Computing Quasi-Steady State Reductions

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Abstract

There is a systematic approach to the computation of quasi-steady state reductions, employing the classical theory of Tikhonov and Fenichel, rather than the commonly used ad-hoc method. In the present paper we discuss the relevant case that the local slow manifold is a vector subspace, give closed-form expressions for the reduction and compare these to the ones obtained by the customary method. As it turns out, investment of more theory pays off in the form of simpler reduced systems. Applications include a number of standard models for reactions in biochemistry, for which the reductions are extended to the fully reversible setting. In a short final section we illustrate by example that a QSS assumption may be erroneous if the hypotheses for Tikhonov's theorem are not satisfied.

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1 Introduction

The mathematical description and analysis of reacting systems in chemistry and biochemistry frequently leads to a slow-fast separation for the associated differential equations. This slow-fast separation may involve slow and fast reactions or, frequently, slow and fast variables (i.e. concentrations). In the latter case one speaks of quasi-steady state (QSS) for the slow variables. The purpose of this paper is to propose that QSS reductions should be computed in a way different from the commonly used direct ("ad hoc") approach, and to illustrate that

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and how this can be done. As an additional benefit, this alternative approach will yield simpler reduced equations in many cases.

In the ad hoc approach certain rates of change (for certain variables designated "slow") are set equal to zero. Examples for the ad hoc method can be found in standard textbooks like Atkins and de Paula [1], and Berg, Tymovzko and Stryer [2]. In the monographs by Keener and Sneyd [8] and Murray [10], scaling and singular perturbation theory act as guidelines, but effectively this may lead to ad-hoc computations.

There are two main reasons for choosing an approach that is more closely tied to the singular perturbation theory of Tikhonov [19], Fenichel [5] and others. From a mathematical perspective, a procedure which invokes the theory not just as a principle but as a toolkit for actual computations, ensures consistency and takes care of convergence problems. From a purely pragmatic perspective the systematic approach via Tikhonov and Fenichel is preferable because the reduced equations generally are easier to compute and to handle than those obtained by the ad-hoc procedure.

A straightforward application of Tikhonov's theorem (see e.g. Verhulst [21], Theorem 8.5) to a QSS scenario is generally impossible since the theorem applies to systems in a certain standard form. This fact may have been one reason for Segel and Slemrod, in their extensive study [17] of the Michaelis-Menten reaction, to derive and prove asymptotic properties directly. On the other hand, all the necessary groundwork for the computation of a reducing map was laid by Fenichel [5]. Schauer and Heinrich [15] used the singular perturbation approach for slow and fast reactions, citing Vasil'eva [20] and explicitly using a linear transformation to Tikhonov standard form. In Heinrich and Schuster [7], Chapter 4, one finds a clear statement of Tikhonov's theorem, and the necessity to transform a given system to standard form is pointed out. But in applications (in contrast to [15]) the authors use singular perturbation arguments only to simplify certain expressions for reaction rates, and stop short of determining explicit reduced differential equations. (Similar remarks apply to Keener and Sneyd [8].) Duchêne and Rouchon [4], and Stiefenhofer [18] directly appeal to Fenichel's results and use them to determine reduced equations for particular systems from chemistry and biochemistry. A general approach to the explicit computation of Tikhonov-Fenichel reductions, which was recently presented in [13], leads to a reduced system with rational right-hand side whenever the original system has a polynomial or rational right-hand side. In particular, reduction of equations for chemical reactions with mass action kinetics will yield equations with rational right-hand side.

In Section 2 of the present paper we discuss in detail the practically important case that the local slow manifold is a (vector or affine) subspace: In this scenario there is an efficient shortcut for the necessary computations, and the structure of the reduced system becomes transparent. The main result is Theorem 2, which provides a readily applicable formula for the computation of a reduced system. In particular this provides insight under what conditions Tikhonov-Fenichel reduction and the ad-hoc method will yield the same reduced system (e.g. in several relevant cases when certain reactions are irreversible),

and under what conditions the reduction procedures provide different results (e.g. when fully reversible versions of the above systems are considered). In Section 3 we will apply our results to a number of reaction equations of practical interest; on purpose we are choosing standard examples, mostly from Keener and Sneyd [8]. To our knowledge, the general form of the reduced systems has not been available in the literature so far. In a final section we briefly discuss, for the relevant example of reverse quasi-steady state (rQSS) in the Michaelis-Menten system, the importance of the hypotheses in Tikhonov's and Fenichel's theorems for QSS. It turns out that time scale heuristics may erroneously suggest QSS in some settings.

2 QSS reduction to subspaces

This section contains the mathematical groundwork and the main results. We introduce the setting and notation first: Consider a differential equation with analytic right-hand side, depending on a ("small") parameter ε:

(1) $\qquad \dot{x} = h(x, \varepsilon) = h^{(0)}(x) + \varepsilon h^{(1)}(x) + \cdots, \ x \in U \subset \mathbb{R}^{n+m}.$

Here both n and m are positive integers. The right hand side may depend on additional parameters; these will usually be suppressed in the notation. The subset U of $\mathbb{R}^{n+m}$ is assumed to have nonempty interior, and h will be defined in some neighborhood of $U \times [0, \varepsilon_0]$, for some positive ε_0.

The most relevant application in our context will be to differential equations which model chemically reacting systems with mass action kinetics. Hence the right-hand side of (1) will be polynomial or even, if only first and second order reactions take place, polynomial of degree ≤ 2. But the main results will be stated and proven in a general framework.

In the following, let Y be the zero set of $h^{(0)}$.

Basic assumptions.
(i) There is an $y_0 \in Y$ and a neighborhood M_0 of y_0 in Y such that $Dh^{(0)}(y)$ admits the eigenvalue 0 with geometric and algebraic multiplicity n, for all $y \in M_0$.
(ii) Moreover, there is a constant $\mu > 0$ such that for every $y \in M_0$ the remaining eigenvalues of $Dh^{(0)}(y)$ have real part $\leq -\mu$.

By the implicit function theorem and linear algebra one sees:

Lemma 1. *If basic assumption* (i) *holds then M_0 is a local submanifold of dimension n. Moreover, $\mathbb{R}^{n+m}$ is the direct sum of kernel and image of $Dh^{(0)}(y)$, for every $y \in M_0$.*

We denote by π_y the projection onto the kernel of $Dh^{(0)}(y)$ with respect to the kernel-image decomposition.

The following result on asymptotic reduction goes back, in principle, to Fenichel [5]; see [13] for a more extensive account.

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Theorem 1. (a) *Given the basic assumptions* (i) *and* (ii) *there exists a reduced system for* (1) *on M_0, in the sense of Tikhonov and Fenichel. Thus, there is a neighborhood of x_0 in U and $T > 0$ such that every solution of the time scaled version*

$$\varepsilon^{-1}\dot{x} = h^{(0)}(x) + \varepsilon h^{(1)}(x) + \cdots$$

of (1) *starting in this neighborhood converges to a solution of the time scaled reduced system on the interval $(0, T)$, as $\varepsilon \to 0$.*
(b) *The reduced system on M_0 (with no time scaling) is given by*

$$\dot{x} = \varepsilon \cdot p(x), \ \ with \ p(x) = \pi_x(h^{(1)}(x)).$$

As was pointed out in [13], one can explicitly compute this projection of $h^{(1)}(x)$ onto the kernel along the image of $Dh^{(0)}(x)$, with $x \in Y$. The procedure proposed in [13] starts from the minimum polynomial of $Dh^{(0)}(x)$, and leads straightforwardly to the reduced system, albeit possibly at considerable computational expense. Examples show that the procedure is feasible at least for low-dimensional systems. But the question remains whether computations can be facilitated at least for special classes of systems. Moreover, other approaches may provide more insight into the structure of the reduced system.

In the present paper we will resolve the problem of efficient computation and structural insight for the case that M_0 is (a relatively open subset of) a subspace of $\mathbb{R}^{n+m}$. While this is a rather simple setting, it already poses nontrivial problems, and it is of substantial interest for many QSS reductions in biochemistry.

We start with some preparations.

Lemma 2. (a) *Up to a linear (resp. affine) coordinate transformation, every n-dimensional vector subspace (or affine subspace) V of $\mathbb{R}^{n+m}$ can be represented by the equations $y = 0$, with*

$$\begin{pmatrix} x \\ y \end{pmatrix} \in \mathbb{R}^{n+m}, \ \ y \in \mathbb{R}^m.$$

(b) *Given the setting of part (a), any (polynomial or analytic) vector field q on $\mathbb{R}^{n+m}$ for which every point of V (near 0) is stationary has the form*

$$q(\begin{pmatrix} x \\ y \end{pmatrix}) = \begin{pmatrix} B(x)y + \sum_{|i|\geq 2} b_i(x)y^i \\ A(x)y + \sum_{|i|\geq 2} a_i(x)y^i \end{pmatrix}.$$

Here we employ the standard abbreviations

$$i = (i_1, ..., i_m) \in \mathbb{N}_0^m, \ \ |i| = i_1 + \cdots + i_m, \ \ y^i = y_1^{i_1} \cdots y_m^{i_m},$$

moreover A and B are analytic matrix-valued functions of x, and a_i, b_i are analytic vector-valued functions of x, each of appropriate size.
(c) *The matrix $Dq(x, y)$ satisfies the basic assumption* (i) *in a neighborhood of 0 if and only if $A(0)$ is invertible, and it satisfies the basic assumption* (ii) *in a neighborhood of 0 if and only if all eigenvalues of $A(0)$ have negative real parts.*

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Proof. Part (a) is linear algebra, while part (b) follows directly from rearranging the power series expansion and using $q(x, 0) = 0$. Finally, part (c) is a consequence of

$$Dq(0,0) = \begin{pmatrix} 0 & B(0) \\ 0 & A(0) \end{pmatrix}.$$

$\square$

We now will consider the reduction problem for equation (1) in the case that M_0 is a neighborhood of 0 in a subspace $V \subseteq \mathbb{R}^{n+m}$. We may - and will - assume that V is given as in Lemma 2(a), and

$$(2) \qquad h^{(0)} : U \to \mathbb{R}^{n+m}, \quad \begin{pmatrix} x \\ y \end{pmatrix} \mapsto \begin{pmatrix} B(x)y + \sum\limits_{|i|\geq 2} b_i(x)y^i \\ A(x)y + \sum\limits_{|i|\geq 2} a_i(x)y^i \end{pmatrix}$$

with A, B, a_i and b_i as in Lemma 2(b). Furthermore we fix notation by setting

$$(3) \qquad h^{(1)} : U \to \mathbb{R}^{n+m}, \quad \begin{pmatrix} x \\ y \end{pmatrix} \mapsto \begin{pmatrix} u(x) + \sum\limits_{|i|\geq 1} u_i(x)y^i \\ v(x) + \sum\limits_{|i|\geq 1} v_i(x)y^i \end{pmatrix}$$

with u, v, u_i and v_i analytic vector-valued functions of appropriate size.

Theorem 2. *Let system* (1) *be such that $h^{(0)}$ is as in* (2), *and $h^{(1)}$ is as in* (3). *Assume that* (i) *and* (ii) *hold in a neighborhood of 0 (equivalently, every eigenvalue of $A(0)$ has real part < 0). Then the Tikhonov-Fenichel reduction of the system with respect to the small parameter ε is given by*

$$(4) \qquad \dot{x} = \varepsilon \cdot \left(u(x) - B(x)A(x)^{-1}v(x) \right), \quad x \text{ near } 0, \text{ on } y = 0.$$

Proof. Let V denote the subspace defined by $y = 0$. On this subspace the Jacobian of $h^{(0)}$ is given by

$$(5) \qquad Dh^{(0)}(x,0) = \begin{pmatrix} 0 & B(x) \\ 0 & A(x) \end{pmatrix}.$$

Because (i) and (ii) are satisfied near 0, Theorem 1 is applicable for x near 0, and there remains to compute the kernel-image decomposition.

Because $A(x)$ has full rank m, and the first n columns of $Dh^{(0)}(x, 0)$ are identically zero, the rank of $Dh^{(0)}(x, 0)$ is also equal to m and the dimension of its kernel equals n. The standard basis vectors $e_1, ..., e_n$ are elements of the kernel, and therefore

$$\mathrm{Ker}\left(Dh^{(0)}(x,0) \right) = \langle e_1, ..., e_n \rangle.$$

Moreover the image is given by

$$\mathrm{Im}\left(Dh^{(0)}(x,0) \right) = \left\{ \begin{pmatrix} B(x) \\ A(x) \end{pmatrix} \cdot w; \; w \in \mathbb{R}^m \right\}.$$

Given an arbitrary $z := \begin{pmatrix} u^* \\ v^* \end{pmatrix} \in \mathbb{R}^{n+m}$, there exist uniquely determined $z_0 \in \langle e_1, ..., e_n \rangle$ und $w_0 \in \mathbb{R}^m$ such that

(6) $$\begin{pmatrix} u^* \\ v^* \end{pmatrix} = z_0 + \begin{pmatrix} B(x) \cdot w_0 \\ A(x) \cdot w_0 \end{pmatrix}$$

is the kernel-image decomposition of z with respect to $Dh^{(0)}(x, 0)$.

Since the last m entries of $z_0 = \begin{pmatrix} z_0^* \\ 0 \end{pmatrix}$ vanish, one may rewrite the last m entries of (6) to obtain

$$v^* = A(x)w_0 \Leftrightarrow w_0 = A(x)^{-1}v^*.$$

Using this identity, the first n entries of (6) yield

$$\begin{aligned} u^* &= z_0^* + B(x)w_0 \\ &= z_0^* + B(x)A(x)^{-1}v^*. \end{aligned}$$

Thus the desired projection with respect to $Dh^{(0)}(x, 0)$ is given by

$$z_0^* = u^* - B(x)A(x)^{-1}v^*.$$

Substitution of $h^{(1)}(x, 0)$ leads to the expression (4) for the reduced system. $\square$

Remarks. (a) In particular this result justifies a QSS assumption for the variables y as $\varepsilon \to 0$: Locally, after a short initial phase, the reduced system provides a good approximation to the dynamics of (1) for an extended time interval, and one has $y \approx 0$ as well as $\dot{y} \approx 0$. But it should be emphasized that we did not assume the variables y to be "fast" at the outset. Indeed, no initial distinction was made between slow and fast variables: Theorem 2 requires an assumption concerning the "small parameter", and an assumption that one component of the zero set of $h^{(0)}$ is a vector subspace. The variables x and y are then chosen with respect to this subspace, according to Lemma 2.
(b) In the present paper we will not address the question how a QSS assumption can lead to the identification of a suitable "small parameter". It should be emphasized that this is an important and nontrivial first step in the analysis. See for instance Segel and Slemrod [17], Schauer and Heinrich [14], and also [11], [12]. But one should note that QSS assumptions have to be tested for consistency, and this topic will be taken up in the present paper.
(c) The ad hoc QSS reduction of (1) with respect to the slow variable set y, given (2) and (3), thus setting "$\dot{y} = 0$", requires an explicit solution of the implicit equation

(7) $$0 = A(x)y + \sum_{|i| \geq 2} a_i(x)y^i + \varepsilon \cdot v(x) + \sum_{|i| \geq 1} v_i(x)y^i + \ldots$$

for y, which is then substituted into the differential equation for x in (1). Solving this equation may not be possible or feasible.

Generally the ad-hoc procedure will yield a reduction different from the Tikhonov-Fenichel approach. The procedure suffers from the theoretical problem of unresolved convergence issues as well as frequently from the practical drawback of having to solve equation (7). However, in some settings (including, as it turns out, very familiar ones) the Tikhonov-Fenichel approach and the ad hoc method provide the same reduction. We will discuss this in the application-relevant setting of reaction equations involving only first and second order reactions. Thus we consider system (1) in the following special form:

$$(8) \qquad \begin{aligned} \dot{x} &= B(x)y + p(y) + \varepsilon \cdot (u(x) + \widetilde{u}(x,\,y))\,, \\ \dot{y} &= A(x)y + q(y) + \varepsilon \cdot (v(x) + \widetilde{v}(x,\,y)) \end{aligned}$$

with p and q homogeneous polynomials of degree 2, u and v polynomials of degree ≤ 2, and $\widetilde{u}$, $\widetilde{v}$ polynomials of degree ≤ 2 with $\widetilde{u}(x,0) = \widetilde{v}(x,0) = 0$. The Tikhonov-Fenichel reduction of this system is still given by

$$\dot{x} = \varepsilon \cdot \big(u(x) - B(x)A^{-1}(x)v(x)\big)\,.$$

Corollary 1. *Assume the setting of* (8)*. If $p = 0$, $q = 0$, $\widetilde{u} = 0$ and $\widetilde{v} = 0$ then the ad-hoc reduction from the QSS assumption for y and the Tikhonov-Fenichel reduction coincide.*

Proof. To obtain the ad-hoc reduction, solve (7), which simplifies to

$$0 = A(x)y + \varepsilon \cdot v(x),$$

substitute and compare results. $\qquad\square$

Remark. One should emphasize that Corollary 1 describes an exceptional case. An ad-hoc reduction can generally not be computed in closed form. Even if a closed-form computation is manageable, the result is usually more complicated than the one given by Tikhonov-Fenichel.

It is a frequent phenomenon that ad hoc and Tikhonov yield the same reduction if certain (product forming) reactions are irreversible, but not in the reversible setting (which is more realistic and more appropriate; see e.g. Keener and Sneyd [8]). Corollary 1 provides a partial explanation. In the following sections we will discuss a number of relevant examples and derive reduced equations for the reversible setting; in most cases the expressions for the reduced systems seem to be unavailable in the literature.

3 Applications

In this section we discuss a number of applications. As the reader will notice, all of these are related to well-known standard models, mostly taken from the monograph by Keener and Sneyd [8]. This is a deliberate choice, to make the point that Theorem 2 is relevant and provides new information even in familiar settings. We will not include a discussion of appropriate small parameters here,

but rather start with a working hypothesis for ε, to be justified a posteriori. Readers will notice that we do not employ the customary method of introducing scaled variables. There is no doubt that this is an important tool, but it seems more relevant for quantitative estimates than for limiting processes. Moreover, scaling and limiting processes need to be defined carefully, as is indicated by section 4 below.

3.1 Reversible Michaelis-Menten

The Michaelis-Menten reaction was treated in detail via the Tikhonov-Fenichel approach in [13]. We use it here as a benchmark example, and also to discuss variants and modifications that are used to justify the familiar QSS reduction. The equations for substrate s and complex c are given by

$$(9) \qquad \begin{aligned} \dot{s} &= - && k_1 e_0 s &+&& (k_1 s + k_{-1})c, \\ \dot{c} &= && k_1 e_0 s &-&& (k_1 s + k_{-1} + k_2)c + k_{-2}(e_0 - c)(s_0 - s - c), \end{aligned}$$

with nonnegative initial values $c(0) = 0$ and $s(0) = s_0$. The rate constants k_i will all be assumed > 0 (except for $k_{-2} \geq 0$), and $s_0 > 0$, resp. $e_0 > 0$ denote initial concentrations for substrate, resp. enzyme.

If one considers QSS for the complex C then a suitable "small parameter" (found heuristically by Segel and Slemrod [17] via time scale arguments) in the irreversible case $k_{-2} = 0$ is given by

$$\varepsilon = \frac{e_0}{s_0 + M} \text{ with } M = \frac{k_{-1} + k_2}{k_1}.$$

The discussion in [12] shows that this remains an appropriate choice when k_{-2} is small. If one considers only scenarios with bounded s_0 (and M), and one is primarily interested in the limiting case $\varepsilon \to 0$, then one may just as well choose $\varepsilon = e_0$. Rewriting (9) yields

$$\begin{aligned} \dot{s} &= (k_1 s + k_{-1})c + \varepsilon(-k_1 s), \\ \dot{c} &= -(k_1 s + k_{-1} + k_2 - k_{-2}(s_0 - s))c + k_{-2}c^2 + \varepsilon(k_1 s + k_{-2}(s_0 - s - c)). \end{aligned}$$

In the irreversible case (i.e., $k_{-2} = 0$) Corollary 1 applies, and the familiar result of the ad hoc method coincides with the Tikhonov-Fenichel reduction. But for $k_{-2} > 0$ the ad hoc method leads to a quadratic equation for c as a function of s, hence the right-hand side of the reduced equation involves square roots. On the other hand the reduction according to (4) yields a reduced equation with rational right-hand side. It is given by

$$\dot{s} = -e_0 \cdot \frac{s(k_1 k_2 + k_{-1} k_{-2}) - k_{-1} k_{-2} s_0}{k_1 s + k_{-1} + k_2 + k_{-2}(s_0 - s)}.$$

We refer to [13] for more details. The right-hand side of the reduced equation corresponds to eq. (2.20) ff. in Heinrich and Schuster [7], p. 17, which (in our notation) is given by

$$\dot{s} = -e_0 \cdot \frac{k_1 k_2 s - k_{-1} k_{-2} p}{k_1 s + k_{-1} + k_2 + k_{-2} p}.$$

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This reduces to the previous equation if one uses (heuristically) the conservation law $s+c+p = s_0$ and recalls that $c = 0$ on the slow manifold. But such a step is not mentioned in [7], and thus Heinrich and Schuster do not arrive at a reduced differential equation for one function. The authors note, however, that in the case of zero product concentration one obtains the expression for irreversible Michaelis-Menten.

Keener and Sneyd [8], in Section 1.4.5 obtain the same relation and the same conclusion for the irreversible expression, and then justify setting $p = 0$ by invoking continuous removal of product. But it may be seen as problematic to invoke product removal a posteriori, and it seems more natural to incorporate it in the model ab initio. We will do so in the following, starting with the full system of equations

$$
\begin{aligned}
\dot{e} &= -k_1 es + (k_{-1} + k_2)c - k_{-2}pe, \\
\dot{s} &= -k_1 es + k_{-1}c, \\
\dot{c} &= k_1 es - (k_{-1} + k_2)c + k_{-2}pe, \\
\dot{p} &= k_2 c - k_{-2}pe - \alpha p
\end{aligned}
$$

with the product removal rate being assumed proportional to p with a parameter $\alpha > 0$. The system admits the first integral $e + c = e_0$ (but there exists no second, independent linear first integral), and we obtain the three-dimensional system

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

Designating the small parameter e_0, this fits into the mold of equation (8); we obtain

$$
\dot{s} = \begin{pmatrix} k_{-1} + k_1 s & 0 \end{pmatrix} \begin{pmatrix} c \\ p \end{pmatrix} + e_0(-k_1 s),
$$

$$
\begin{aligned}
\begin{pmatrix} \dot{c} \\ \dot{p} \end{pmatrix} &= \begin{pmatrix} -k_1 s - k_{-1} - k_2 & 0 \\ k_2 & -\alpha \end{pmatrix} \begin{pmatrix} c \\ p \end{pmatrix} + \begin{pmatrix} -k_{-2}cp \\ k_{-2}cp \end{pmatrix} \\
&\quad + e_0 \left(\begin{pmatrix} k_1 s \\ 0 \end{pmatrix} + \begin{pmatrix} k_{-2}p \\ -k_{-2}p \end{pmatrix} \right).
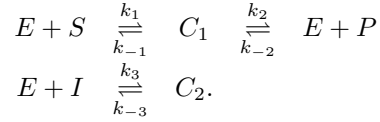\end{aligned}
$$

By Theorem 2 we obtain the reduced system

$$
\dot{s} = -k_1 s - \begin{pmatrix} k_{-1} + k_1 s & 0 \end{pmatrix} \begin{pmatrix} -k_1 s - k_{-1} - k_2 & 0 \\ k_2 & -\alpha \end{pmatrix}^{-1} \begin{pmatrix} k_1 s \\ 0 \end{pmatrix}
$$

and a straightforward computation shows that this is indeed the same expression as for the irreversible Michaelis-Menten reaction. Note that the factor α cancels out in the course of the computation. One also readily verifies that a nonlinear removal rate function $\rho(p) = \alpha \cdot p + \ldots$, with the dots standing for higher order terms in p, leads to the same reduction. Thus the conclusion stated in [8] does indeed hold, and it is not necessary to invoke product removal a posteriori. One also sees that a QSS assumption for both c and p is justified (cf. Remark (a) following Theorem 2). However, reduction by the ad hoc method would again produce different results.

3.2 Competitive inhibition

The reaction mechanism of competitive inhibition (see Keener and Sneyd [8], Subsection 1.2.3) is given as follows:

$$E + S \quad \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \quad C_1 \quad \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} \quad E + P$$

$$E + I \quad \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} \quad C_2.$$

Thus in addition to the basic Michaelis-Menten ingredients there is a substance I (inhibitor) which can also bind to the enzyme. Since an enzyme-inhibitor complex can no longer bind substrate, formation of product is impeded.

We are only interested in nonnegative solutions. The relevant initial data are given by $e(0) = e_0 > 0$, $s(0) = s_0 > 0$, $i(0) = i_0 > 0$ and $c_1(0) = c_2(0) = p(0) = 0$. Assuming mass action kinetics and employing the conservation laws $e + c_1 + c_2 = e_0$, $s + c_1 + p = s_0$ and $i + c_2 = i_0$ to eliminate e, i and p, one obtains the following system:

$$\dot{s} = \begin{pmatrix} k_{-1} + k_1 s & k_1 s \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \end{pmatrix} + e_0(-k_1 s),$$

$$\begin{pmatrix} \dot{c_1} \\ \dot{c_2} \end{pmatrix} = \begin{pmatrix} -k_1 s - k_{-1} - k_{-2}(s_0 - s) - k_2 & -k_1 s - k_{-2}(s_0 - s) \\ -k_3 i_0 & -k_3 i_0 - k_{-3} \end{pmatrix} \begin{pmatrix} c_1 \\ c_2 \end{pmatrix}$$
$$+ \begin{pmatrix} k_{-2}(c_1^2 + c_1 c_2) \\ k_3(c_1 c_2 + c_2^2) \end{pmatrix} + e_0 \left(\begin{pmatrix} k_1 s - k_{-2}(s - s_0) \\ k_3 i_0 \end{pmatrix} + \begin{pmatrix} -k_{-2} c_1 \\ -k_3 c_2 \end{pmatrix} \right).$$

We designate $\varepsilon = e_0$ as small parameter. Then the zero set Y of $h^{(0)}$ contains as a component the subspace

$$V = \{(s, 0, 0); \ s \in \mathbb{R}\}$$

and Theorem 2 is applicable. Since one has $s \leq s_0$ by initial conditions and conservation laws, the matrix

$$A(s) = \begin{pmatrix} -k_1 s - k_{-1} - k_{-2}(s_0 - s) - k_2 & -k_1 s - k_{-2}(s_0 - s) \\ -k_3 i_0 & -k_3 i_0 - k_{-3} \end{pmatrix}$$

has negative trace and positive determinant

$$\det A(s) = k_{-3}(k_1 s + k_{-1}) + (k_2 + k_{-1})k_3 i_0 + k_2 k_{-3} + k_{-2} k_{-3}(s_0 - s).$$

By a familiar special case of the Hurwitz-Routh criterion (generally see e.g. Gantmacher [6], Chapter V (6)) one sees that both eigenvalues of $A(s)$ have negative real parts. We obtain the reduced system

$$\dot{s} = e_0 \cdot \left(-k_1 s - (k_{-1} + k_1 s, \ k_1 s) \cdot A(s)^{-1} \cdot \begin{pmatrix} k_1 s - k_{-2}(s - s_0) \\ k_3 i_0 \end{pmatrix} \right)$$
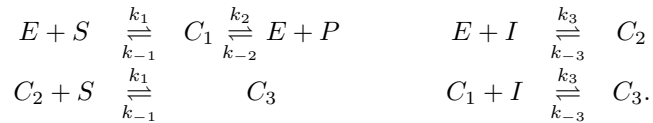
or

$$\dot{s} = -\frac{e_0 \left(k_{-3} \left(-k_{-2}k_{-1}(s_0 - s) + k_2 k_1 s\right)\right)}{\det A(s)}$$

for $0 \leq s \leq s_0$. For $k_{-2} = 0$ this expression specializes to the one derived by the ad-hoc method in [8] (as guaranteed by Corollary 1), while the general reduction for the non-reversible case seems to be new.

A posteriori one sees that the choice of the small parameter e_0 is consistent with Tikhonov-Fenichel, and one also sees that a quasi-steady state assumption for complexes C_1 and C_2 is justified for small e_0. We note that in case $k_{-2} > 0$ the ad hoc method leads to a rather complicated system of quadratic equations for c_1 and c_2 as functions of s. Although this system can be solved in principle by deriving a degree four equation for c_1 and using Cardano's formula, and similarly for c_2, the discussion of the reduced equation seems hardly feasible. In contrast, the reduction determined via Tikhonov-Fenichel is easy to discuss qualitatively, and the role of the parameters is transparent: The terms containing i_0 increase the denominator of the right-hand side, and thus substrate degradation will be delayed. The effect of $k_{-2} > 0$ leads essentially to a shift of the stationary point from 0 to a positive value. We note that incorporating product removal, as in the Michaelis-Menten example, will lead to the expression for the irreversible case.

3.3 Allosteric inhibition

The underlying reaction scheme of allosteric inhibition is as follows:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_1 \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P \qquad\qquad E + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_2$$

$$C_2 + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} C_3 \qquad\qquad C_1 + I \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} C_3.$$

See Keener and Sneyd [8], Subsection 1.2.3 for more details. As above, in contrast to [8] we include reversible product formation, allowing $k_{-2} \geq 0$. In this model, an inhibitor bound to the enzyme does not affect the binding of substrate. The catalytic formation of product is slowed down, however, because the enzyme-substrate-inhibitor complex cannot degrade directly to yield product. Again we choose e_0 as "small parameter".

The relevant initial conditions are given by $e(0) = e_0 > 0$, $s(0) = s_0 > 0$, $i(0) = i_0 > 0$ and $c_1(0) = c_2(0) = c_3(0) = p(0) = 0$. With mass action kinetics one obtains a seven-dimensional differential equation. Elimination of e, p and i via the conservation laws $s + p + c_1 + c_3 = s_0$, $i + c_2 + c_3 = i_0$ and

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$e + c_1 + c_2 + c_3 = e_0$ yields

$$\dot{s} = (k_{-1} + k_1 s,\ 0,\ k_1 s + k_{-1}) \begin{pmatrix} c_1 \\ c_2 \\ c_3 \end{pmatrix} + e_0(-k_1 s)$$

$$\begin{pmatrix} \dot{c_1} \\ \dot{c_2} \\ \dot{c_3} \end{pmatrix} = A(s) \cdot \begin{pmatrix} c_1 \\ c_2 \\ c_3 \end{pmatrix} + \begin{pmatrix} k_{-2}(c_1 + c_2 + c_3)(c_1 + c_3) + k_3 c_1 (c_2 + c_3) \\ k_3(c_1 + c_2 + c_3)(c_2 + c_3) \\ -k_3 c_1 (c_2 + c_3) \end{pmatrix}$$
$$+ e_0 \left(\begin{pmatrix} k_1 s + k_{-2}(s_0 - s) \\ k_3 i_0 \\ 0 \end{pmatrix} + \begin{pmatrix} -k_{-2}(c_1 + c_3) \\ -k_3(c_2 + c_3) \\ 0 \end{pmatrix} \right)$$

The matrix

$$A(s) = \begin{pmatrix} \alpha_{11}(s) & -k_1 s - k_{-2}(s_0 - s) & -k_1 s - k_{-2}(s_0 - s) + k_{-3} \\ -k_3 i_0 & -k_3 i_0 - k_{-3} - k_1 s & -k_3 i_0 + k_{-1} \\ k_3 i_0 & k_1 s & -k_{-3} - k_{-1} \end{pmatrix}$$

where we abbreviated

$$\alpha_{11}(s) = -k_1 s - k_{-1} - k_{-2}(s_0 - s) - k_2 - k_3 i_0,$$

admits the eigenvalue $-(k_3 i_0 + k_{-3})$, as direct inspection shows. The characteristic polynomial is equal to

$$\chi(\tau) = (\tau + k_{-3} + k_3 i_0) \cdot (\tau^2 + a_1 \tau + a_2),$$

with abbreviations

$$\begin{aligned} a_1 &= k_3 i_0 + k_{-3} + 2(k_1 s + k_{-1}) + k_2 + k_{-2}(s_0 - s), \\ a_2 &= (k_1 s + k_{-1})(k_3 i_0 + k_{-3} + k_1 s + k_{-1}) + (k_1 s + k_{-1} + k_{-3}) \cdot \\ &\quad (k_2 + k_{-2}(s_0 - s)) \end{aligned}$$

Since $s \leq s_0$ throughout the reaction, a_1 and a_2 are positive, and by the Hurwitz-Routh criterion the quadratic polynomial admits only zeros with negative real part. One has

$$\begin{aligned} \det A(s) &= -(k_3 i_0 + k_{-3})((k_1 s + k_{-1})(k_3 i_0 + k_{-3} + k_2 + k_1 s + k_{-1}) + \\ &\quad k_2 k_{-3} + (s_0 - s)k_{-2}(k_1 s + k_{-3} + k_{-1})). \end{aligned}$$

In particular we have $\det A(s) < 0$ for $0 \leq s \leq s_0$. Theorem 2 is applicable and we obtain the reduced equation

$$\dot{s} = \frac{e_0 k_{-3}(k_2 k_1 s - k_{-2} k_{-1}(s_0 - s))(k_{-1} + k_{-3} + k_1 s + k_3 i_0)}{\det A(s)}, \quad 0 \leq s \leq s_0.$$
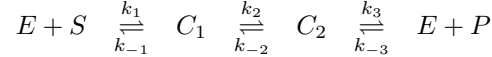
on the subspace $V = \{(s, 0, 0, 0);\ s \in \mathbb{R}\}$ which is a component of the zero set Y of $h^{(0)}$. This equation has rational right-hand side with quadratic numerator and denominator. For the irreversible case $k_{-2} = 0$ this reduced equation is the same as given in Keener and Sneyd [8], Section 1.5, by the ad hoc method (due

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to Corollary 1). While computations are a bit more involved here, the procedure is still straightforward. The ad-hoc approach seems no longer manageable in the reversible scenario.

Again one obtains an a posteriori justification for QSS of the three complexes when e_0 is small, and again incorporating product removal ab initio will lead to the expression for the irreversible case.

3.4   A variant of the Michaelis-Menten model

The following three-stage mechanism for an enzyme-catalyzed reaction, a variant of the basic Michaelis-Menten model, can for instance be found in Heinrich and Schuster [7], p. 18f. In contrast to the basic model one distinguishes an enzyme-substrate complex C_1 and an enzyme-product complex C_2 which change to each other reversibly. Thus we have the scheme

$$E + S \quad \overset{k_1}{\underset{k_{-1}}{\rightleftharpoons}} \quad C_1 \quad \overset{k_2}{\underset{k_{-2}}{\rightleftharpoons}} \quad C_2 \quad \overset{k_3}{\underset{k_{-3}}{\rightleftharpoons}} \quad E + P$$

At the start of the reaction we assume that only enzyme $e(0) = e_0 > 0$ and substrate $s(0) = s_0 > 0$ are present. Again, the small parameter will be chosen as e_0.

Mass action kinetics in conjunction with the conservation laws $e + c_1 + c_2 = e_0$ and $s + c_1 + c_2 + p = s_0$ leads to the following differential equation system for s, c_1 and c_2:

$$
\begin{aligned}
\dot{s} &= (k_{-1} + k_1 s, k_1) \begin{pmatrix} c_1 \\ c_2 \end{pmatrix} + e_0(-k_1 s) \\
\begin{pmatrix} \dot{c_1} \\ \dot{c_2} \end{pmatrix} &= A(s) \cdot \begin{pmatrix} c_1 \\ c_2 \end{pmatrix} + \begin{pmatrix} 0 \\ k_{-3}(c_1 + c_2)^2 \end{pmatrix} \\
&+ e_0 \left(\begin{pmatrix} k_1 s \\ -k_{-3}s + k_{-3}s_0 \end{pmatrix} + \begin{pmatrix} 0 \\ -k_{-3}(c_1 + c_2) \end{pmatrix} \right).
\end{aligned}
$$

Since $s \leq s_0$ throughout the course of the reaction, the matrix

$$A(s) := \begin{pmatrix} -k_1 s - k_{-1} - k_2 & -k_1 s + k_{-2} \\ k_2 - k_{-3}s_0 + k_{-3}s & -k_{-2} - k_{-3}s_0 + k_{-3}s - k_3 \end{pmatrix}$$

has negative trace and positive determinant

$$\det A(s) = k_{-3}(s_0 - s)(k_{-1} + k_2 + k_{-2}) + (k_3 + k_{-2})(k_1 s + k_{-1}) + k_1 k_2 s.$$

The zero set Y of $h^{(0)}$ has the subspace $V := \{(s, 0, 0,) \in \mathbb{R}^3\}$ as a component, and Theorem 2 is applicable. The reduced equation is given by
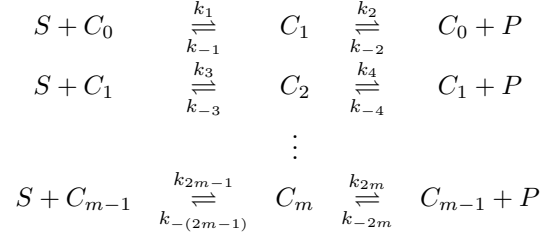
$$\dot{s} = -e_0 \frac{-k_{-3}k_{-1}k_{-2}(s_0 - s) + k_3 k_1 k_2 s}{\det A(s)}$$

for $0 \leq s \leq s_0$. The structure of this reduced equation (the right-hand side is a rational function with linear numerator and denominator) is the same as

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for the basic Michaelis-Menten mechanism; this is also noted in [7]. (As in the case of the basic Michaelis-Menten system, Heinrich and Schuster stop short of determining a reduced system explicitly.) Again, a QSS assumption for both complexes is justified for small e_0.

3.5   Cooperativity

In this section we consider an extension of the Michaelis-Menten model to an enzyme with m identical binding sites. We denote by $C_0 = E$ the enzyme with no substrate bound, while the enzyme with $1, \ldots, m$ substrate molecules bound will be denoted by $C_1, \ldots, C_m$ respectively. The irreversible system with two binding sites is discussed in Keener and Sneyd [8], Subsection 1.2.4, in a manner similar to the Michaelis-Menten system. The extension to reversible product formation in this particular case was given in [13], Section 4. Generally, with reversible product formation we have the following reaction scheme.

$$
\begin{array}{ccccc}
S + C_0 & \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} & C_1 & \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} & C_0 + P \\
S + C_1 & \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} & C_2 & \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} & C_1 + P \\
& & \vdots & & \\
S + C_{m-1} & \underset{k_{-(2m-1)}}{\overset{k_{2m-1}}{\rightleftharpoons}} & C_m & \underset{k_{-2m}}{\overset{k_{2m}}{\rightleftharpoons}} & C_{m-1} + P
\end{array}
$$

By mass action kinetics one obtains the following system of differential equations.

$$
\begin{aligned}
\dot{s} &= \sum_{j=0}^{m-1} \left(k_{-(2j+1)} c_{j+1} - k_{2j+1} c_j s \right), \\
\dot{c}_0 &= k_{-1} c_1 - k_1 c_0 s + k_2 c_1 - k_{-2} c_0 p, \\
\dot{c}_l &= k_{2l-1} c_{l-1} s - k_{-(2l-1)} c_l - k_{2l} c_l + k_{-(2l+1)} c_{l+1} - k_{2l+1} c_l s \\
& \quad + k_{2(l+1)} c_{l+1} + k_{-2l} c_{l-1} p - k_{-2(l+1)} c_l p \qquad \text{for } 1 \le l \le m-1, \\
\dot{c}_m &= k_{2m-1} s c_{m-1} - k_{-(2m-1)} c_m - k_{2m} c_m + k_{-2m} c_{m-1} p, \\
\dot{p} &= \sum_{j=0}^{m-1} \left(k_{2j+2} c_{j+1} - k_{-2(j+1)} c_j p \right).
\end{aligned}
$$

We assume that initially only the concentrations of unbound enzyme and substrate are nonzero: $c_0(0) = e_0 > 0$ and $s(0) = s_0 > 0$. Again e_0 will be our working choice of small parameter. Moreover we use the conservation laws to eliminate

$$
c_0 = e_0 - \sum_{j=1}^{m} c_j \quad \text{und} \quad p = s_0 - s - \sum_{j=1}^{m} j c_j.
$$

We introduce some abbreviations: Set

$$
A_1(s) = \begin{pmatrix}
-k_{-1} - k_2 - k_3 s & k_{-3} + k_4 & -k_1 s & -k_1 s & \cdots & -k_1 s \\
k_3 s & -k_{-3} - k_4 - k_5 s & k_{-5} + k_6 & 0 & \cdots & 0 \\
0 & k_5 s & -k_{-5} - k_6 - k_7 s & k_{-7} + k_8 & \cdots & 0 \\
0 & 0 & \ddots & \ddots & \ddots & 0 \\
\vdots & \vdots & \ddots & \ddots & \ddots & \vdots
\end{pmatrix}
$$

and

$$A_2(s) = \begin{pmatrix} -(k_{-2}+k_{-4})(s_0-s) & -k_{-2}(s_0-s) & \cdots & \cdots & \cdots & -k_{-2}(s_0-s) \\ k_{-4}(s_0-s) & -k_{-6}(s_0-s) & 0 & 0 & \cdots & 0 \\ 0 & k_{-6}(s_0-s) & -k_{-8}(s_0-s) & 0 & \cdots & 0 \\ 0 & 0 & \ddots & \ddots & \ddots & 0 \\ \vdots & \vdots & \ddots & \ddots & \ddots & \vdots \end{pmatrix},$$

and

$$A(s) = A_1(s) + A_2(s).$$

Moreover we define

$$B(s) = \begin{pmatrix} (k_{-1}-k_3 s) & (k_{-3}-k_5 s) & \cdots & k_{-2m+1} \end{pmatrix}, \quad u(s) = -k_1 s$$

and

$$v(s) = \begin{pmatrix} k_1 s + k_{-2}(s_0-s) \\ 0 \\ \vdots \\ 0 \end{pmatrix}, \quad \tilde{v}(c) = \begin{pmatrix} -k_{-2}\sum_{j=1}^m jc_j \\ 0 \\ \vdots \\ 0 \end{pmatrix}$$

and finally

$$q(c) = \begin{pmatrix} k_{-4}c_1\sum_{j=1}^m jc_j + k_{-2}\left(\sum_{j=1}^m jc_j\right)\left(\sum_{j=1}^m c_j\right) \\ (k_{-6}c_2-k_{-4}c_1)\sum_{j=1}^m jc_j \\ \vdots \\ (k_{-2(m+1)}c_m - k_{-2m}c_{m-1})\sum_{j=1}^m jc_j \end{pmatrix}, \quad c = \begin{pmatrix} c_1 \\ \vdots \\ c_m \end{pmatrix}$$

Then the system can be written in the form

$$\begin{aligned} \dot{s} &= B(s) \cdot c + e_0 \cdot u(s) \\ \dot{c} &= A(s) \cdot c + q(c) + e_0 \cdot (v(s) + \tilde{v}(c)). \end{aligned}$$

It seems arduous to discuss the spectrum of $A(s)$ for all $0 \le s \le s_0$, but one can readily see that, at least for s_0 not too big, all eigenvalues of $A(s)$ do have negative real part for small s: Indeed, $A_1(0)$ is an upper triangular matrix with negative diagonal elements, and $A_2(0) = s_0 \cdot A_2^*(0)$, whence $A(0)$ has only eigenvalues with negative real part for sufficiently small s_0. We will be satisfied with this relatively weak result, since it ensures consistency and local applicability of the reduction. The relevant component of the zero set Y of $h^{(0)}$ is the subspace

$$\{(s, 0, \ldots, 0); \, s \in \mathbb{R}\}$$

and the reduction is given by

$$\dot{s} = e_0 \cdot \left(u(s) - B(s)A(s)^{-1}v(s)\right)$$

$0 \le s \le s_0$. Since all entries of $A(s)$, $B(s)$, $u(s)$ and $v(s)$ are of degree ≤ 1 in s, one sees that the right-hand side of this equation is rational, with denominator $\det A(s)$ generically of degree m, and numerator of degree $\le m+1$. (For small m computations show that the numerator is actually of degree $\le m$; this is likely the case for all m.) Once more, incorporating product removal in the model ab initio will lead to the same reduction as the irreversible system.

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4 QSS without Tikhonov?

It is not obvious whether a quasi-steady state assumption, if justified, must necessarily lead to a singular perturbation setting. The initial quasi-steady state arguments were (understandably, from a historical perspective) not based on Tikhonov's theory, or even on small parameter considerations. Schauer and Heinrich [14] use a line of argument which is essentially different from "slow-fast" considerations (see also [12]). It seems that only with the work of Segel and Slemrod [17] time scale estimates and time scale arguments became the established method to identify "small parameters" for QSS.

In this short section we look at one example to indicate that the hypotheses for Tikhonov's theorem should carefully be taken into account. We discuss the *reverse QSSA* (rQSSA) (see Segel and Slemrod [17], Schnell and Maini [16]) for the irreversible Michaelis-Menten system

$$\begin{aligned} \dot{s} &= - \quad k_1 e_0 s + (k_1 s + k_{-1})(s_0 - (s + p)), \\ \dot{p} &= \quad k_2(s_0 - (s + p)). \end{aligned}$$

In rQSS substrate is assumed to be in steady state after a short initial phase, and appreciable formation of product should begin only after this initial phase. From a time scale discussion, Segel and Slemrod [17] obtain the condition

$$\delta := \frac{k_{-1}}{k_1 e_0} \ll 1$$

for rQSS to hold. In [13] it was shown that the hypotheses of Tikhonov's theorem are satisfied (after time scaling) if $1/e_0 \to 0$ while k_{-1} remains bounded and k_1 remains bounded away from 0. But it should be emphasized that $\delta \to 0$ generally implies neither the hypotheses for Tikhonov-Fenichel nor quasi-steady state for substrate. Indeed, assume that k_1 and e_0 remain bounded and bounded away from 0, and consider the case $k_{-1} \to 0$. This implies $\delta \to 0$, but $\varepsilon = k_{-1}$ is now the "small parameter". Rewriting the system in the appropriate form

$$\begin{aligned} \dot{s} &= - \quad k_1 e_0 s + k_1 s(s_0 - (s + p)) + \varepsilon(s_0 - (s + p)), \\ \dot{p} &= \quad k_2(s_0 - (s + p)), \end{aligned}$$

one sees that $h^{(0)}$ now has only an isolated stationary point $(0, s_0)$, and the hypotheses for Tikhonov-Fenichel are not fulfilled. Moreover, no rQSS is detectable in the following (arbitrarily chosen) numerical example, where all parameters were set equal to 1, except for $k_{-1} = 10^{-6}$. Figure 1 shows that, first and foremost, degradation of substrate and formation of product occur at about the same rate, and substrate does not quickly approach quasi-steady state prior to appreciable product formation. Second, one sees that the "approximate" product formation rate according to the ad hoc reduction, is hardly useful.

A general cautionary comment can be drawn from this example: Scaling frequently involves the lumping of several parameters into one "small parameter" (such as $\delta = k_{-1}/(k_1 e_0)$). This may cause problems, since various ways of letting δ approach 0 may lead to different results. By extension, this also
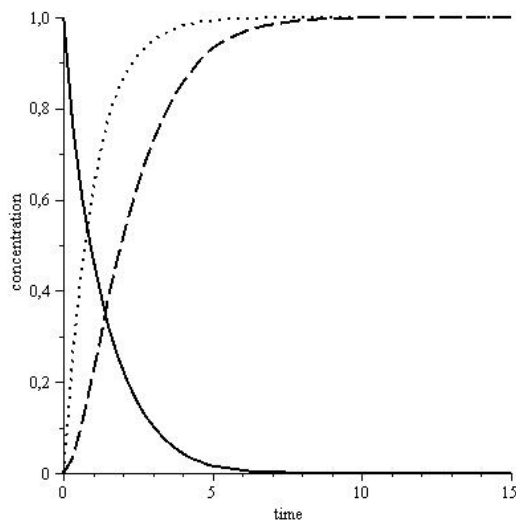
Figure 1: Numerical example with $e_0 = s_0 = 1$, $k_1 = k_2 = 1$ and $k_{-1} = 10^{-6}$. The graphs are for substrate (full), product according to the two-dimensional system (dashed), and product according to the ad-hoc reduction (dotted).

applies to the *total QSSA* introduced by Borghans et al. [3]. A more detailed case-by-case investigation seems necessary in such scenarios.

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