J Math Chem (2013) 51:1467–1477
DOI 10.1007/s10910-013-0160-9

ORIGINAL PAPER

Single-substrate enzyme kinetics: the quasi-steady-state approximation and beyond

Sharmistha Dhatt · Kamal Bhattacharyya

Received: 8 January 2013 / Accepted: 9 March 2013 / Published online: 19 March 2013

Abstract We analyze the standard model of enzyme-catalyzed reactions at various substrate-enzyme ratios by adopting a different scaling scheme and computational procedure. The regions of validity of the quasi-steady-state approximation are noted. Certain prevalent conditions are checked and compared against the actual findings. Efficacies of a few other measures, obtained from the present work, are highlighted. Some recent observations are rationalized, particularly at moderate and high enzyme concentrations.

Keywords Enzyme kinetics · Quasi-steady state approximation · Total QSSA · Padé approximants

1 Introduction

The kinetics of enzyme-catalyzed reactions is usually modeled by a set of coupled differential equations, exact solutions of which are not obtainable in closed forms. However, in view of the importance of such reactions involving biochemical systems, simplifying assumptions are often made. One celebrated result of such endeavors is the standard Michaelis–Menten (MM) form, based on the quasi-steady-state approximation (QSSA). Crucial in this treatment is the assumption that, after a *short* transient, the concentration of the enzyme–substrate complex remains approximately constant. The impact of the MM form is still quite significant [1].

S. Dhatt · K. Bhattacharyya (✉)
Department of Chemistry, University of Calcutta, Kolkata 700 009, India
e-mail: pchemkb@yahoo.com; pohemkb@gmail.com

The two-step model is symbolized by the reaction scheme

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

On the basis of (1), various aspects of the QSSA have been studied from time to time. An early study [2] revealed that *any* of the following four conditions is necessary:

$$\begin{aligned} &(a)\ s_0/e_0 >> 1; \\ &(b)\ s_0/e_0 << 1; \\ &(c)\ k_1 s_0/(k_{-1} + k_2) << 1; \\ &(d)\ k_1 e_0/(k_{-1} + k_2) << 1. \end{aligned} \tag{2}$$

In (2), s_0 and e_0 refer to the initial concentrations of substrate and enzyme, respectively. Later, Laidler et al. [3] also noted how the product profile gives a clear signature of the validity of QSSA. Most authors [4–11], however, opine in favor of condition (2*a*) only. A somewhat different condition for the validity of QSSA [12,13] reads as

$$e_0/(s_0 + K_m) << 1, \tag{3}$$

where K_m is the Michaelis constant, defined by

$$K_m = (k_{-1} + k_2)/k_1. \tag{4}$$

Borghans et al. [14] distinguished different types of QSSA as standard QSSA (s-QSSA), reverse QSSA (r-QSSA) and total QSSA (t-QSSA), depending on whether the ratio s_0/e_0 is large, small or arbitrary. Several works [15–17] then concentrated on moderate to high enzyme–substrate ratios. Tzafriri et al. [17] remarked that in case of large e_0/s_0, (3) should be modified as

$$s_0/(e_0 + K_m) << 1. \tag{5}$$

Analyses over a wide range of the ratio s_0/e_0 were pursued in several recent theoretical works [18–21] with interesting experimental relevance [22]. A route to calculate rate constants also followed [23].

A few problems, however, remained unsolved. Thus, following an earlier work [17], Kargi [20] mainly focused attention on variable s_0/e_0 ratio to conclude again that t-QSSA can be implemented in *any* situation. A very recent work [21], on the other hand, maintained that QSSA is valid, if at all, only for a *short* time-interval when e_0/s_0 is *large* and that the region of validity is considerably larger for large s_0/e_0.

In view of the above remarks, our purpose is to explore whether (i) QSSA is valid *irrespective* of the value of the ratio s_0/e_0, (ii) the steady-state region, if exists, is *smaller* for large e_0/s_0 ratio, (iii) the initial transient period is *small* when QSSA holds, (iv) the set of conditions (2), or (3) or (5) works sensibly in *predicting* the legitimacy of the QSSA, and (v) *better* measures of the applicability of QSSA exist.

Answers to such questions can hopefully settle the issue of applicability of QSSA for moderate-to-large ratio of e_0/s_0 [14–20].

The primary reason behind the emergence of varied criteria in the present context lies in different forms of attack to the problem with diverse scaled variables [24]. The basic issue, however, can be resolved by invoking a straightforward strategy. For example, the standard power-series method with Padé approximants (PA) [25] and a very different scaling scheme can handle the problem quite efficiently, as may be seen below.

2 The method

On the basis of (1), the following differential equations emerge:

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES], \tag{6}$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_2[ES], \tag{7}$$

$$\frac{d[E]}{dt} = -k_1[E][S] + k_{-1}[ES] + k_2[ES], \tag{8}$$

$$\frac{d[P]}{dt} = k_2[ES]. \tag{9}$$

In addition, we have two mass conservation equations

$$\begin{aligned} e_0 &= [E] + [ES], \\ s_0 &= [S] + [ES] + [P]. \end{aligned} \tag{10}$$

To solve the above equations, we employ the following set of new dimensionless variables:

$$\alpha = \frac{[E]}{e_0}, \quad \beta = \frac{[S]}{e_0}, \quad \gamma = \frac{[ES]}{e_0}, \quad \delta = \frac{[P]}{e_0}, \quad \tau = k_2 t. \tag{11}$$

Then, the principal kinetic equations, out of (6–9), may be compactly written as

$$\frac{d\alpha}{d\tau} = \frac{d\beta}{d\tau} + (1-\alpha), \tag{12}$$

$$\frac{d\beta}{d\tau} = -K_1\beta\alpha + K_2(1-\alpha), \tag{13}$$

with initial conditions

$$\alpha_0 = 1, \ \beta_0 = s_0/e_0. \tag{14}$$

The constants K_1 and K_2 in (13) are given by

$$K_1 = k_1 e_0/k_2, \quad K_2 = k_{-1}/k_2. \tag{15}$$

The conservation equations (10) read now as

$$\alpha + \gamma = 1, \quad \beta + \gamma + \delta = \beta_0. \tag{16}$$

The above system of non-linear equations (12, 13), with the aid of (16), can be solved analytically using the traditional power series method. Expressing the concentrations of the participating species in power series of τ, *viz.*,

$$\alpha_\tau = \sum_{j=0} \alpha_j \tau^j, \quad \beta_\tau = \sum_{j=0} \beta_j \tau^j, \quad \gamma_\tau = \sum_{j=0} \gamma_j \tau^j, \quad \delta_\tau = \sum_{j=0} \delta_j \tau^j, \tag{17}$$

inserting them suitably into (12) and (13), and collecting similar powers of τ, the unknown parameters of the expansions are obtained. Note that our scaling is very different from others [15,24]. Moreover, to tackle the expansions in (17) at large τ, we construct three types of PA, $[N/N]$, $[(N+1)/N]$ and $[N/(N+1)]$, as found useful in a variety of contexts (see, e.g. [26–28] and refs. quoted therein).

Numerical stability of our computations is checked via two routes. First, the estimates are considered reliable when all three varieties of the above Padé sequences agree. Secondly, one finds from (12), (13) and (16) that

$$d\gamma/d\tau = K_1\beta - (K_1\beta + K_2 + 1)\gamma. \tag{18}$$

Therefore, the point τ_c at which γ attains its maximum value (γ_c) is obtainable from (18) as

$$\gamma_c = K_1\beta_c/(K_1\beta_c + K_2 + 1). \tag{19}$$

Hence, from the computed temporal profiles of γ and β, we verify that (19) is obeyed. Indeed, one can thus go well beyond the region of adequacy of QSSA, and hence can assess the quality of the steady state, if there is any.

3 Legitimacy of QSSA

Theoretically, QSSA is best identified by noting the $\gamma - \tau$ plot. Usually, there is a sharp rise to a maximum within the transient phase, followed by a steady phase that may or may not last over a longer time scale. Equation (18) shows

$$\begin{aligned}(d\gamma/d\tau)_{\tau_2} - (d\gamma/d\tau)_{\tau_1} = {} & K_1\left(\beta_{\tau_2} - \beta_{\tau_1}\right) - K_1\left(\beta_{\tau_2}\gamma_{\tau_2} - \beta_{\tau_1}\gamma_{\tau_1}\right) \\ & -(K_2+1)(\gamma_{\tau_2} - \gamma_{\tau_1}).\end{aligned} \tag{20}$$

For an observable region over which QSSA will be valid, one needs to consider $\tau_2 > \tau_1 > \tau_c$. It is also apparent that, if such a region exists up to τ_2, one would have

$$(d\gamma/d\tau)_{\tau_2} \approx (d\gamma/d\tau)_{\tau_1} \approx 0; \quad \gamma_{\tau_2} \approx \gamma_{\tau_1}. \tag{21}$$

Condition (21) is satisfied by (20) only if

$$K_1 << 1. \tag{22}$$

Thus, (22) turns out to be one necessary condition.

Another deductive analysis arises out of the observation that β_τ shows a linear fall-off beyond $\tau = \tau_c$ up to the range of validity of QSSA. Thus, we can write

$$\beta_\tau = \beta_c + \bar{\beta}(\tau - \tau_c), \quad \tau > \tau_c. \tag{23}$$

From (12), however, one notes that

$$(d\beta/d\tau)_{\tau=\tau_c} = -\gamma_c. \tag{24}$$

Therefore, one obtains from (23) and (24) that

$$\bar{\beta} = -\gamma_c. \tag{25}$$

We now pay attention to (13) and write

$$-\gamma_c \approx -K_1\beta_\tau(1 - \gamma_\tau) + K_2\gamma_\tau. \tag{26}$$

Putting (23) and (25) in (26), a rearrangement leads to

$$\gamma_\tau \approx \gamma_c + (\gamma_c - 1)\,\frac{K_1\gamma_c}{K_1\beta_c + K_2}(\tau - \tau_c), \tag{27}$$

correct up to first order in $(\tau - \tau_c)$. Now, if γ has to show a maximum at $\tau = \tau_c$, then we expect no first-order term in the expansion (27). In other words, we have the condition for QSSA

$$|K_1\gamma_c/(K_1\beta_c + K_2)| << 1. \tag{28}$$

Since β_c or γ_c can vary arbitrarily, a sufficient condition for the satisfaction of (28) is

$$K_2/K_1 >> 1. \tag{29}$$

However, (29) may be relaxed when (28) holds by virtue of very small γ_c or large β_c.

In effect, therefore, we have arrived at two conditions here for the validity of QSSA. The first one is (22) and the second one is (28); both are necessary conditions, but (29) is a sufficient one to validate (28).

Table 1 Characteristics of sets with varying ratios of s_0/e_0 and K_2/K_1 and at several K_1 values

s_0, e_0	τ_c	(k_1, k_{-1}, k_2)	Conditions						K_1	K_2/K_1	Observed
			2(a)	2(b)	2(c)	2(d)	3	5			
10, 1	0.9	(0.05, 0.05, 1.0)	Y	N	N	Y	Y	–	0.05	1.0	No QSSA
	0.6	(0.1, 10.0, 1.0)	Y	N	Y	Y	Y	–	0.1	100.0	QSSA
1, 1	0.9	(1.0, 0.8, 1.0)	N	N	N	N	N	–	1.0	0.8	No QSSA
	0.9	(0.01, 10.0, 1.0)	N	N	Y	Y	Y	–	0.01	1000.0	QSSA
1, 10	1.0	(0.1, 0.1, 1.0)	N	Y	Y	N	N	Y	1.0	0.1	No QSSA
	4.4	(0.001, 0.1, 1.0)	N	Y	Y	Y	Y	Y	0.01	10.0	QSSA

The observations correspond to Figs. 1, 2 and 3

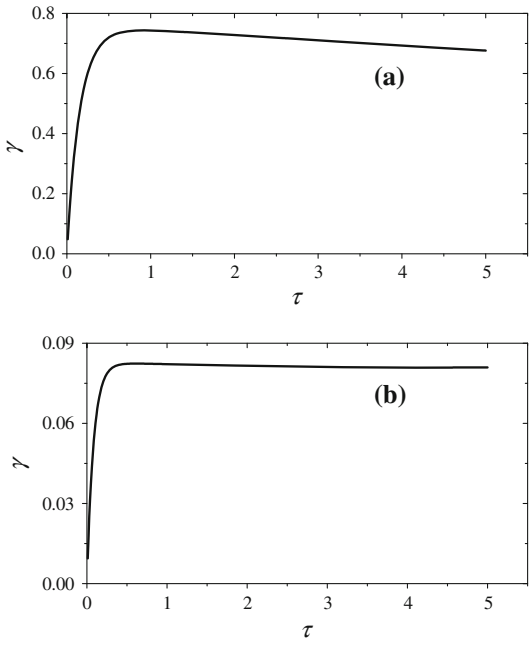

Fig. 1 Plots of the scaled complex concentration γ as a function of scaled time τ at $s_0/e_0 = 10$. Note that only a small value of K_1 does not guarantee QSSA and that a small γ_c is not mandatory

4 Results and discussion

We now examine the adequacy of our analysis. Table 1 shows a total of six sample results studied via our method. For a fixed ratio of s_0/e_0, two distinct situations are shown; the first entries do not conform to QSSA, while the second ones do. The corresponding $\gamma - \tau$ plots are shown in Figs. 1, 2 and 3 with cases (a) referring to sets where QSSA is not obeyed, and cases (b) in tune with QSSA. Particularly interesting here is Fig. 3, with $K_2/K_1 = 10$ only. Had this value been larger, one would find a still longer steady region. Indeed, cases (b) of Figs. 2 and 3 support a few earlier works [18–20] beyond doubt.

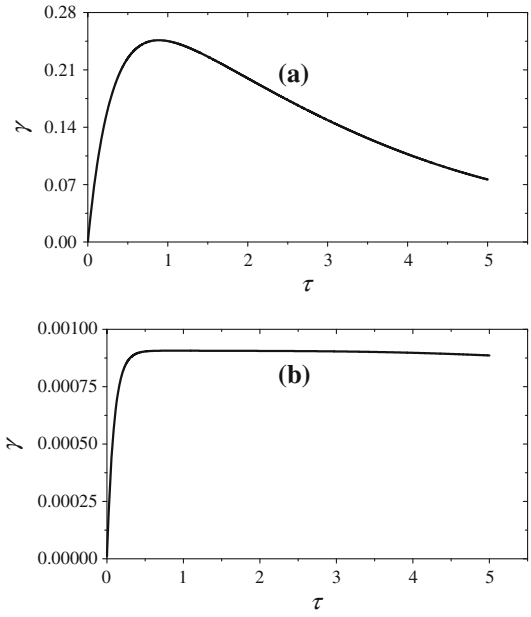

Fig. 2 Plots of the scaled complex concentration γ as a function of scaled time τ at $s_0/e_0 = 1$. Note that a small τ_c is never an indicator of QSSA

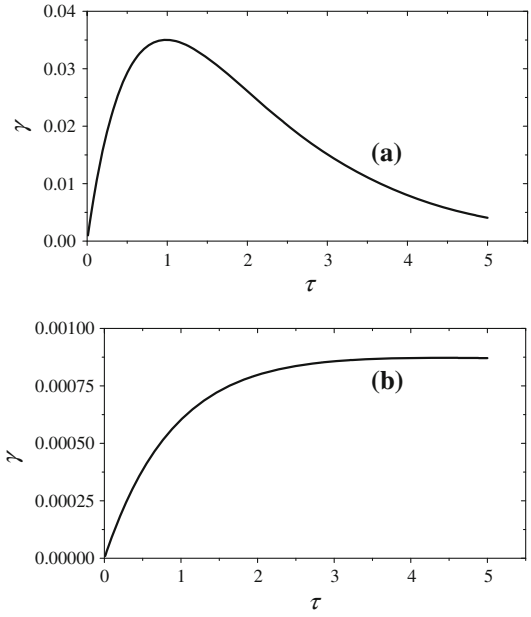

Fig. 3 Plots of the scaled complex concentration γ as a function of scaled time τ at $s_0/e_0 = 1/10$. Note again that a small τ_c cannot ensure QSSA. Also, here γ_c is small, but τ_c is large. This is opposite to the findings of Fig. 1

The figures reveal, in addition, that a small value of neither γ_c nor τ_c guarantees t-QSSA. Moreover, these two quantities do not run parallel in the sense that a small γ_c never ensures that τ_c would be small.

Table 2 Predictions based on (22) and (29) for sets chosen in ref. [21] with varying ratios of s_0/e_0 and K_2/K_1 and at several K_1 values

s_0, e_0	(k_1, k_{-1}, k_2)	Conditions						K_1	K_2/K_1	Expected
		2(a)	2(b)	2(c)	2(d)	3	5			
10, 1	(0.1, 1, 100)	Y	N	Y	Y	Y	–	0.001	10.0	QSSA
	(0.1, 100, 1)	Y	N	Y	Y	Y	–	0.1	1,000.0	QSSA
	(1, 0.1, 100)	Y	N	Y	Y	Y	–	0.01	0.1	QSSA
	(1, 100, 0.1)	Y	N	Y	Y	Y	–	10.0	100.0	No QSSA
	(100, 0.1, 1)	Y	N	N	N	Y	–	100.0	0.001	No QSSA
	(100, 1, 0.1)	Y	N	N	N	Y	–	1,000.0	0.01	No QSSA
	(1, 1, 1)	Y	N	N	N	Y	–	1.0	1.0	Borderline
1, 1	(0.1, 1, 100)	N	N	Y	Y	Y	–	0.001	10.0	QSSA
	(0.1, 100, 1)	N	N	Y	Y	Y	–	0.1	1,000.0	QSSA
	(1, 0.1, 100)	N	N	Y	Y	Y	–	0.01	0.1	QSSA
	(1, 100, 0.1)	N	N	Y	Y	Y	–	10.0	100.0	No QSSA
	(100, 0.1, 1)	N	N	N	N	N	–	100.0	0.001	No QSSA
	(100, 1, 0.1)	N	N	N	N	N	–	1,000.0	0.01	No QSSA
	(1, 1, 1)	N	N	N	N	N	–	1.0	1.0	No QSSA
1, 10	(0.1, 1, 100)	N	Y	Y	Y	Y	Y	0.01	1.0	QSSA
	(0.1, 100, 1)	N	Y	Y	Y	Y	Y	1.0	100.0	No QSSA
	(1, 0.1, 100)	N	Y	Y	Y	Y	Y	0.1	0.01	Borderline
	(1, 100, 0.1)	N	Y	Y	Y	Y	Y	100.0	10.0	No QSSA
	(100, 0.1, 1)	N	Y	N	N	N	Y	1,000.0	0.0001	No QSSA
	(100, 1, 0.1)	N	Y	N	N	N	Y	10,000.0	0.001	No QSSA
	(1, 1, 1)	N	Y	N	N	N	Y	10.0	0.1	No QSSA

The sets are defined by (k_1, k_{-1}, k_2) [21]

Table 1 displays also the various criteria used by several authors from time to time (see text). To test, we have fixed here a standard. If a quantity 'q' is said to obey $q << 1$, we accept a value of 1/10 or lower. Similarly, we allow $q \geq 10$ to imply $q >> 1$. In Table 1, satisfaction of a condition is reported by 'yes/no' type response. Since condition (5) is said to work only for large e_0, we show its worth only at appropriate places. But, no single condition has come up as a right criterion. Even, one cannot go by majority. As an alternative, if we stick to our criteria of low K_1 and high K_2/K_1, the observations quoted in the table can all be rationalized.

In Table 2, we predict definitive fates of the diverse sets chosen in a very recent study [21]. Note that, in the excess enzyme case, it would be unwise to employ the last set to observe a steady state [21]. Better, one should have chosen a different set of values for (k_1, k_{-1}, k_2) like (0.1, 10, 10), or still better (0.1, 100, 100). One is also not sure about the adequacy of QSSA for the last set in the $s_0/e_0 = 10$ case either, as reported [21]. Only, a large β_c in (28) can somehow favor the situation.

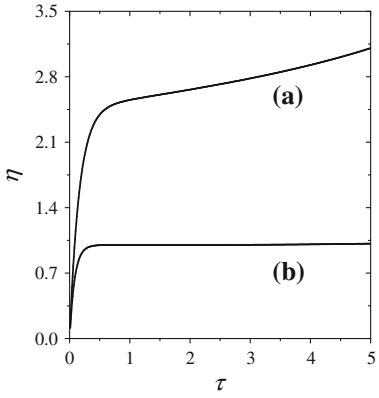

Fig. 4 Temporal plots of η [see (32)] at $s_0/e_0 = 10$ for the cases *a* and *b*; QSSA is obeyed only in the latter case

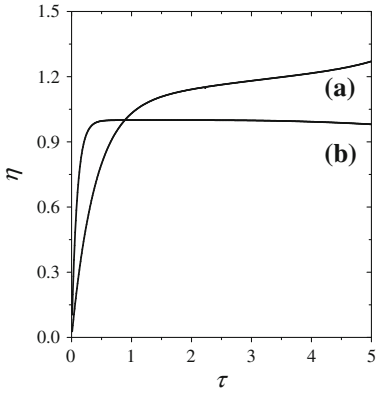

Fig. 5 Temporal plots of η [see (32)] at $s_0/e_0 = 1$ for the cases *a* and *b*; QSSA is obeyed only in the latter case

Conventionally, the MM kinetics is given by

$$d\delta/d\tau = K_1\beta/(K_1\beta + K_2 + 1) \tag{30}$$

and, if QSSA is valid, we find that

$$d\delta/d\tau = \gamma_c. \tag{31}$$

Therefore, satisfaction of the condition

$$\eta = \gamma_c(K_1\beta + K_2 + 1)/K_1\beta = 1 \tag{32}$$

would ensure the validity of QSSA in justifying the MM form. We show in Figs. 4, 5 and 6 how far (32) is obeyed for the cases described in Table 1. They reveal, one can confide on our analysis.

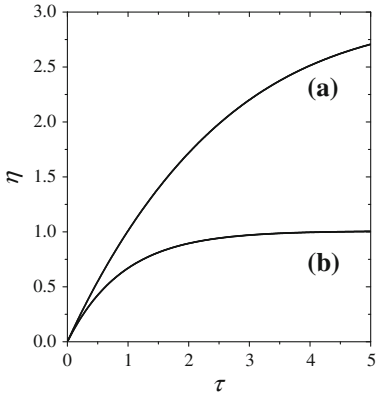

Fig. 6 Temporal plots of η [see (32)] at $e_0/s_0 = 10$ for the cases *a* and *b*; QSSA is obeyed only in the latter case

5 Conclusion

In brief, we have thus found that QSSA (i) can be acceptable at both moderate and large e_0/s_0 under conditions similar to those applicable to large s_0/e_0, (ii) can hold over a good span of time at large K_2/K_1, (iii) can show up even when γ_c or τ_c is not small enough, and (iv) requires validity of conditions (22) and (28). We have also settled the issue of the adequacy of t-QSSA [18–20] for large e_0/s_0 versus its alleged inadequacy [9,10,21]. Thus, MM kinetics *can* hold *irrespective* of the enzyme–substrate ratio, provided *proper* rate constants are chosen. In fine, our work examines the kinetic equation without any restriction on the enzyme–substrate ratio to encompass both classical in-vitro as well as biotechnologically-pro in-vivo situations.

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

References

1. K.A. Johnson, R.S. Goody, Biochemistry **50**, 8264 (2011)
2. K.J. Laidler, Can. J. Chem. **33**, 1614 (1955)
3. L. Ouellet, K.J. Laidler, Can. J. Chem. **34**, 146 (1956)
4. F.A. Hommes, Arch. Biochem. Biophys. **96**, 28 (1962)
5. J. Bowen, A. Acrivos, A. Oppenheim, Chem. Eng. Sci. **18**, 177 (1963)
6. J.T. Wong, J. Am. Chem. Soc. **87**, 1788 (1965)
7. M.M. Stayton, H.J. Fromm, J. Theor. Biol. **78**, 309 (1979)
8. L.A. Segel, Bull. Math. Biol **50**, 579 (1988)
9. S.M. Hanson, S. Schnell, J. Phys. Chem. A **112**, 8654 (2008)
10. K.R. Sanft, D.T. Gillespie, L.R. Petzold, IET Syst. Biol. **5**, 58 (2011)
11. A.M. Bersani, G. Dell'Acqua, J. Math. Chem. **50**, 335 (2012)
12. L.A. Segel, M. Slemrod, SIAM Rev. **31**, 446 (1989)
13. S. Schnell, C. Mendoza, J. Theor. Biol. **189**, 207 (1997)
14. J.A.M. Borghans, R.J. De Boer, L.A. Segel, Bull. Math. Biol. **58**, 43 (1996)
15. S. Schnell, P.K. Maini, Bull. Math. Biol. **2**, 483 (2000)
16. A.R. Tzafriri, Bull. Math. Biol. **65**, 1111 (2003)
17. A.R. Tzafriri, E.R. Elderman, J. Theor. Biol. **226**, 303 (2004)
18. A.R. Tzafriri, E.R. Elderman, J. Theor. Biol. **245**, 737 (2007)
19. M.G. Pedersen, A.M. Bersani, E. Bersani, G. Cortese, MATCOM **79**, 1010 (2008)

20. F. Kargi, Biochem. Biophys. Res. Commun. **382**, 57 (2009)
21. Z. Bajer, E. Strehler, Biochem. Biophys. Res. Commun. **417**, 982 (2012)
22. S.G. Tsodikova, I.A. Shkel, O.V. Tsodikov, Anal. Biochem. **387**, 276 (2009)
23. B. Li, B. Li, Y. Shen, J. Math. Chem. **50**, 752 (2012)
24. J.D. Murray, *Mathematical Biology* (Springer, New York, 2002)
25. G.A. Baker Jr, P. Graves-Morris, *Padé Approximants* (Cambridge University Press, Cambridge, 1996)
26. K. Bhattacharyya, Phys. Rev. A **39**, 6124 (1989)
27. F.M. Fern'andez, J. Phys. A: Math. Gen. **39**, 1683 (2006)
28. S. Dhatt, K. Bhattacharyya, Int. J. Quantum Chem. **113**, 916 (2013)