be solved analytically using the standard power series method. Hence, we express the concentrations of the participating species in powers of τ, *viz*.

$$\beta_\tau = \sum_{j=0} \beta_j \tau^j, \ \gamma_\tau = \sum_{j=0} \gamma_j \tau^j, \tag{13}$$

etc., insert them suitably into (9) and (10), and collect similar powers of τ. Thus, the unknown parameters of the expansions in (13) are obtained in terms of β_0, K_1 and K_2. The other

concentration terms can then be obtained simply by invoking (8). A few results of future interest are the following:

$$\begin{aligned} \beta_1 &= -K_1\beta_0, \\ \beta_2 &= K_1\beta_0(K_1\beta_0 + \beta_0 + K_2)/2. \end{aligned} \tag{14}$$

$$\begin{aligned} \gamma_1 &= K_1\beta_0, \\ \gamma_2 &= -K_1\beta_0(K_1\beta_0 + \beta_0 + K_2 + 1)/2. \end{aligned} \tag{15}$$

By using equations (8), one can get similar expansion coefficients for α and δ. In case of δ, a better alternative is to directly integrate (5) that now takes the form

$$d\delta/d\tau = \gamma. \tag{16}$$

Let us also note that, while γ rises from zero linearly during the initial phase of the reaction, it would finally tend to zero again. Hence, there exists at least one maximum in γ - τ plot. Indeed, one finds from (9) and (10) that

$$d\gamma/d\tau = K_1\beta - (K_1\beta + K_2 + 1)\gamma. \tag{17}$$

It shows, the point where $d\gamma/d\tau$ becomes zero is unique and, at this point (τ_c), γ would read as

$$\gamma_c = K_1\beta_c/(K_1\beta_c + K_2 + 1). \tag{18}$$

Therefore, there appears yet another possibility of expansions like (13). If one obtains τ_c and the corresponding concentrations γ_c and β_c, then the new pair of expansions takes the form

$$\beta_\tau = \sum_{j=0} \beta_{cj}(\tau - \tau_c)^j, \ \gamma_\tau = \sum_{j=0} \gamma_{cj}(\tau - \tau_c)^j. \tag{19}$$

Putting (19) in (9) and (10), we obtain the first few terms as

$$\begin{aligned} \beta_{c0} &= \beta_c, \\ \beta_{c1} &= -\gamma_c, \\ \beta_{c2} &= K_1\gamma_c(1-\gamma_c)/2; \end{aligned} \tag{20}$$

$$\begin{aligned} \gamma_{c0} &= \gamma_c, \\ \gamma_{c1} &= 0, \\ \gamma_{c2} &= -K_1\gamma_c(1-\gamma_c)/2. \end{aligned} \tag{21}$$

We shall see the usefulness of these terms later.

2.3. Numerical stability

An obvious problem with the expansions like (13) is their inability to yield reliable results for large τ. We circumvent here this problem by constructing the PA [38,39]. The PA has been found to be quite faithful in perturbation theory involving divergent Taylor expansions [40,41] (see, e.g., [41] and references quoted therein) and quite a few other contexts [42–44].

Here, we construct three types of PA, the diagonal [N/N] ones, and the two nearest off-diagonal [(N+1)/N] and [N/(N+1)] varieties. The agreement among values of such varieties points to the adequacy of the scheme. More specifically, we have taken the first 21 terms in (13) to obtain the sequences of these approximants. They suffice our purpose [35] as long as K_1 and K_2 are not large enough. Otherwise, one has to routinely increase the number of terms in order to get gradually improved results, or over a wider range of time τ at a fixed accuracy level.

Another way to check the numerical stability of our computed data is to compare the left and right sides of (18) from the PA sequences for γ and β at $\tau = \tau_c$. Indeed, this is the point at which rate of product formation attains its maximum value and, therefore, it possesses an experimental relevance too.

By following the above two checks, we noted that one can go well beyond the region of adequacy of QSSA. It may be pointed out that τ_c exists irrespective of whether QSSA is satisfied or not. Hence, the quality of steady state can be nicely assessed, if there is any, once the numerical scheme is known to be stable.

A different kind of possibility of extending the temporal regime is to first get γ_c and β_c via (13) and then employ (19). The rest of the scheme proceeds as before. After matching the coefficients, in the way we arrive at (20) and (21), one can construct the types of PA quoted above. However, in the present work, we did not require any use of (19) for numerical purposes.

3. ANALYSIS

3.1. Behavior of the concentration profiles

We consider first the case of γ. In the small-τ regime, it turns out that

$$\gamma = \gamma_1 \tau + \gamma_2 \tau^2 + \ldots , . \tag{22}$$

where the coefficients are given by (15). It shows the initial linear rise, with a slope of $K_1\beta_0$. After the transient time τ_c, however, it is expressible as

$$\gamma = \gamma_c + \gamma_{c2} (\tau - \tau_c)^2 + \ldots , \tag{23}$$

in view of (19) and (21). Accepting that the quick linear rise is opposed by the quadratic term in (22) to yield a maximum, one can write

$$\gamma_c \approx \gamma_1 \tau_c + \gamma_2 \tau_c^{\,2} \tag{24}$$

and it can be solved for τ_c, yielding

$$\tau_c \approx \gamma_c / \gamma_1 = \beta_c / \left(\beta_0 (K_1 \beta_c + K_2 + 1) \right). \tag{25}$$

Result (25) should be approximately true for small τ_c.

Initial fall-off of β is linear too, with a slope of $K_1\beta_0$. Moreover, if K_1 is small, which we shall later see to turn out as a condition for QSSA, one can write

$$\beta \approx \beta_0 + \beta_1\tau \approx \beta_0 \exp[-K_1\tau] \tag{26}$$

over a good range. Now, if it so happens that $K_1 << 1$, and the transient phase (0 to τ_c) is small, then the RSA [21, 33] follows. Another characteristic parameter of some use [13, 18 – 24] is τ_s, the time required for maximum change in β. For $\beta_0 << 1$, the initial decay is very slow. Hence, from (26), on the basis of initial decay, τ_s is the lifetime. Thus, we have a different timescale

$$\tau_s = 1/K_1. \tag{27}$$

This attaches a physical meaning to K_1. Note, however, that β_2 tends to oppose the fall-off. Around τ_c, on the other hand, we find

$$\beta = \beta_c - \gamma_c(\tau - \tau_c) - \gamma_{c2}(\tau - \tau_c)^2 + ... \tag{28}$$

that reveals again a linear fall-off unless $|\gamma_{c2}|$ is large. We shall see later how this result becomes useful.

Turning attention to δ, we notice from (15), (16) and (22) that

$$d\delta / d\tau = \gamma \approx K_1\beta_0\tau + \boldsymbol{O}(\tau^2). \tag{29}$$

It tells, the initial rise of the product is always parabolic in time [45]. However, unless τ_c is large, the parabolic nature may not show up significantly. Again, from (16), (21) and (23), one arrives at the temporal behavior of the product beyond τ_c as

$$\delta = \delta_c + \gamma_c(\tau - \tau_c) + \gamma_{c2}(\tau - \tau_c)^3/3 + ... , \tag{30}$$

showing a linear rise for small enough $|\gamma_{c2}|$.

3.2. Workability of the QSSA

It is now appropriate to remark on the conditions so far put forward concerning the workability of QSSA. One of the earliest ones is given by Laidler [26]. Stated in terms of our parameters, his four conditions are

$$\begin{aligned} &(a)\ \beta_0 >> 1; \\ &(b)\ \beta_0 << 1; \\ &(c)\ K_1\beta_0/(1+K_2) << 1; \\ &(d)\ K_1/(1+K_2) << 1. \end{aligned} \tag{31}$$

Either of these is a necessary condition. Additionally, it is agreed that, if (31a) holds, then τ_c would be small [11,26]. As stated earlier, most authors favor (31a) only. Some authors [16,17]

still maintain that QSSA would fail under condition (31*b*). A few other works [18,20] replace (31*a*) by

$$K_1 / (K_1\beta_0 + K_2 + 1) << 1, \tag{32}$$

highlighting it as the sole criterion for the validity of QSSA. It has also been remarked [19] that QSSA is tenable even when $\beta_0 \approx 1$, but then the Michaelis constant k_m should obey

$$k_m = e_0(1 + K_2) / K_1 >> 1. \tag{33}$$

An extension [22–24] of the earlier work [19] revealed that (32) would apply if $\beta_0 >> 1$; in the converse case, one has to ensure whether

$$K_1\beta_0 / (K_1 + K_2 + 1) << 1 \tag{34}$$

is satisfied and this condition validates QSSA. Let us remark here that conditions (32) and (34) may better be viewed as extensions of (31*d*) and (31*c*), respectively. A thorough check [35] shows, however, that none of these conditions (31), (32) and (34) withstand a close scrutiny.

In terms of timescales, another idea [18–24] is to check the ratio τ_s/τ_c. For $\beta_0 >> 1$, QSSA (or, s-QSSA) is said to be valid if

$$\tau_s / \tau_c >> 1, \tag{35}$$

with

$$\tau_s = \beta_0 + (K_2 + 1) / K_1, \ \tau_c = 1 / (K_1\tau_s). \tag{36}$$

At the other extreme (r-QSSA) of $\beta_0 << 1$, condition (35) is replaced by

$$\tau_s / \tau_c << 1, \tag{37}$$

where

$$\tau_c = 1, \tau_s = 1 / K_1. \tag{38}$$

Workability of such conditions will be surveyed in the next section. We only remark here that when $\beta_0 >> 1$, $\beta_0 \approx \beta_c$ is obeyed and hence (25) agrees with τ_c in (36), while the expression for τ_s in (27) matches with the same in (38) under r-QSSA condition.

In the present work, it is transparent from (21) and (23) that, if γ needs to retain an approximate constancy (*i.e.*, $\gamma \approx \gamma_c$) over a considerable range of $\tau > \tau_c$, as is required for QSSA, then we should have

$$\left|\gamma_{c2}/\gamma_c\right| << 1. \tag{39}$$

But, (21) shows that a sufficient condition for (39) to hold is

$$K_1 << 1. \tag{40}$$

It now also explains the adequacy of the constant-β approximation [33] discussed below (26). More appropriately, however, (39) yields

$$\left|\gamma_{c2}/\gamma_c\right| = K_1(1-\gamma_c)/2 << 1. \tag{41}$$

Thus, (41) turns out to be a condition for QSSA.

Using (18), one finds from (41) that

$$\lambda_c = K_1(K_2+1)/\left(2(K_1\beta_c + K_2 + 1)\right) << 1. \tag{42}$$

For convenience, we call the quantity at the left side of (42) by λ_c. However, its value depends on β_c that may not be known *a priori*. So, we also define a quantity λ_0 by

$$\lambda_0 = K_1(K_2+1)/\left(2(K_1\beta_0 + K_2 + 1)\right). \tag{43}$$

Then, while $\lambda_c > \lambda_0$, if one can ensure that $\lambda_0 << 1$, one may not be far from the applicability of QSSA. We shall later check how such a condition performs. Condition (43) looks in part like (31*c*) or (31*d*) and partly like (32). One can conclude from (43) that (i) a very small K_1 is sufficient for QSSA, as found before, but (ii) if K_1 is not small enough, we can still satisfy inequality (43) by requiring that $K_1\beta_c >> (K_2 + 1)$. Further, for large β_0, we may replace the preceding inequality by $K_1\beta_0 >> (K_2 + 1)$. Thus, it is neither true [9–17] that QSSA is always valid for $\beta_0 >> 1$, nor false [17, 46] that QSSA is always invalid for $\beta_0 << 1$.

3.3. Conventional MM kinetics

The popular representation [7, 8] of the MM kinetics reads for the rate r as

$$r = r_M s/(s+k_m),\ r_M = k_2 e_0, \tag{44}$$

obtained from the condition

$$dc/dt = 0. \tag{45}$$

In (44), r_M is the maximum rate. Sometimes, (44) is written more simply as (s-QSSA)

$$r = r_M s_0/(s_0+k_m). \tag{46}$$

But, we must note that, while (46) predicts a constant rate, (44) does not. Moreover, by virtue of (45), r is never the *initial rate*. The initial rate is always zero. Indeed, (44) or (46) refers to the *maximum rate* for a given run and r_M stands for the overall maximum of all such maxima. In terms of our variables, (44) reduces to

$$R = d\delta/d\tau = \gamma = K_1\beta/(K_1\beta + K_2 + 1), \tag{47}$$

where R stands for the rate in our terms. Form (47) [equivalently (44)] has another immediate problem. Putting the expansion (28) for β at the right hand part of (47), one can check that there

exists a non-zero first-order term in γ. Hence, this form cannot respect (23) or (45), which tells that there is a maximum of γ in the γ - τ plot. On the other hand, if one goes along with (47) and argues that γ remains stationary only in an approximate sense [$\gamma_{c1} \approx 0$ in (21)], the first-order factor associated with (τ - τ_c) should be very small. This leads to

$$\mu_c = K_1(K_2+1)/(K_1\beta_c + K_2 + 1)^2 << 1. \tag{48}$$

Since the second part of (48) is naturally less than unity, one notes from here that, either a large β_c (or, approximately, β_0) or a large K_2/K_1 would suffice. Indeed, the first requirement here corresponds closely to condition (31*a*) and the second one exactly to (31*d*). Otherwise, we may like to satisfy $K_1\beta_c >> (K_2 + 1)$. Calling the left side of (48) by μ_c and defining, like (43), the quantity

$$\mu_0 = K_1(K_2+1)/(K_1\beta_0 + K_2 + 1)^2. \tag{49}$$

one can test the performance of this criterion. However, we should admit that no strict theoretical basis of (47) exists.

3.4. MM kinetics in absence of QSSA

From (16), we get an exact equation for the rate of product formation. Form (23), along with the validity of the QSSA condition (39), would imply

$$\left| d\gamma / d\tau \right| \approx 0 \tag{50}$$

over a considerable region beyond τ_c. This choice yields

$$R = d\delta / d\tau \approx \gamma_c = K_1\beta_c / (K_1\beta_c + K_2 + 1) \tag{51}$$

by virtue of (18). The rate is thus constant and (51) is the correct form of MM kinetics [17], admitting a linear growth of the product up to a certain range, and is in keeping with our discussion below (30). A different look on this point involves the mass balance equation (8b). At τ_c, it reads as $\beta_0 = \beta_c + \gamma_c + \delta_c$. But, beyond τ_c, balance is provided by first order effects from (28) and (30) up to the range of validity of QSSA. Explicitly, one can write the conservation as

$$\beta_0 = [\beta_c - \gamma_c(\tau - \tau_c)] + \gamma_c + [\delta_c + \gamma_c(\tau - \tau_c)], \tag{52}$$

rendering γ constant at γ_c. Indeed, such a linear decay of β and the concomitant linear rise in δ is the hallmark of the MM kinetics as well as QSSA.

Even when QSSA is not valid, we can still use (51) as

$$R_m = K_1\beta_c / (K_1\beta_c + K_2 + 1). \tag{53}$$

This means, the maximum rate is *always* given by (53). Translated to the parent form, it yields

$$r_m \equiv r_c = r_M s_c / (s_c + k_m),\ r_M = k_2 e_0. \tag{54}$$

Therefore, one needs to note the point τ_c at which rate attains the maximum value. The substrate concentration at this point is measured. Then, (54) offers a way to obtain the reaction constants via the familiar Lineweaver-Burk (LB) plot. The greatest advantage here is that, one *need not care* about QSSA. Hence, MM kinetics may be freed from the domain of validity of QSSA. We shall see the success of this endeavor later.

4. RESULTS AND DISCUSSION

4.1. Concentration profiles and validity of QSSA

The most direct way to check the validity of QSSA is to examine the $\gamma - \tau$ plot. However, one may

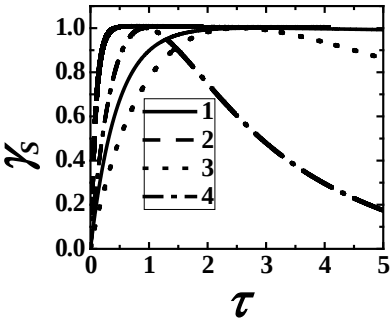

Figure 1. Scaled concentration profiles for the complex (γ_S): Set 9 (1); set 23 (2); Set 56 (3); Set 55 (4). Curve 1 depicts validity of the QSSA, but curves 3 and 4 do not. Curve 2 holds an intermediate position.

need to choose very different scales to detect characteristic changes in β, γ or δ for different sets. So, we have used an additional scaling. In place of a variable *x*, we employ

$$x_S = (x - x_{\min}) / (x_{\max} - x_{\min}) \tag{55}$$

over the range under consideration. This does not affect the qualitative character of a plot, but different sets can be accommodated in the same graph. Figure 1 shows 4 representative plots. Curve 1 is best in respect of satisfaction of the QSSA, curve 4 is worst. Case 3 also does not obey the QSSA and case 2 maintains an intermediate position. Based on such observations, one can classify various sets. The sets already mentioned in the figure correspond to the parameters summarized in Table 1.

Table 1. Behavior of a variety of sets defined by different reaction constants relative to k_2 (k_2 = 1, by choice) and with gradually lower substrate-enzyme ratios. The last column displays validity of QSSA for each set (Y: yes; N: no; I: intermediate), as evident from the concentration profiles of the complex.

Set	s_0	e_0	k_1	k_{-1}	t_c	s_c	c_c	Remark
1	100	1	1/200	10	0.89	99.922	0.0434	Y
2	100	1	1/20	1	1.17	98.561	0.7113	Y
3	80	1	1/20	1	1.28	78.597	0.6627	Y
4	60	1	1/20	1	1.43	58.672	0.5946	Y
5	50	1	1/20	1	1.52	48.736	0.5492	Y
6	30	1	1/20	1	1.73	28.967	0.4200	Y
7	100	5	1/50	10	0.58	98.852	0.7617	Y
8	100	5	1/25	1	1.05	93.828	3.2618	I
9	100	5	1/500	1	2.86	98.468	0.4482	Y
10	20	1	1/10	1/10	1.82	18.427	0.6262	I
11	20	1	1/20	1	1.87	19.172	0.3240	Y
12	10	1	1/10	1/1000	2.20	8.713	0.4653	N
13	10	1	1/100	10	0.85	9.984	0.0090	Y
14	10	1	1/10	1	1.64	9.261	0.3165	I
15	10	1	1/100	1/1000	4.48	9.593	0.0874	Y
16	10	1	1/2	1/10	1.00	8.533	0.7950	N
17	10	1	1/5	1/10	1.59	8.599	0.6099	I
18	10	1	1/100	1/100	4.45	9.598	0.0868	Y
19	10	1	1/100	1/10	4.24	9.639	0.0806	Y
20	10	1	1/10	1/50	2.19	8.729	0.4611	I
21	10	1	1/100	1	2.92	9.837	0.0469	Y
22	10	1	1/100	20	0.51	9.993	0.0047	Y
23	10	1	1	1	0.58	8.783	0.8145	I
24	10	1	1	4/5	0.59	8.750	0.8294	I
25	10	1	1/10	1/500	2.20	8.713	0.4651	N
26	10	1	1/10	1/10	2.12	8.797	0.4444	N
27	10	1	1/20	1	2.02	9.490	0.1918	I
28	10	1	1/10	1/100	2.20	8.720	0.4633	N
29	8	1	1	4/5	0.65	6.773	0.7901	N
30	6	1	1	4/5	0.72	4.833	0.7286	N
31	5	1	1/10	1/10	2.30	4.224	0.2774	I
32	5	1	1/20	1	2.11	4.714	0.1054	I
33	5	1	1	4/5	0.76	3.888	0.6835	N
34	3	1	1	4/5	0.84	2.101	0.5386	N
35	10	5	1/10	1	1.09	7.480	1.3615	N
36	10	6	1/5	1	0.79	6.270	2.3124	N
37	10	8	1/30	2/3	1.49	7.712	1.0696	N

38	1	1	1	4/5	0.89	0.588	0.2462	N
39	1	1	1/100	10	0.85	0.998	0.0009	Y
40	1	1	1/100	20	0.51	0.999	0.0005	Y
41	1	1	1/100	1/1000	4.63	0.955	0.0094	Y
42	1	1	1/100	1/10	4.38	0.961	0.0086	Y
43	1	1	1/10	1/100	2.52	0.789	0.0725	N
44	1	1	1/10	1/10	2.43	0.806	0.0682	N
45	1	1	1	1/2	0.94	0.545	0.2665	N
46	1/2	1	1/10	1/10	2.45	0.400	0.0351	N
47	1/2	1	1	4/5	0.89	0.277	0.1336	N
48	5	10	1/50	1	1.53	4.134	0.3970	N
49	2/5	1	1	4/5	0.89	0.219	0.1086	N
50	1	10	1/1000	1/100	4.63	0.955	0.0094	Y
51	1	10	1/1000	1/10	4.40	0.960	0.0087	Y
52	1	10	1/1000	10	0.85	0.998	0.0009	Y
53	1	10	1/10	4/5	0.89	0.528	0.2851	N
54	1	10	1/100	10	0.64	0.990	0.0090	Y
55	1	10	1/10	1/2	0.93	0.480	0.3099	N
56	1	10	1/100	1/100	2.55	0.778	0.0764	N
57	5	100	1/500	1	1.54	4.106	0.4090	N
58	5	100	1/1000	10	0.64	4.930	0.0448	Y
59	5	100	1/10000	1/10	4.39	4.800	0.0436	Y
60	1	100	1/10000	1	2.99	0.9827	0.0049	Y

Indeed, Table 1 displays in a nutshell all the results of our numerical experiments. The relevant constants in terms of primitive symbols are given. The last column summarizes our observations on the validity of the QSSA [Y: yes; N: no; I: intermediate], based on features of the $\gamma - \tau$ plots. In this respect, we follow the outcomes of Figure 1 and classify the sets. Note that the sets vary widely in terms of the starting concentration ratios of the substrate and the enzyme, and the rate constants. We have maintained $k_2 = 1$ throughout and thus varied really the relative rate constants. This is what actually matters.

The table additionally shows the following general features: (i) It is *not* true that a low t_c and a low c_c are *necessary* for the satisfaction of QSSA, though such a condition may be *sufficient*. A comparison of sets 13 and 15 is worthwhile in this respect. (ii) It is also *not* true that a low value of t_c would imply a low c_c, or *vice-versa*, even when QSSA is valid. Sets 39 – 42 would make the point clear. (iii) A high value of t_c with moderately large c_c may not *invalidate* the QSSA. Sets 15, 18 and 19 acknowledge this fact. (iv) If s_0/e_0 is large, it is *more* usual to find that QSSA is valid, unless $k_{-1} << k_1$. Only in the latter situation, one notices the breakdown. A

number of sets would make the point clear. (v) When e_0/s_0 is large, invalidation of QSSA is commonplace. It is satisfied only if $k_{-1} >> k_1$ is obeyed. (vi) The most complex case concerns the condition $s_0 \approx e_0$. Here, QSSA holds either with very small k_1 and the condition $k_{-1} < k_1$ or with moderate k_1 and the condition $k_{-1} > k_1$. Sets 38 – 45 provide evidence to such rationalizations.

We explore next whether the validity of QSSA has anything to do with the features of the β - τ plots. Figure 2 shows 4 such plots. One may note that curves 1 and 2 do show a faster linear fall-off initially, but the reduction becomes slower soon, though the decrease remains still linear. Let us

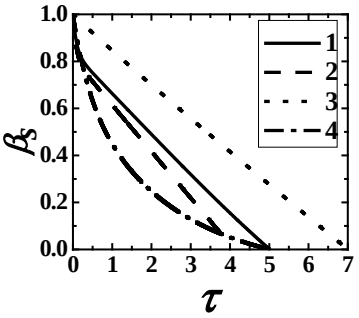

Figure 2. Scaled concentration profiles for the substrate (β_S): Set 58 (1); set 7 (2); Set 59 (3); Set 53 (4). Curves 1, 2 and 3 ensure validity of the QSSA, but curve 4 does not.

note that this is a hallmark if QSSA is valid. Otherwise, a less-than-linear decay is observed, as in case 4. Curve 3 shows, on the contrary, a sharp, linear drop. QSSA is valid in this situation too. Similar plots are found for sets 15, 18, 19, 41, 42, 50 and 51. From Table 1, we detect that all such sets have high t_c, very small K_1 and small K_2. Under such conditions, we see from (26) that

$$d\beta / d\tau = -K_1\beta_0, \tau \to 0, \tag{56}$$

which is valid even for reasonably large times, while (28) gives

$$d\beta / d\tau = -\gamma_c \tag{57}$$

for τ around τ_c and well beyond, as required by the validity of QSSA. However, (18) shows that

$$\gamma_c \approx K_1\beta_c \approx K_1\beta_0. \tag{58}$$

Hence, the two slopes coincide to give a single linear decay curve.

The δ - τ plot similarly contains signature of the validity of QSSA. Figure 3 shows again 4 plots. In accordance with our observations around (29), (30) and (52), we notice that δ starts

with a quadratic rise but soon follows linearity. The linear region is large when QSSA is obeyed. In case QSSA fails to work, the growth rate gets reduced soon to yield a sigmoid profile. Cases 1 and 2 reveal typical linear regimes in support of QSSA; others show how such plots look when QSSA ceases to be obeyed. From an experimentalist's point of view, Figure 2 or 3 can serve as a fingerprint to conclude whether QSSA is valid or invalid for a chosen system, or whether it admits of a borderline behavior.

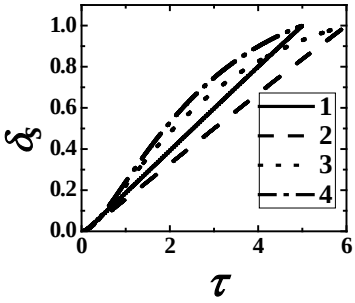

Figure 3. Scaled concentration profiles for the product (δ_S): Set 39 (1); set 52 (2); Set 38 (3); Set 45 (4). Curves 1 and 2 ensure validity of the QSSA, but curves 3 and 4 do not.

An alternative to the individual concentration plots is to go for triangular plots. We note from (8b) that

$$(\beta / \beta_0) + (\gamma / \beta_0) + (\delta / \beta_0) = 1 . \qquad (59)$$

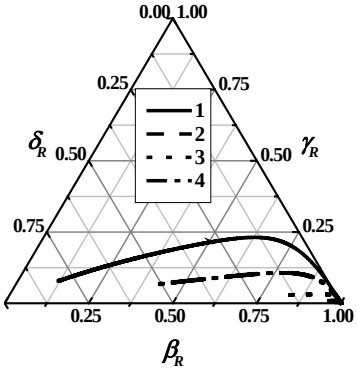

Figure 4. Reduced concentration profiles for substrate (β_R), complex (γ_R) and product (δ_R) in triangular plots: Set 36 (1); set 54 (2); Set 14 (3); Set 37 (4).

Therefore, calling these variables as β_R, γ_R and δ_R, we show the characteristics of situations that approve QSSA. Cases 1 and 4 in Figure 4 show complete breakdown of QSSA, whereas case 2 supports QSSA better than case 3. Indeed, after an initial rise from the right, if the line remains parallel to the β_R axis, we note that QSSA is obeyed in such a case. In the best of cases, however, the plot looks much like a point. There are indeed systems for which such a characteristic is observed and these obey QSSA more securely than case 2 of this figure. Numerical evidence of this sort of betterment is provided by measures λ_c in (42) and μ_c in (48). Form the quoted values of these quantities in Table 5, one can ascertain how good is the QSSA for sets considered in Table 1.

4.2. Role of timescales

The validity of QSSA is often checked through timescales τ_c and τ_s. The first one has a definite experimental basis, but the other one is a purely theoretical construct. So, we put such relations to test. Table 2 shows how the theoretical measure fares for the substrate-excess [s-QSSA] cases. The observed values are ordered and taken from Table 1, but the order breaks down miserably in case of calculated values. Scaling cannot save us in such situations. A similar problem is encountered with r-QSSA measure, as displayed in Table 3. As a result, some prediction based on

Table 2. Calculated and observed values of τ_c for a few sets with substrate in excess.

Set	τ_c (obs)	τ_c (calc*)
34	0.84	0.21
13	0.85	0.09
4	1.43	0.20
37	1.49	0.50
11	1.87	0.33
43	2.52	0.90
21	2.92	0.48
15	4.48	0.91

*See eq. (36)

(35) or (37) will become misleading. This is precisely seen in Table 4. We find that, whereas the adequacy of s-QSSA is rightly guessed by condition (35) with the expressions (36), the same is not true of r-QSSA.

Table 3. Calculated and observed values of τ_c for a few sets with enzyme in excess.

Set	τ_c (obs)	τ_c (calc*)
58	0.64	1
52	0.85	1
55	0.93	1
48	1.53	1
56	2.55	1
60	2.99	1
59	4.39	1
50	4.63	1

*See eq. (38)

Table 4. Prediction of the validity of s-QSSA and r-QSSA on the basis of the ratio τ_s/τ_c. The left column conforms to s-QSSA [see (35)], the right to r-QSSA [see (37)].

Set	τ_s/τ_c		τ_s/τ_c
1	25287.36	56	10
2	979.02	57	5
3	718.56	58	10
4	500.00	59	100
5	405.41	60	100

The right part of Table 4 shows the fate of r-QSSA predictions that come out of (37), aided by (38). To check the inconsistency explicitly, one may compare sets 56, 58, 59 and 60.

4.3. Criteria to Justify QSSA

It was mentioned earlier that the various criteria for the validity of QSSA [*e.g.*, (31), (32) and (34)] do not stand [35] a close scrutiny. We have just seen that the condition based on τ_s/τ_c ratio

Table 5. Estimates of the characteristic constants λ_0 and λ_c [see eqs. (43) and (42)], and μ_0 and μ_c [see eqs. (49) and (48)], for all the sets quoted in Table 1. Low values predict the validity of QSSA.

	λ_0	λ_c	μ_0	μ_c	Set	λ_0	λ_c	μ_0	μ_c
1	0.0024	0.0024	0.0004	0.0004	31	0.0344	0.0361	0.0430	0.0475
2	0.0007	0.0007	0.0020	0.0021	32	0.0222	0.0224	0.0198	0.0200
3	0.0083	0.0084	0.0028	0.0028	33	0.1324	0.1582	0.0389	0.0556
4	0.0100	0.0101	0.0040	0.0041	34	0.1875	0.2307	0.0781	0.1183
5	0.0111	0.0113	0.0049	0.0051	35	0.1667	0.1820	0.1111	0.1324
6	0.0143	0.0145	0.0082	0.0084	36	0.3000	0.3688	0.1500	0.2267
7	0.0423	0.0424	0.0065	0.0065	37	0.1111	0.1155	0.1111	0.1201

8	0.0333	0.0348	0.0111	0.0120	38	0.3214	0.3769	0.2296	0.3156
9	0.0045	0.0046	0.0041	0.0041	39	0.0050	0.0050	0.0009	0.0009
10	0.0177	0.0187	0.0114	0.0127	40	0.0050	0.0050	0.0005	0.0005
11	0.0167	0.0169	0.0111	0.0114	41	0.0050	0.0050	0.0098	0.0098
12	0.0250	0.0267	0.0250	0.0286	42	0.0050	0.0050	0.0089	0.0089
13	0.0050	0.0050	0.0009	0.0009	43	0.0455	0.0464	0.0820	0.0852
14	0.0333	0.0342	0.0222	0.0234	44	0.0458	0.0466	0.0764	0.0789
15	0.0045	0.0046	0.0083	0.0083	45	0.3000	0.3667	0.2400	0.3587
16	0.0451	0.0512	0.0148	0.0191	46	0.0478	0.0482	0.0832	0.0846
17	0.0355	0.0390	0.0229	0.0277	47	0.3913	0.4333	0.3403	0.4173
18	0.0045	0.0046	0.0082	0.0083	48	0.0952	0.0960	0.0907	0.0922
19	0.0046	0.0046	0.0076	0.0077	49	0.4091	0.4458	0.3719	0.4416
20	0.0252	0.0272	0.0250	0.0285	50	0.0050	0.0050	0.0099	0.0099
21	0.0048	0.0048	0.0045	0.0045	51	0.0050	0.0050	0.0091	0.0091
22	0.0050	0.0050	0.0005	0.0005	52	0.0050	0.0050	0.0009	0.0009
23	0.0833	0.0927	0.0139	0.0172	53	0.4737	0.4858	0.4986	0.5243
24	0.0763	0.0853	0.0129	0.0162	54	0.0500	0.0500	0.0091	0.0091
25	0.0250	0.0267	0.0250	0.0286	55	0.4688	0.4845	0.5859	0.6260
26	0.0262	0.0278	0.0249	0.0281	56	0.0495	0.0496	0.0971	0.0975
27	0.0200	0.0202	0.0160	0.0163	57	0.0995	0.0996	0.0990	0.0992
28	0.0251	0.0268	0.0250	0.0285	58	0.0500	0.0500	0.0091	0.0091
29	0.0918	0.1050	0.0187	0.0245	59	0.0050	0.0050	0.0091	0.0091
30	0.1154	0.1357	0.0296	0.0409	60	0.0050	0.0050	0.0050	0.0050

or even τ_c alone does *not* work unambiguously. So, it is now time to test the criteria that emerged from the present work. Specifically, we inquire about the smallness of λ_c in (42) and μ_c in (48). However, these quantities involve β_c that is not known *a priori*. So, we test, in addition, the performance-levels of λ_0 and μ_0. Table 5 presents the results. A glance at it reveals that the QSSA is obeyed if both λ_c and μ_c are less than 0.01. Roughly, the same criteria hold for λ_0 and μ_0. On the other hand, when λ_0, $\mu_0 > 0.1$, one is sure that QSSA will not be obeyed at all. In short, we thus find the following conditions:

$$\text{QSSA: } \lambda_c(\lambda_0), \mu_c(\mu_0) < 0.01$$

$$\text{No QSSA: } \lambda_c(\lambda_0), \mu_c(\mu_0) > 0.1$$

Cases for which $0.1 > \lambda_0$, $\mu_0 > 0.01$ show mostly a borderline behavior. This is how one can rationalize all our observations. Notice that these criteria have succeeded in explaining all our observations displayed in Table 1.

4.4. MM Kinetics and LB Plots

We shall now see the effectiveness of the LB plots corresponding to the MM equations (53) or (54). First, we choose sets 2 – 6, 11, 27 and 32 of Table 1. These sets are such that QSSA is generally obeyed in these cases. Only, the last few sets show intermediate behavior with respect to QSSA. This is also apparent from our criteria, with the values furnished in Table 5. A glance at the parameters of the concerned sets given in Table 1 reveals that the LB equation applied to these sets takes the form

$$1/R_m = 1/r_m = 1 + (k_m / s_c),\ r_M = 1. \qquad (60)$$

Conventional linear plots, however, employ s_0 in place of s_c [see, *e.g.*, (53) and (54) with the associated discussion], as shown in Figure 5(a). The data-set makes it also clear that the slope should turn out to be $k_m = 40$, with unit intercept. Figure 5 shows the plots with two choices, the latter [Figure 5(b)] being ours. While both of them appear linear, calculations show that the deviation from linearity is much larger in case (a). This is reflected in estimations of reaction constants. Since k_2 has been fixed at unity for all the sets, we need to evaluate k_m. A least-squares-fit yields the following results:

Case (a): slope = 42.519; intercept = 0.971;

Case (b): slope = 40.043; intercept = 0.997.

We note happily that our case (b) offers much better values than the conventional plot (a).

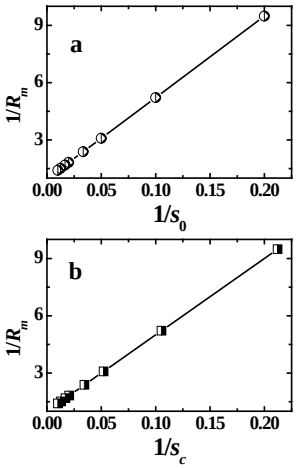

Figure 5. LB plots for the sets 2, 3, 4, 5, 6, 11, 27 and 32 corresponding to choices (a) initial substrate concentration and (b) substrate concentration at t_c. All such sets have the same reaction constants and, except for the last few, they obey QSSA.

The second choice refers to the sets 24, 29, 30, 33, 34, 38, 47 and 49. None of the sets chosen here obey the QSSA. The first few satisfy QSSA intermediately, but most of them violate the same badly. To notice this readily, one may consult Table 5 for values of both λ_c and μ_c of the sets under study in Figures 5 and 6. Normally, for sets under consideration in Figure 6, one *never* goes for LB plots to estimate the reaction constants. But, if we are ready to fit an equation like (60), with k_m = 9/5 (see Table 1 and check that k_m is the same for all these sets), Figure 6 comes into sight. For the same two choices as above, *viz.* s_0 and s_c, one obtains here the results given below:

Case (a): slope = 3.344; intercept = 0.804;

Case (b): slope = 1.800; intercept = 1.000.

Note that here plot (a) shows some visible deviation from linearity at high substrate concentration, though an overall approximate linearity is maintained. But, plot (b) is now relatively much more accurate. The slope and intercept values are almost rightly evaluated from this plot, with little deviations. Thus, the quoted estimates are correct here up to three decimal places. The power of our approach is clear. Hence, the idea of employing MM kinetics results to estimate the reaction constants in conditions defying QSSA is supported beyond any doubt. Such findings may be important in deciphering whether the MM mechanism is really operative in a large variety of cases [34] or not.

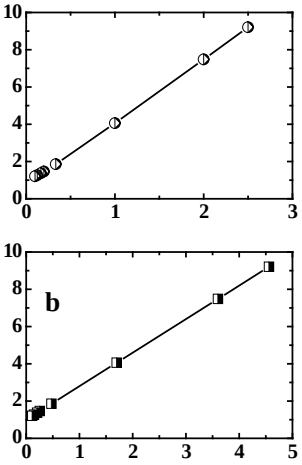

Figure 6. LB plots for the sets 24, 29, 30, 33, 34, 38, 47 and 49 with choices (a) initial substrate concentration and (b) substrate concentration at t_c. All such sets have the same reaction constants and none obeys QSSA.

5. CONCLUSION

To summarize, we have studied here 60 sample cases with widely different reaction constants and substrate-enzyme ratios to check the conditions of validity of the QSSA. Our scheme is simple, but efficient. We have found that a reduced concentration profile of either the substrate [Figure 2] or the product [Figure 3] can also identify whether a given enzyme-substrate system obeys QSSA. This should be particularly useful to experimentalists because more often the concentration profile of the complex is difficult to follow. We have additionally found the theoretical importance of triangular plots [Figure 4] in deciphering a case of QSSA. In view of the limited success [35] of a number of prevalent criteria to check *a priori* the adequacy of QSSA, two new measures [Eqs. (42), (43) and (48), (49)] have been put forward. They emerged from our analytical considerations. We checked thoroughly their efficacy [Table 5] and found them quite satisfactory. Most importantly, we have established that LB plots corresponding to the MM kinetics equations can be wisely employed to find the reaction constants even when QSSA ceases to hold. Figure 6 and the corresponding results establish our assertion beyond doubt.

It may be mentioned that several numerical approaches to study the reaction scheme (1) exist and a recent exposition [47] highlights quite a few earlier works. Certain endeavors [48, 49] consider a catalytic cycle to handle (1). A detailed analysis [48] by casting the relevant equations in terms of a single nonlinear second order differential equation reveals some interesting features of the problem. A subsequent work [49] led to the emergence of an equation for the substrate concentration profile without invoking QSSA. However, the goals of such formulations differ from ours. MM kinetics is also of interest in stochastic simulation studies [50 – 52], electrocatalysis [53], etc. The relevance of QSSA in such a context [54] has drawn attention as well. Finally, numerous quantum-chemical computational studies of specific enzyme-catalyzed reactions have appeared from time to time and an excellent review on such works is available [55]. We hope that the present endeavor may be useful in these backgrounds.

Acknowledgement: SD is thankful to CSIR, India for a research fellowship.

REFERENCES

[1] E. H. Flach, S. Schnell, Use and abuse of the quasi-steady-state approximation, *Syst. Biol. IEE Proc.* **153** (2006) 187-191.

[2] M. G. Pedersen, A. M. Bersani, E. Bersani, G. Cortese, The quasi-steady-state approximation for complex enzyme reactions, *Math. Comput. Simul.* **79** (2008) 1010-1019.

[3] M. G. Pedersen, A. M. Bersani, E. Bersani, Steady-state approximations in intracellular signal transduction – a word of caution, *J. Math. Chem.* **43 (**2008**)** 1318-1344.

[4] S. G. Tsodikova, I. A. Shkel, O. V. Tsodikov, Exact and user-friendly kinetic analysis of a two step rapid equilibrium Michaelis-Menten mechanism, *Anal. Biochem.* **387** (2009) 276-279.

[5] M. G. Pedersen, A. M. Bersani, Introducing total substrates simplifies theoretical analysis at non-negligible enzyme concentrations: pseudo first-order kinetics and the loss of zero-order ultrasensitivity, *J. Math. Biol.* **60** (2010) 267-283.

[6] L. Michaelis, M. L. Menten, Die kinetic der invertinwirkung, *Biochem. Z.* **49** (1913) 333-369.

[7] G. E. Briggs, J. B. S. Haldane, A note on the kinetics of enzyme action, *Biochem. J.* **19** (1925) 338-339.

[8] K. A. Johnson, R. S. Goody, The original Michaelis constant: Translation of the 1913 Michaelis-Menten paper, *Biochem.* **50** (2011) 8264-8269.

[9] F. A. Hommes, Integrated Michaelis-Menten equation, *Arch. Biochem. Biophys.* **96** (1962) 28-31.

[10] J. Bowen, A. Acrivos, A. Oppenheim, Singular perturbation refinement to quasi-steady-state approximation in chemical kinetics, *Chem. Eng. Sci.* **18** (1963) 177-188.

[11] J. T. Wong, On steady-state method of enzyme kinetics, *J. Am. Chem. Soc.* **87** (1965) 1788-1793.

[12] M. M. Stayton, H. J. Fromm, Computer analysis of the validity of integrated Michaelis-Menten equation, *J. Theor. Biol.* **78** (1979) 309-323.

[13] L. A. Segel, On the validity of steady state assumption of enzyme kinetics, *Bull. Math. Biol.* **50** (1988) 579-593.

[14] C. Walter, Quasi steady state in general enzyme system, *J. Theor. Biol*. **11** (1966) 181-206.

[15] K. R. Sanft, D. T. Gillespie, L. R. Petzold, Legitimacy of the stochastic Michaelis-Menten approximation, *IET Syst. Biol.* **5** (2011) 58-69.

[16] A. M. Bersani, G. Dell'Acqua, Is there anything left to say on enzyme kinetic constant and quasi steady state approximation? *J. Math. Chem.* **50** (2012) 335-344.

[17] Z. Bajer, E. Strehler, About and beyond the Henri-Michaelis-Menten rate equation for single substrate enzyme kinetics, *Biochem. Biophys. Res. Comm.* **417** (2012) 982-985.

[18] L. A. Segel, M. Slemrod, The quasi-steady-state assumption: A case study in perturbation, *SIAM Review* **31** (1989) 446-477.

[19] J. A. M. Borghans, R. J. De Boer, L. A. Segel, Extending the quasi steady-state approximation by changing variables, *Bull. Math. Biol*. **58** (1996) 43-63.

[20] S. Schnell, C. Mendoza, Closed form solution for time dependent enzyme kinetics, *J. Theor. Biol.* **189** (1997) 207-212.

[21] S. Schnell, P. K. Maini, Enzyme kinetics at high enzyme concentration, *Bull. Math. Biol.* **62** (2000) 483-499.

[22] A. R. Tzafriri, Michaelis-Menten kinetics at high enzyme concentration, B*ull. Math. Biol.* **65** (2003) 1111-1129.

[23] A. R. Tzafriri, E. R. Elderman, The total quasi-steady-state approximation is valid for reversible enzyme kinetics, *J. Theor. Biol.* **226** (2004) 303-313.

[24] A. R. Tzafriri, E. R. Elderman, Quasi steady-state kinetics at enzyme and substrate concentration in excess of the Michaelis-Menten constant, *J. Theor. Biol.* **245** (2007) 737-748.

[25] F. Kargi, Generalised rate equation for single-substrate enzyme catalysed reactions, *Biochem. Biophys. Res. Comm.* **382** (2009) 157-159.

[26] K. J. Laidler, Theory of the transient phase in kinetics, with special reference to enzyme system, *Can. J. Chem.* **33** (1955) 1614-1624.

[27] S. J. Fraser, Double perturbation series in the differential equations of enzyme kinetics, *J. Chem. Phys.* **109** (1998) 411-423.

[28] G. Dell'Acqua, A. M. Bersani, A perturbation solution of Michaelis–Menten kinetics in a "total" framework, *J. Math. Chem.* **50** (2012) 1136-1148.

[29] J. H. He, Variational iteration method – a kind of non-linear analytical technique: some examples, *Int. J. Nonlin. Mech.* **34** (1999) 699-708.

[30] J. H. He, X. H. Wu, Construction of solitary solution and compacton-like solution by variational iteration method, *Chaos Solitons Fractals* **29** (2006) 108-113.

[31] J. H. He, Variational iteration method — some recent results and new interpretations, *J. Comput. Appl. Math.* **207** (2007) 3-17.

[32] A. Meena, A. Eswari, L. Rajendran, Mathematical modelling of enzyme kinetics reaction mechanism and analytical solutions of non-linear equations, *J. Math. Chem.* **48** (2010) 179-186.

[33] S. M. Hanson, S. Schnell, Reactant stationary approximation in enzyme kinetics, *J. Phys. Chem. A* **112** (2008) 8654-8658.

[34] C. M. Hill, R. D. Waight, W. G. Bardsley, Does any enzyme follow the Michaelis-Menten equation? *Mol. Cell. Biochem.* **15** (1977) 173-178.

[35] S. Dhatt, K. Bhattacharyya, Single-substrate enzyme kinetics: the quasi-steady-state approximation and beyond, *J. Math. Chem.* **51** (2013) 1467-1477.

[36] J. A. C. Bispo, C. F. S. Bonafe, M. G. B. Koblitz, C. G. S. Silva, A. R. de Souza, Substrate and enzyme concentration dependence of the Henri–Michaelis–Menten model probed by numerical simulation, *J. Math. Chem.* **51** (2013) 144-152.

[37] J. D. Murray, *Mathematical Biology*, Springer, New York, 2002.

[38] G. A. Baker, Jr., P. Graves-Morris, *Padé Approximants*, Cambridge Univ. Press, Cambridge, 1996.

[39] C. M. Bender, S. A. Orszag, *Advanced Mathematical Methods for Scientists and Engineers*, Springer, New York, 1999.

[40] K. Bhattacharyya, Asymptotic response of observables from divergent power-series expansions, *Phys. Rev. A* **39** (1989) 6124-6128.

[41] S. Dhatt, K. Bhattacharyya, Embedding scaling relations in Padé approximants: Detours to tame divergent perturbation series, *Int. J. Quantum Chem.* **113** (2013) 916-924.

[42] B. Sarkar, K. Bhattacharyya, On the Padé method for sequence acceleration and calculation of Madelung constants, *Chem. Phys. Lett.* **150** (1988) 419-423.

[43] K. Bhattacharyya, On the temperature dependence of the partition function: Molecular free internal rotation, *Chem. Phys. Lett.* **159** (1989) 40-44.

[44] B. Sarkar, K. Bhattacharyya, Accurate calculation of Coulomb sums: Efficacy of Padé-like methods, *Phys. Rev. B* **48** (1993) 6913-6918.

[45] L. Ouellet, K. J. Laidler, Theory of the transient phase in kinetics, with special reference to enzyme systems: II. The case of two enzyme-substrate complexes, *Can. J. Chem.* **34** (1956) 146-150.

[46] S. Schnell, P. K. Maini, Enzyme kinetics far from the standard quasi-steady-state and equilibrium approximations, *Math. Comput. Model.* **35** (2002) 137-144.

[47] G. Gonzalez-Parra, L. Acedo, A. Arenas, Accuracy of analytical-numerical solutions of the Michaelis-Menten equation, *Comput. Appl. Math.* **30** (2011) 445-461.

[48] M. N. Berberan-Santos, A general treatment of Henri-Michaelis-Menten enzyme kinetics: Exact series solution and approximate analytical solutions, *MATCH Commun. Math. Comput. Chem.* **63** (2010) 283-318.

[49] C. Baleizão, M. N. Berberan-Santos, Enzyme kinetics with a twist, *J. Math. Chem.* **49** (2011) 1949-1960.

[50] E. A. Mastny, E. L. Haseltine, J. B. Rawlings, Two classes of quasi-steady-state model reductions for stochastic kinetics, *J. Chem. Phys.* **127** (2007) 094106 (1-16).

[51] S. MacNamara, A. M. Bersani, K. Burrage, R. B. Sidje, Stochastic chemical kinetics and the total quasi-steady-state assumption: application to the stochastic simulation algorithm and chemical master equation, *J. Chem. Phys.* **129** (2008) 095105 (1-13).

[52] S. Park, N. Agmon, Theory and simulation of diffusion-controlled Michaelis-Menten kinetics for a static enzyme in solution, *J. Phys. Chem. B* **112** (2008) 5977-5987.

[53] M. Puida, A. Malinauskas, F. Ivanauskas, Modeling of electrocatalysis at chemically modified electrodes: a combination of second-order and Michaelis-type chemical kinetics, *J. Math. Chem.* **50** (2012) 2001-2011.

[54] R. Grima, Noise-induced breakdown of the Michaelis-Menten equation in steady-state conditions, *Phys. Rev. Lett.* **102** (2009) 218103 (1-4).

[55] K. E. Ranaghan, A. J. Mulholland, Investigations of enzyme-catalysed reactions with combined quantum mechanics/molecular mechanics (QM/MM) methods, *Int. Rev. Phys. Chem*. **29** (2010) 65-133.