# The unreasonable effectiveness of the total quasi-steady state approximation, and its limitations

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**Abstract**

In this short note, we investigate the applicability and clarify the role of the total quasi-steady-state approximation for the Michaelis–Menten reaction mechanism. We show that small initial reduced substrate concentration, $s_0/K_M \to 0$, is the crucial criterion for validity.

**Keywords:** Michaelis–Menten reaction, Quasi-steady-state approximation, validity, eigenvalues, singular perturbation, timescales

## 1 Introduction

Enzyme assays serve multiple objectives. For catalytic reactions with established reaction mechanisms, these assays aim to assess the enzyme's catalytic efficiency toward a substrate and to quantify the physical constants that define the catalytic process.

Within the realm of quantitative reaction analysis, unwavering rigor, precision, and accuracy in measuring these physical constants are essential. Achieving this necessitates the development of mathematical approximations for the rate laws that govern the reaction mechanisms. Furthermore, a profound comprehension of the experimental conditions that validate such approximations is indispensable [5].

The irreversible single-substrate, single-enzyme Michaelis–Menten reaction mechanism

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C \xrightarrow{k_2} E + P \tag{1}$$

has been extensively explored. Numerous approximations for the rate law governing substrate depletion or product formation have been derived. On certain occasions, these approximations have been accompanied by stipulations that outline the conditions under which they are valid.

The ordinary differential equations that describe the time courses of the substrate, $s$, enzyme, $e$, and substrate-enzyme intermediate complex, $c$, concentrations are

$$\begin{aligned} \dot{s} &= -k_1 e_0 s + (k_1 s + k_{-1})c \\ \dot{c} &= k_1 e_0 s - (k_1 s + k_{-1} + k_2)c \end{aligned} \tag{2}$$

with parameters $e_0, s_0, k_1, k_{-1}, k_2$.[1]

The standard quasi-steady state assumption (sQSSA) for the Michaelis–Menten reaction mechanism assumes that the fast chemical species, the complex, is in quasi-steady state (QSS) with respect to the slow chemical species, the substrate. From this, one derives the familiar Michaelis–Menten equation

$$\dot{s} = -\frac{k_1 k_2 e_0 s}{k_{-1} + k_2 + k_1 s} = -\frac{k_2 e_0 s}{K_\mathrm{M} + s} \tag{3}$$

with the Michaelis constant $K_\mathrm{M} = (k_{-1} + k_2)/k_1$. This reduction is based on the assumption of low initial reduced enzyme concentration, $e_0/K_M \to 0$ [2].

Moreover, in case of initial reduced substrate concentration $s_0/K_M$ being sufficiently small, one may further replace this by the linear approximation

$$\dot{s} = -\frac{k_2 e_0 s}{K_\mathrm{M}}. \tag{4}$$

This linear approximation is of critical importance to estimate a fundamental kinetic parameter, $k_2/K_\mathrm{M}$: the apparent second-order rate constant for the enzyme-catalyzed reaction. It is also known as specificity constant, because it determines the catalytic efficiency (or specificity) of the enzyme for each substrate. The higher the value of $k_2/K_\mathrm{M}$ the more specific the enzyme is for a given substrate.

In recent work Kim & Tyson [3], and Vu et al. [7, Eq. (8)], keeping the assumption of small $s_0/K_M$, but putting no restriction on initial enzyme $e_0$, proposed to replace

[1] $k_1$, $k_2$ and $k_{-1}$ are rate constants, $s_0$ is the initial substrate concentration, and $e_0$ is the initial enzyme concentrations.

this equation by

$$\dot{s} = -\frac{k_2 e_0 s}{K_\mathrm{M} + e_0}, \tag{5}$$

claiming a universal range of validity for this reduction. Their argument is based on the total quasi-steady-state assumption (tQSSA), as first proposed by Borghans et al. [1] and followed up by Tzafriri [6], and relies on a series of heuristics used in these papers.

From the physico-chemical point of view, there is a challenge with the linearization (5). The specificity constant, $k_2/(K_\mathrm{M} + e_0)$, exhibits a fundamental distinction from the standard case due to its reliance on the initial enzyme concentration. This noteworthy aspect has not yet been observed in experimental settings.

This prompts intriguing questions. Is there a contradiction between the sQSSA and tQSSA? If there is no contradiction, does this mean that the tQSSA is not univesally valid as claimed in the literature? Is there an alternative facet of the specificity constant under the tQSSA framework?

The main purpose of the present note is to provide a solid mathematical foundation for the approximation (5), given small initial substrate concentration, and also to clarify its limitations. We will use some results and arguments from [2] to set a solid foundation to the tQSSA.

## 2 Conditions to justify the quasi-steady state assumptions

To address the above questions, we need to reconsider the conditions for the validity of the quasi-steady state reductions. These reductions are characterized by distinguished parameters which guarantee (or are supposed to guarantee) their validity.

For the case of the sQSSA, Segel and Slemrod [4] obtained the dimensionless parameter

$$\varepsilon_{SSl} := \frac{e_0}{K_\mathrm{M} + s_0} \tag{6}$$

from partly heuristical timescale arguments, to ensure sQSS for complex concentration $c$. They proved the existence of a singular perturbation reduction as $\varepsilon_{SSl} \to 0$, assuming some upper bound on $s_0$.

Extending the timescale arguments introduced in [4], and using some heuristics for estimates, Borghans et al. [1] established the small parameter

$$\varepsilon_{BdBS} := \frac{K e_0}{(K_\mathrm{M} + e_0 + s_0)^2} \tag{7}$$

to ascertain tQSS, where $K = k_2/k_1$ is the Van Slyke-Cullen constant.

From a different approach taken in [2], we have the parameter

$$\varepsilon_{EVR} := \frac{K e_0}{(K_\mathrm{M} + e_0)^2} \tag{8}$$

which measures the eigenvalue ratio of the linearizaton at the stationary point (see, for details, [2, Proposition 1 and Remark 2]). Note that $\varepsilon_{BdBS}$ tends to $\varepsilon_{EVR}$ as $s_0/K_M \to 0$. As evident from the analysis in [2], one can therefore argue that tQSSA is not universally valid, as claimed by some, but valid in the limiting case of low initial reduced substrate concentration. Note that this assumption on reduced substrate forms the foundation of Kim & Tyson [3] and Vu et al. [7].

We will show that low initial reduced substrate concentration is the decisive feature to explain the apparent "unreasonable effectiveness" (adopting a well known phrase by Wigner [8] from a different context) of the tQSSA application. Thus, actually $\varepsilon_{EVR}$ is the relevant parameter, rather than $\varepsilon_{BdBS}$. We will arrive at this conclusion by a careful analysis of the linearization of (2) at the stationary point.

# 3 The linearization of the reaction dynamics and tQSSA

Consider the linearization of the governing equation for the Michaelis–Menten reaction mechanism (1) at the stationary point 0

$$\begin{aligned} \dot{s} &= -k_1 e_0 s + k_{-1} c \\ \dot{c} &= k_1 e_0 s - (k_{-1} + k_2) c. \end{aligned} \tag{9}$$

Looking at this linearization is justified since Vu et al. [7] are interested in small substrate concentrations only[2]. The eigenvalues of the matrix in (9) are

$$\lambda_{1,2} = k_1 \frac{K_M + e_0}{2} \cdot \left( -1 \pm \sqrt{1 - \frac{4 K e_0}{(K_M + e_0)^2}} \right),$$

with $\lambda_1$ of smaller absolute value. With the corresponding eigenvectors $v_{1,2}$, every solution of the differential equation has the form

$$\begin{pmatrix} s \\ c \end{pmatrix} = \gamma_1 \exp(\lambda_1 t) v_1 + \gamma_2 \exp(\lambda_2 t) v_2,$$

with constants $\gamma_{1,2}$ determined by the initial values. Now when $|\lambda_1/\lambda_2| \ll 1$ (equivalently $\varepsilon_{EVR} \ll 1$), then the second term in the above sum will vanish quickly, and after a short initial phase we have a good approximation by

$$\begin{pmatrix} s \\ c \end{pmatrix} \approx \gamma_1 \exp(\lambda_1 t) v_1.$$

Loosely speaking, the dominating timescale for the degradation of any species (regardless of the initial conditions) is $|\lambda_1|^{-1}$, given the above condition on $\varepsilon_{EVR}$. In

[2] A more complete justification will be presented in forthcoming work.

particular, it is acceptable to go with a reduced equation in the linear regime

$$\dot{s} = \lambda_1 s, \tag{10}$$

with initial value to be determined.

The remaining (seeming) discrepancy between (10) and (5) will be taken care of right now. Given small $\varepsilon_{EVR}$, one has

$$\sqrt{1 - \frac{4Ke_0}{(K_M + e_0)^2}} \approx 1 - \frac{2Ke_0}{(K_M + e_0)^2}$$

by standard Taylor approximation, which implies

$$\lambda_1 \approx -\frac{k_2 e_0}{K_M + e_0},$$

and thus we have agreement with (5).

# 4 Discussion

In the mathematical enzymology community, it is generally accepted that the tQSSA is universally valid [6, for an example]. The validity has been explored through heuristic approaches and numerical simulations. In recent work, it appears that Vu et al. [7] do not explicitly address the necessity of small $\varepsilon_{EVR}$ for the validity of the tQSSA. They might be presuming that this condition is universally fulfilled, or perhaps they noted by inspection of [2, especially Figure 2] that the condition holds true for the most of parameters (in a well-defined sense). This explains the "unreasonable effectiveness" of the application of tQSSA in the literature.

In this note, we provide solid foundation for the tQSSA approximation (5), and show its validity as $s_0/K_M \to 0$. Interestingly, it is easy to see that there is no contradiction between the tQSSA approximation (5) and the sQSSA approximation (4) as (5) tends (4) for $e_0 \to 0$. However, the tQSSA predicts a specificity constant dependent on the initial enzyme concentration at high enzyme concentrations. This is an interesting theoretical prediction, which should be explored experimentally.

We could have taken a more general approach, without a presumption on small $\varepsilon_{EVR}$. Using the generally valid inequalities

$$1 - \rho \leq \sqrt{1 - \rho} \leq 1 - \rho/2 \quad (0 \leq \rho \leq 1),$$

we obtain that

$$-2\frac{k_2 e_0}{K_M + e_0} \leq \lambda_1 \leq -\frac{k_2 e_0}{K_M + e_0},$$

and thus may work with differential inequalities rather than with (5). Thus one can estimate the timescale within a factor of 2. Unfortunately, this would be of little value when the eigenvalue ratio is close to 1 (i.e. $\varepsilon_{EVR}$ is close to 1). The possibility of

eigenvalue ratios close to 1 is a notable limitation of the tQSSA heuristic approach, which has not been previously reported.

There is another notable, and principal, limitation. By the above general approach, we obtain linear timescale estimates, but starting values for $s$ at the beginning of the slow regime (following the initial fast transient of the reaction) are a different matter. This requires resorting to more detailed mathematical arguments to calculate estimates for substrate (or product) concentrations.

We will present these arguments and a more complete justification for the tQSSA – not just for points near the origin – in a forthcoming mathematically oriented paper.

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# Declarations

## Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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