Use and abuse of the Quasi-Steady-State Approximation

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Abstract

We examine the transient kinetic behaviour of an open single enzyme, single substrate reaction. The reaction follows the van Slyke-Cullen mechanism, a special case of the Michaelis-Menten reaction. We perform the analysis both with and without applying the Quasi-Steady-State Approximation. The analysis of the full system shows conditions for biochemical pathway coupling which yield sustained oscillatory behaviour in the enzyme reaction. The reduced model does not demonstrate this behaviour. Our results have important implications in the analysis of open biochemical reactions and the modelling of metabolic systems.

Keywords: single enzyme-substrate reaction, open reactions, quasi-steady-state approximation, model reduction, modelling metabolic pathways

1 Introduction

The aim of modelling reaction mechanisms and biochemical pathways is to clarify the nature of reaction intermediates and their interactions (how they react with, or transform into, each other), and to determine the rates of these transformations. Mechanisms are investigated mathematically through two distinct approaches: stoichiometric and kinetic models. The stoichiometric model is based on the time-invariant characteristics of the reactions and describes the system pathway, known as its connectivity [1]. The kinetic model is based on both the stoichiometry and reaction rates. Here, the individual interactions in the mechanism are assigned appropriate kinetic properties, known as rate laws [1, 2].

Of the two, therefore, kinetics models are more complete and provide a greater understanding of the system behaviour. To produce such models, however, we require knowledge
of the reaction stoichiometry and detailed information about the kinetics of a reaction pathway. Consider the following pathway scheme:

$$
\begin{align*}
S_1 & \xrightarrow{v_1} S_2 \quad S_2 & \xrightarrow{v_2} S_3 \quad S_3 & \xrightarrow{v_3} 2S_4 \quad S_4 & \xrightarrow{v_4} S_5 \quad S_5 & \xrightarrow{v_5} S_6
\end{align*}
$$

In this scheme we show a biochemical pathway in which a product $S_6$ is synthesised from a substrate $S_1$ through the four intermediates $S_2$, $S_3$, $S_4$ and $S_5$. The pathway has negative feedback in the production of $S_2$, caused by the later intermediate $S_5$. Based on the stoichiometry, we can write the mass balance equations [1, 2, 3]. The rate of change of intermediate $S_4$, for example, is

$$
\frac{ds_4}{dt} = 2v_3 - v_4,
$$

where $v_i$ are the reaction rates, or fluxes, and $s_4$ is the concentration of the intermediate species $S_4$. In this paper we will use upper case notation to represent the names of different chemical species, and lower case letters to represent their corresponding concentrations. Here we consider that the system is well-mixed, or there is no mass flow due to convection or diffusion. The algebraic expression for the reaction rates $v_i$ will depend on the kinetics under consideration. Many biochemists employ phenomenological rate laws derived empirically [4]. There are several common approaches used to derive kinetic functions: mass action kinetics, Michaelis-Menten type and allosteric kinetics, and the power-law approximation, which we have recently summarised [1, 5].

Ideally, the mechanisms underlying biochemical pathways are described by incorporating all of the elementary steps of the enzyme-substrate association-dissociation, isomerisation of intermediates and formation of products. A major problem with this method of modelling complex biochemical pathways is that it produces systems of highly-nonlinear differential equations with many kinetic and stoichiometric parameters [1]. Typically these systems are stiff and have multiple timescales [6]. Consequently their numerical solutions are computationally demanding. This also makes data-fitting difficult [7, for a general discussion].

The kinetic modelling of enzymatic reactions can be simplified considerably if the overall reaction is studied with the aid of the Quasi-Steady-State Approximation (QSSA), sometimes referred to as the Pseudo-Steady-State Hypothesis. This originates from the empirical observation that, in some cases, the reaction intermediates are short-lived relative to the other reactants. Successful application of the QSSA requires there to be separation of timescales between the fast and slow reacting species [8, 9]. Employing the QSSA simplifies the model by reducing the dimension of the system of equations. We set the differential equation governing the reaction intermediates equal to zero, which produces an algebraic expression. This application of the QSSA has been a commonly used tool in the modelling of biochemical networks since the late 1960s [10]. In fact, the QSSA has been systematically employed in chemical kinetics for more than 80 years [11]. Metabolic control theory, a widely use sensitivity analysis technique for biochemical systems, also relies on the application of the QSSA [2, 12]. We can see, then, that the identification of the appropriate timescales and ranges of validity for the simplification of enzyme kinetics is of great importance. Over the last ten years, we have determined the timescales of the
reacting species and validity of the QSSA in isolated enzyme reactions [8, 11, 13, 14]. Recently, the effects of substrate input on the validity of the QSSA for open enzyme reactions has also been considered [15, 16].

The coupling of an enzyme–substrate reaction to metabolic pathways may lead to the appearance of weakly damped or even sustained oscillations [17, 18]. However, the dynamical consequences of reducing open biochemical systems using the QSSA has not been systematically explored. In this paper, we examine the transient kinetic behaviour of an open single enzyme, single substrate reaction following the Van Slyke-Cullen mechanism [19] (Sections 3–4). This is a very similar mechanism to the Henri-Michaelis-Menten one, differing only in that there is no reverse reaction from the complex back to the substrate. The dynamics of both are similar; we choose the simpler one for our demonstration. We perform the analysis both with and without applying the QSSA (Section 5). We show that the two analyses can produce qualitatively different results, in which case one must be cautious in the application of the QSSA. The paper is concluded with a brief discussion (Section 6).

2 The Van Slyke-Cullen reaction mechanism

We consider an isolated enzyme reaction following the Van Slyke-Cullen mechanism [19]. This is the simplest single enzyme, single substrate reaction mechanism. Here we have an enzyme \(E\) and a substrate \(S\) which bind, irreversibly, to form an intermediate enzyme-substrate complex \(C\). This complex then breaks down, irreversibly, to form a product \(P\):

\[
\begin{align*}
\xrightarrow{v_1} & S + E & \xrightarrow{k_1} & C & \xrightarrow{k_2} & E + P \xrightarrow{v_2} \\
\end{align*}
\]

Here \(k_1\) and \(k_2\) are positive rate constants, \(v_1\) and \(v_2\) are rate functions. Applying the law of mass action, we obtain the following system of ordinary differential equations:

\[
\begin{align*}
\frac{ds}{dt} &= v_1(p) - k_1se , \\
\frac{de}{dt} &= -k_1se + k_2c , \\
\frac{dc}{dt} &= k_1se - k_2c , \\
\frac{dp}{dt} &= k_2c - v_2(p) .
\end{align*}
\]

In this system, the variables \(s, e, c\) and \(p\) are mathematical representations of the concentrations of the reaction molecules \(S, E, C\) and \(P\). The enzyme is conserved: \(e' + c' = 0\), which integrates to give \(e + c = \text{constant} \equiv e_0\). Here \(e_0\) denotes the total enzyme concentration. We use substitute \(e_0 - c\) for \(e\) and thus simplify the system to three equations:

\[
\begin{align*}
\frac{ds}{dt} &= v_1(p) - k_1se_0 , \\
\frac{dc}{dt} &= k_1se_0 - k_2c , \\
\frac{dp}{dt} &= k_2c - v_2(p) .
\end{align*}
\]

We rescale the variables in order to remove the rate constants \(k_1\) and \(k_2\) from the system:

\[
s = \frac{k_2}{k_1} \bar{s} , \quad e = \frac{k_2}{k_1} \bar{e} , \quad p = \frac{k_2}{k_1} \bar{p} , \quad t = \frac{1}{k_2} .
\]
The resultant form has only one explicit parameter, \( \bar{e}_0 = k_1 e_0 / k_2 \):

\[
\begin{align*}
\dot{s} &= \bar{v}_1(p) - s(\bar{e}_0 - \bar{c}) , \\
\dot{c} &= s(\bar{e}_0 - \bar{c}) - \bar{c} , \\
\dot{p} &= \bar{c} - \bar{v}_2(\bar{p}) .
\end{align*}
\]

The rescaled functions are \( \bar{v}_i(p) = k_1 v_i(p) / k_2^2 \). We drop the bars and revert to dashes instead of dots to refer to differentiation in this rescaled system.

For general \( v_i \), this is the simplest form and the system remains three-dimensional. There are some specific cases where further reduction is possible, which we consider before we embark on the full analysis.

3 Flux considerations of the reaction

In some circumstances the product does not have any influence over the reaction kinetics of the reactants. This yields a far simpler system – a two-dimensional one – with a limited range of dynamics possible. There are two causes of this situation for our example, but here they are not entirely distinct.

3.1 Mass conservation

We expect mass to be conserved within the system, over time. For general \( v_i \) this remains an implicit consideration: \( \int_0^\infty s' + c' + p' = 0 \). Without knowing the system solution, we cannot evaluate this integral. There is, however, a case where we can perform the integral without having found the solution explicitly: for \( v_1 - v_2 = \text{constant} \equiv v \) we have \( s' + c' + p' = v \). This constant must then be zero, and thus \( v_1 = v_2 \). However, for realistic \( v_i \) we could expect \( dv_2/dp \geq 0 \), and for negative feedback (creating a homeostatic environment) \( dv_1/dp \leq 0 \). In this case, the net result is \( dv_1/dp = 0 = dv_2/dp \), which means we end up with \( v_1 = v_2 = \text{constant} \). Since mass conservation holds explicitly the system reduces to two dimensions by substituting \( s_0 - s - c \) for \( p \).

The limiting case of this constraint is \( v_i \equiv 0 \). We refer to this as the closed form, since there is no input or output.

It is possible to imagine functions \( v_i \) that are equal and faintly realistic, without being constant. An example might be \( v_i(p) = 1 - \cos(p) \) [16]. This is difficult to justify biologically: the supply and sink functions matching exactly would either be a strange coincidence or a strongly connected system.

3.2 No feedback

In the case \( v_1(p) = \text{constant} \), \( p \) is separable: the dynamics of \( s \) and \( c \) do not depend on \( p \). The dynamic of \( p \) does however depend on \( s \) and \( c \) and so its behaviour is evaluated once theirs is known. We write \( p(s, c) \) and we see that the system is effectively reduced to two dimensions: \( s \) and \( c \). This is the same result as the mass conservation consideration above, achieved with a weaker constraint (which encompasses the earlier constraint).

3.3 Analysis

One of the best ways to understand the behaviour of chemical reactions is by using dynamical systems techniques to study their evolution in the phase space [14, 6, 20, 21].
Examining the nullsurfaces is a good first step in analysing the phase space of the system. Here we have

\[ s = \frac{v_1}{e_0 - c} \quad \text{for} \quad s' = 0 , \]
\[ c = \frac{e_0 s}{1 + s} \quad \text{for} \quad c' = 0 . \] (8)

The intersection of the two nullsurfaces give the steady state: \( \left( \frac{v_1}{e_0 - v_1}, v_1 \right) \). We see that \( 0 < v_1 < e_0 \). If we revert to the original coordinate system we see that the steady state has quantitative dependence on the rate constants \( k_i \):

\( \left( \frac{v_1}{k_1(e_0 - v_1)^2}, \frac{v_1}{k_2} \right) \).

We investigate the local behaviour around this steady-state using local stability analysis. We linearise the core system by calculating the Jacobian:

\[ \mathcal{L}s' = \begin{pmatrix} -e & s \\ e & -s - 1 \end{pmatrix} \mathcal{L}s . \] (9)

In using \( e \) here we intend the rescaled one, but still as defined previously: \( e = e_0 - c \). At the steady state we have:

\[ \mathcal{L}s' = \begin{pmatrix} -e_* & v_1/e_* \\ e_* & -e_0/e_* \end{pmatrix} \mathcal{L}s . \] (10)

We look for the eigenvalues:

\[ \lambda^2 + \left( \frac{e_0}{e_*} + e_* \right) \lambda + e_* = 0 . \] (11)

The eigenvalues are real and negative, which indicates a stable node.

The phase space trajectories for system (7) with \( v_1, v_2 \) constant, and its \( c' = 0 \) nullsurface are shown in Figure 1. Note that the trajectories departing from \( c = 0 \) horizontally cross the \( c' = 0 \) nullsurface. The trajectory flow is attracted by a unique trajectory, a slow invariant manifold, and all trajectories tend to the point \( \left( \frac{v_1}{e_0 - v_1}, v_1, s_0 \right) \), which is the chemical equilibrium (a stable node), as \( t \to \infty \). For the Michaelis–Menten reaction mechanism, after an initial transient, trajectories eventually lie close to the slow invariant manifold, which is confined to the region bounded between the nullsurfaces [14, 20, 21, 22]. In Figure 1, we do not show the \( s \) nullsurface.

The QSSA is the idea that, after an initial rapid transition, the reaction will settle close to the \( c \) nullsurface. Although time is not apparent in the phase diagram, it seems that the QSSA is a good approximation for the latter stages of the reaction with constant flow, as is the case for the Michaelis–Menten reaction [20, 23].

4 Analysis of the full system

Having considered the reduced form, we now examine the general system. Again we consider the nullsurfaces. For \( p' = 0 \), we find that \( c = v_2(p) \), with \( s \) unconstrained.
Figure 1: Phase space for the Van Slyke-Cullen reaction (7) with constant flow, given by numerical solution. Solid curves starting with circles are the trajectories. The surface is the QSSA null surface ($c' = 0$). We see the trajectories initially heading directly towards the null surface. As each trajectory approaches the surface it alters direction so as to remain close to it, while $s$ continues to diminish, and $p$ continues to increase. Note that the steady state is node-like, not spiral. The rescaled parameters are here $e_0 = 2$ and $v_i = 1$. 
The $c' = 0$ plane is the same as for the previous system: $c = \frac{e_0s}{1 + s}$ with $p$ free.

The $s'$ nullsurface is a little more complex: $\left( \frac{v_1(p)}{e_0 - c}, c, p \right)$. We find steady states for $p_*$ such that $v_1(p_*) = v_2(p_*) \equiv v_*: \left( \frac{v_*}{e_0 - v_*}, v_*, p_* \right)$. In the original system this is $\left( \frac{v_*}{k_1(e_0 - v_*)}, \frac{v_*}{k_2}p_* \right)$. Note that at the steady state, the net system flow is zero – thus we see the system is well defined.

We evaluate the Jacobian at the steady state:

$$J = \begin{pmatrix}
-e_* & v'_* / e_* & v'_1 \\
e_* & -e_0 / e_* & 0 \\
0 & 1 & -v'_2
\end{pmatrix}.$$  \hspace{1cm} (12)

We find the eigenvalue equation for the solution:

$$\lambda^3 + \left( e_* + \frac{e_0}{e_*} + v'_2 \right) \lambda^2 + \left( e_* + \frac{e_0}{e_*} \right) v'_2 + e_* \lambda + e_* (v'_2 - v'_1) = 0.$$  \hspace{1cm} (13)

We know that $e_*$ is positive, since it is the steady state of the free enzyme concentration, in the rescaled system. We gave properties of $v'_i$ which tell us that $v'_2 - v'_1 \geq 0$. Thus every constant in the polynomial is positive. This means that there are no real positive roots, so we must have at least one negative real root. It is possible that there are complex roots with positive real parts, in fact this arises for $-v'_1 > \frac{f}{e_*} \left( v'_2 + f v'_2 + e_* \right)$, where $f \equiv e_* + \frac{e_0}{e_*}$.

In this case we have a local unstable spiral in a plane about the steady state. The phase space trajectories for system (7) and its $c' = 0$ nullsurface are shown in Figure 2. We choose $v_1 = 2 - p$ to satisfy both $v_1 \geq 0$ (for $p \leq 2$) and $dv_1/dp = \leq 0$. Similarly, $v_2 = 1$ gives $v_2 \geq 0$ and $dv_2/dp = \geq 0$. We see that the trajectories departing from $c = 0$ cross the $c' = 0$ nullsurface almost perpendicularly, and then remain close to it. This figure shows that the overall behaviour of this model can be qualitatively different to that of the Van Slyke-Cullen reaction mechanism with constant flow, shown in Figure 1. Here we see that damped oscillations occur close to the QSSA surface (the $c$ nullsurface). In this case all trajectories eventually approach the steady state. This illustrates only one possible behaviour of the general system (7).

The simple source and sink functions chosen for this example are not valid for all initial conditions. Consideration of initial conditions is difficult to motivate in an open system: long-term behaviour is primarily of interest.

5 Applying the QSSA to the open mechanism

The standard practice in the modelling of complex biochemical pathways (which are usually open enzyme-substrate reactions) is applying the QSSA to reduce the number of differential equations in the system. Alternatively, expressions derived using the QSSA (for example, the Michaelis-Menten equation) are employed as reaction rates or fluxes in the mass balance equations [see, for example, Eq. (2)] in the models of biochemical
Figure 2: Phase space for the Van Slyke-Cullen reaction with linear flow given by the numerical solution of (7). Solid curves starting with circles are the trajectories. The surface is the QSSA null surface ($c' = 0$). We see the trajectories initially heading directly towards the null surface. As each trajectory approaches the surface it alters direction so as to remain close to it. Note that the steady state is a spiral. The rescaled parameters are here $e_0 = 2$, $v_1 = 2 - p$ and $v_2 = 1$.

pathways. Whilst we appreciate that this approximation is quite reasonable, we must be careful in the use of it. We will show that the simplified system may be qualitatively different from the original.

Depending on the initial conditions, there can be an initial transient where there is rapid formation of the enzyme-substrate complex. After that, saturation of the complex occurs, and then the reaction becomes more steady. We see that the amount of complex changes very slowly from now on: $dc/dt \approx 0$. Thus the QSSA is valid in the sense that it is close to the actual system behaviour. However, when we simplify the system using an approximation like this (and this is a very strong one, being at the heart of the system’s dynamics) we must be careful not to remove from the model some of the behaviour which we are interested in studying.

We consider the simplified system (7), then we apply the QSSA. This states that $c' \approx 0$. From this we find an expression for $c$ in terms of $s$:

$$c \approx \frac{e_0 s}{1 + s}.$$  \hspace{1cm} (14)

We substitute this back into what remains of the system to give:

$$s' \approx v_1(p) - \frac{e_0 s}{1 + s},$$

$$p' \approx \frac{e_0 s}{1 + s} - v_2(p).$$  \hspace{1cm} (15)

We have reduced the system from three species to two.
Figure 3: Phase space for the Van Slyke-Cullen reaction with linear flow. Solid curves starting with circles are the trajectories. The surface is the QSSA nullsurface \((c' = 0)\). We see the trajectories initially heading towards the null surface. As each trajectory approaches the surface it alters direction so as to remain close to it. We see that the two models exhibit qualitatively different behaviour. The rescaled parameter \(e_0\) here is set to 2, and the flow functions are \(v_1 = 2 - 3.1p\) and \(v_2 = 1\).

5.1 Analysis

The steady state is the same as for the full system. We investigate the stability of the steady state by evaluating the Jacobian there:

\[
\mathcal{J} = \begin{pmatrix}
  -\alpha & v'_1 \\
  \alpha & -v'_2
\end{pmatrix}
\]  

(16)

where \(\alpha = \frac{(e_0 - v_\star)^2}{e_0} > 0\). This then gives the following stability formula:

\[
\lambda^2 + (\alpha + v'_2) \lambda + \alpha (v'_2 - v'_1) = 0
\]  

(17)

We see that the quadratic expression has only positive terms and so the only solution is found for \(\text{Re}(\lambda) < 0\). Thus the steady state is always stable in the system reduced using the QSSA.

We demonstrate this difference with an example. The phase space trajectories for system (7) and the same system with the QSSA applied (15) are shown in Figure 3. The specific substrate supply function here is \(v_1 = 2 - 3.1p\) and the product removal is \(v_2 = 1\). The rescaled parameter \(e_0\) here is set to 2. These settings were the same for both numerical solutions. In the full system we see a stable limit cycle occurring as the limiting behaviour; in the system reduced by the QSSA the complex dynamic of the limit cycle has been replaced with a relatively simple stable spiral.

We notice that there is a special case: \(v'_1 - v'_2 = 0\), which yields \(\lambda = 0\). Here the full analysis cannot give an answer. However, this is the condition which we studied earlier (Section 3).
6 Discussion

The QSSA is probably the most frequently used method for reducing the number of equations governing a complex biochemical pathway. In recent years, special attention has been paid to the conditions for the validity of the QSSA in both closed [9, 11] and open [15, 16] mechanisms. However, there has been little interest in understanding the impact of the QSSA on the analysis of the dynamical behaviour of biochemical pathways.

The dynamical consequences of the application of the QSSA have been explored previously for some large systems. In 1974, a chemical mechanism of 81 species was compared with the reduced version produced by applying the QSSA [24]. Critical differences were observed between the full and reduced models, leading to the conclusion that the QSSA is, in general, not reliable, because it can conceal some aspects of the dynamics. More recently, the non-linear system describing the allosteric reaction in [25] was reduced from twelve equations to two by applying the QSSA. It was shown that using the QSSA changed the perceived dynamical behaviour of the system. Unfortunately these results have not been widely accepted within the biochemical community. There is an assumption that the long-term behaviour of most systems is to reach a steady state. The reduction of the model by the QSSA is considered acceptable because the long-term dynamic is assumed not to change [2, for example]. A single enzyme, single substrate Michaelis–Menten reaction mechanism has demonstrated both weakly damped oscillations (stable spiral) and sustained oscillations (limit cycle) when the reaction mechanism is coupled to other biochemical pathways [17]. Damped oscillations have also been generated in open reactions by coupling very simple mechanisms, such as competitive inhibition of a single enzyme [26].

In this paper, we have investigated the dynamical effect of the QSSA in the simplest open enzyme–substrate reaction. We have shown that it is possible to generate both damped and sustained oscillations in an open Van Slyke–Cullen reaction. We demonstrate that the use of the QSSA produces a reduced version of the model which fails to reveal the full dynamical behaviour of the system. In particular, we saw that the linear feedback mechanism can exhibit instability, including limit cycle behaviour (sustained oscillations), while the reduced version – using the QSSA – cannot (see Figure 3). Our analysis contradicts the general assumption that the use of the QSSA does not affect the long-term dynamics of the model.

This investigation shows that model reduction by the use of the QSSA can fail to reveal some important dynamical properties of the unreduced system. We hope that this demonstrates to the biochemical community that care should be taken when modelling complex pathways. The use of the QSSA in reducing the system, or in deriving reaction rates or fluxes, is contra-indicated.

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