J.D. Murray

Mathematical Biology
I. An Introduction
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6. Reaction Kinetics

6.1 Enzyme Kinetics: Basic Enzyme Reaction

Biochemical reactions are continually taking place in all living organisms and most of them involve proteins called enzymes, which act as remarkably efficient catalysts. Enzymes react selectively on definite compounds called substrates. For example, haemoglobin in red blood cells is an enzyme and oxygen, with which it combines, is a substrate. Enzymes are important in regulating biological processes, for example, as activators or inhibitors in a reaction. To understand their role we have to study enzyme kinetics which is mainly the study of rates of reactions, the temporal behaviour of the various reactants and the conditions which influence them. Introductions with a mathematical bent are given in the books by Rubino (1975), Murray (1977) and the one edited by Segel (1985). Biochemically oriented books, such as Laidler and Bwung (1977) and Roberts (1977), go into the subject in more depth.

The complexity of biological and biochemical processes is such that the development of a simplifying model is often essential in trying to understand the phenomenon under consideration. For such models we should use reaction mechanisms which are plausible biochemically. Frequently the first model to be studied may itself be a model of a more realistic, but still too complicated, biochemical model. Models of models are often first steps since it is a qualitative understanding that we want initially. In this chapter we discuss some model reaction mechanisms, which mirror a large number of real reactions, and some general types of reaction phenomena and their corresponding mathematical realisations; a knowledge of these is essential when constructing models to reflect specific known biochemical properties of a mechanism.

Basic Enzyme Reaction

One of the most basic enzymatic reactions, first proposed by Michaelis and Menten (1913), involves a substrate $S$ reacting with an enzyme $E$ to form a complex $SE$ which in turn is converted into a product $P$ and the enzyme. We represent this schematically by

$$S + E \xrightarrow{k_1} SE \xrightarrow{k_2} P + E.$$  \hspace{1cm} (6.1)

Here $k_1$, $k_2$ and $k_3$ are constant parameters associated with the rates of reaction; they are defined below. The double arrow symbol $\xrightarrow{}$ indicates that the reaction is reversible.
while the single arrow $\rightarrow$ indicates that the reaction can go only one way. The overall mechanism is a conversion of the substrate $S$, via the enzyme catalyst $E$, into a product $P$. In detail it says that one molecule of $S$ combines with one molecule of $E$ to form one of $SE$, which eventually produces one molecule of $P$ and one molecule of $E$ again.

The Law of Mass Action says that the rate of a reaction is proportional to the product of the concentrations of the reactants. We denote the concentrations of the reactants in (6.1) by lowercase letters

$$s = [S], \quad e = [E], \quad c = [SE], \quad p = [P],$$

(6.2)

where $[\cdot]$ traditionally denotes concentration. Then the Law of Mass Action applied to (6.1) leads to one equation for each reactant and hence the system of nonlinear reaction equations

$$\frac{ds}{dt} = -k_1se + Lc, \quad \frac{de}{dt} = -k_2se + (k_3 + k_4)c,$$

$$\frac{dc}{dt} = k_1se - (k_3 + k_2)c, \quad \frac{dp}{dt} = k_2c,$$

(6.3)

The $k$'s, called rate constants, are constants of proportionality in the application of the Law of Mass Action. For example, the first equation for $s$ is simply the statement that the rate of change of the concentration $[S]$ is made up of a loss rate proportional to $[S][E]$ and a gain rate proportional to $[SE]$.

To complete the mathematical formulation we require initial conditions which we take here as those at the start of the process which converts $S$ to $P$, so

$$s(0) = s_0, \quad e(0) = e_0, \quad c(0) = 0, \quad p(0) = 0.$$  

(6.4)

The solutions of (6.3) with (6.4) then give the concentrations, and hence the rate of the reactions, as functions of time. Of course in any reaction kinetics problem we are only concerned with nonnegative concentrations.

The last equation in (6.3) is uncoupled from the first three; it gives the product

$$p(t) = k_2 \int_0^t c(t')dt',$$

(6.5)

once $c(t)$ has been determined, so we need only be concerned (analytically) with the first three equations in (6.3).

In the mechanism (6.1) the enzyme $E$ is a catalyst, which only facilitates the reaction, so its total concentration, free plus combined, is a constant. In this conservation law the concentration $c_0$ for the enzyme also comes immediately from (6.3) on adding the 2nd and 3rd equation, those for the free (e) and combined (c) enzyme concentrations respectively, to get

$$\frac{dc}{dt} + \frac{de}{dt} = 0 \implies c(t) + e(t) = c_0,$$

(6.6)
using the initial conditions \((t, x, c)\). With this, the system of ordinary differential equations reduces to only two, for \(x = \frac{dx}{dt}\) and \(c = \frac{dc}{dt}\), namely,

\[
\frac{dx}{dt} = -k_1 x + (k_1 x + k_{-1}) c, \\
\frac{dc}{dt} = k_2 c - (k_{-1} x + k_2 + k_{-2}) c, \tag{6.7}
\]

with initial conditions

\(x(0) = x_0, \quad c(0) = 0. \tag{6.8}\)

The usual approach to these equations is to assume that the initial stage of the complex, \(c\), formation is very fast after which it is essentially at equilibrium, that is, \(dc/dt \approx 0\) in which case from the second of (6.7) we get \(c\) in terms of \(x\),

\[c(t) = \frac{k_2 x(t)}{k_1 + k_2} \tag{6.9}\]

which on substituting into the first of (6.7) gives

\[
\frac{dx}{dt} = -\frac{k_1 x(t)}{k_1 + k_2} \tag{6.10}
\]

where \(K_m\) is called the Michaelis constant. Since the enzyme is traditionally considered to be present in small amounts compared with the substrate the assumption is that the substrate concentration effectively does not change during this initial transient stage. In this case (the approximate) dynamics is governed by (6.10) with the initial condition \(x = x_0\). This is known as the pseudo- or quasi-steady state approximation. Solving (6.10) with the initial condition on \(x(t)\) we obtain an implicit solution, namely,

\[x(t) + K_m \ln x(t) = x_0 + K_m \ln x_0 \tag{6.11}\]

If we now substitute this into (6.9) we get an expression for the complex \(c(t)\). But this does not satisfy the initial condition on \(c(t)\) in (6.8). However, perhaps it is a reasonable approximation for most of the time; this is the belief in the usual application of this approach. In fact for many experimental situations it is sufficient, but crucially not always. There are in fact two timescales involved in this system: one is the initial transient timescale near \(t = 0\) and the other is the longer timescale when the substrate changes significantly during which the enzyme complex is reasonably approximated by (6.9) with \(x(t)\) from (6.11). This basic reasoning raises several important questions such as (i) how fast is the initial transient; (ii) for what range of the parameters is the approximation (6.9) and (6.11) a sufficiently good one; (iii) if the enzyme concentration is not small compared with the substrate concentration, how do we deal with it? Other questions arise, and are also dealt with, later. As a first step we must clearly nondimensionalise the system. There are several ways this can be done, of course. A key
dimensionless quantity is the time since the basic assumptions above depend on how short the transient period is. The standard way of doing the quasi-steady state analysis is to introduce dimensionless quantities

\[
\tau = \frac{k_1}{m_1} t, \quad \eta(t) = \frac{u(t)}{n_0}, \quad \psi(t) = \frac{v(t)}{n_0}
\]

which is a reasonable nondimensionalisation if \( \epsilon \ll 1 \). Substituting these into (6.7) together with (6.8) gives the dimensionless system for the traditional quasi-steady state approximation

\[
d\eta = -\eta + (u + K - \lambda)\psi, \quad d\psi = u - (u + K)\psi.
\]

\[
a(0) = 1, \quad \psi(0) = 0
\]

Note that \( K - \lambda > 0 \) from (6.2). With the solutions \( \eta(t) \), \( \psi(t) \) we then immediately get \( u \) and \( v \) from (6.6) and (6.5) respectively.

From the original reaction (6.1), which converts \( S \) into a product \( P \), we clearly have the final steady state \( u = 0 \) and \( \psi = 0 \); that is both the substrate and the substrate-enzyme complex concentrations are zero. We are interested here in the time evolution of the reaction so we need the solutions of the nonlinear system (6.13), which we cannot solve analytically in a simple closed form. However, we can see what \( u(t) \) and \( v(t) \) look like qualitatively. Near \( \tau = 0, du/d\tau < 0 \) so \( u \) decreases from \( u = 1 \) and since there \( dv/d\tau > 0 \), \( v \) increases from \( v = 0 \) and continues to do so until \( v = u/(u + K) \), where \( dv/d\tau = 0 \) at which point, from the first of (6.13), \( u \) is still decreasing. After \( v \) has reached a maximum it then decreases ultimately to zero as \( u \), which does so monotonically for all \( \tau \). The dimensional enzyme concentration \( e(t) \) first decreases from \( e_0 \) and then increases again to \( e_0 \) as \( \tau \to \infty \). Typical solutions are illustrated in Figure 6.1 below. Quite often a qualitative feel for the solution behaviour can be obtained from just looking at the equations; it is always profitable to try.

6.2 Transient Time Estimates and Nondimensionalisation

It is widespread in biology that the remarkable catalytic effectiveness of enzymes is reflected in the small concentrations needed in their reactions as compared with the concentrations of the substrates involved. In the Michaelis-Menten model in dimensionless form (6.13); this means \( \epsilon = v_0/so \ll 1 \). However, as mentioned above, it is not always the case that \( v_0/so \ll 1 \). Segel (1988) and Segel and Slemrod (1989) extended the traditional analysis with a new nondimensionalisation which includes this case but which also covers the situation where \( u/n = O(1) \). It is their analysis which we now describe.

We first need estimates for the two timescales, the fast transient, \( \tau_c \), and the longer, or slow, time, \( \tau \), during which \( s(t) \) changes significantly. During the initial transient the
complex $c(t)$ increases rapidly while $s(t)$ does not change appreciably so an estimate of this fast timescale is obtained from the second of (6.7) with $s(t) = s_0$, that is,

$$\frac{dc}{dt} = k_1c(t) - k_1(s_0 + K_m)c.$$  

(6.14)

The solution involves an exponential, the timescale of which is

$$t_c = \frac{1}{k_1(s_0 + K_m)}.$$  

(6.15)

To estimate the long timescale, $t_l$, in which $s(t)$ changes significantly we take the maximum change possible in the substrate, namely, $s_0$, divided by the size of the maximum rate of change of $s(t)$ given by setting $s = s_0$ in (6.10). So,

$$t_l \approx \frac{s_0}{\frac{ds}{dt}_{\text{max}}} = \frac{s_0 + K_m}{k_{2+g}}.$$  

(6.16)

One assumption on which the quasi-steady state approximation is valid is that the fast initial transient time is much smaller than the long timescale when $s(t)$ changes noticeably which means that necessarily $t_c \ll t_l$. With the expressions (6.15) and (6.16), this requires the parameters to satisfy

$$\frac{k_{2+g}}{k_1(s_0 + K_m)^2} \ll 1.$$  

(6.17)

Another requirement of the quasi-steady state approximation is that the initial condition for $s(t)$ can be taken as the first of (6.8). This means that the substrate depletes of $\Delta s(t)$ during the fast transient is only a small fraction of $s_0$ that is, $|\Delta s(t)| \ll 1$. An overestimate of $\Delta s(t)$ is given by the maximum rate of depletion possible from the first of (6.7), which is $k_{2+g}c_0$ multiplied by $t_c$. So, dividing this by $s_0$ gives the following requirement on the parameters,

$$\varepsilon = \frac{c_0}{s_0 + K_m} \ll 1.$$  

(6.18)

But condition (6.17), with $K_m$ from (6.9), can be written as

$$\frac{c_0}{s_0 + K_m} \cdot \frac{1}{1 + (s_1/k_2) + (s_0k_1/k_2)} \ll 1$$  

(6.19)

so the condition in (6.19) is more restrictive than (6.18) which is therefore the condition that guarantees the quasi-steady state approximation. With this condition we see that even if $c_0/s_0 = O(1)$, condition (6.19) can still be satisfied if $K_m$ is large as is actually the case in many reactions.

Since the nondimensionalisation depends crucially on the timescales we are focusing on, we have two timescales, $t_c$ and $t_l$, from which we can choose. Which we use depends on where we want the solution; with $t_c$ we are looking at the region near $t = 0$.
while with $r_i$ we are interested in the long timescale during which $f(t)$ changes significantly. A problem which involves two such timescales is generally a singular perturbation problem for which there are standard techniques (see, for example, the small book by Murray (1984). We carry out in detail, the singular perturbation analysis for such a problem in the following section.

If we use the fast transient timescale $t_e$ from (6.15) we introduce the following nondimensional variables and parameters,

$$u(t) = \frac{f(t)}{t_0}, \quad v(t) = \frac{(s_0 + K_m)c(t)}{s_0} \quad \tau = \frac{t}{t_e} = k_i(s_0 + K_m),$$

$$K_m = \frac{k_1}{k_2}, \quad \rho = \frac{k_2}{k_1}, \quad \sigma = \frac{\tau_0}{K_m}, \quad \tau = \frac{c_0}{K_m + \rho}$$

(6.20)

which on substituting into (6.7) and (6.8) give

$$\frac{du}{d\tau} = \left[ -u + \frac{\sigma}{1 + \sigma} \mu - \frac{\rho}{(1 + \sigma)(1 + \rho)} \right]$$

$$\frac{dv}{d\tau} = \left[ u - \frac{\sigma}{1 + \sigma} \mu^2 - \frac{\rho}{1 + \sigma} \right]$$

$$w(0) = 1, \quad v(0) = 0.$$  

(6.21)

With the long or slow timescale, $t_s$, we nondimensionalise the time by writing

$$T = (1 + \rho)r/T_e = \frac{(1 + \rho)\exp t}{s_0 + K_m} \approx \exp((1 + \rho)k_i T).$$

(6.22)

The reason for the scale factor $(1 + \rho)$ is simply for algebraic simplicity. With the dimensionless form in (6.20) but with the dimensionless time $T$ from the last equation, the model equations (6.7) become

$$\frac{du}{dT} = -(1 + \sigma)u + \sigma \mu v + \frac{\rho}{1 + \rho}$$

$$\frac{dv}{dT} = \left[ (1 + \sigma)u - \sigma \mu v - \nu \right]$$

(6.23)

We should keep in mind that the system we are investigating is (6.7). The three equation systems (6.13), (6.21) and (6.23) are exactly the same; they only differ in the way we nondimensionalised them. Important though this is, they both have the small parameter, $\varepsilon$, but it appears in the equations in a different place. Where a small parameter appears determines the analytical procedure we use. We discuss a specific example in the next section and introduce asymptotic or singular perturbation, techniques. These very powerful techniques provide a uniformly valid approximate solution for all time which is a remarkably good approximation to the exact solution of the system.

Before leaving the topic of nondimensionalisation it is relevant to ask what we must do if the enzyme $u_i$ is in excess such that $\varepsilon$ in (6.20) is not small. This occurs in various enzyme reactions but also arises in a quite different situation involving T-cell proliferation in response to an antigen. This was studied by De Boer and Perelson (1994).
Here the 'substrate' is a replicating cell, the 'enzyme' the site on the antigen-presenting cell and the 'complex' is the bound T-cell and antigen-presenting cell. The kinetics is represented by

\[ E + S \xrightleftharpoons{k_1}{k_2} C + E + 25. \]

Borgans et al. (1996) investigated this reaction system in which the enzymes is in excess and extended the above analysis to obtain a uniformly valid asymptotic solution. They did this by replacing the substrate, \( s \) in equation (6.7) by the equation for the total substrate,

\[ H(t) = h(t) + h(t) \]

which is given by adding the first and third equations in (6.3). The system they studied is this equation for \( s(t) \) and the third of (6.3) written in terms of \( c \) and \( i \), together with the boundary conditions. It is

\[ \frac{di}{dt} = -kc, \quad \frac{dc}{dt} = ki [(e_0 - c(t)) - K_{m}], \quad i(0) = i_0, \quad c(0) = 0. \]

The analysis is a little more involved but the concepts are similar. They derive conditions for an equivalent quasi-steady state approximation and discuss several examples including a general class of predator-prey models.

6.3 Michaelis–Menten Quasi-Steady State Analyis

Here we carry out a singular perturbation analysis on one of the above possible dimensionless equation systems. The technique can be used on any of them but to be specific we carry out the detailed pedagogical analysis on (6.13) to explain the background reasoning for the technique and show how to use it. We thereby obtain a very accurate approximate, or rather asymptotic solution to (6.13) for \( 0 < \varepsilon \ll 1 \). Before doing this we should reiterate that the specific nondimensionalisation (6.12) is only one of several we could choose. In the following section we analyse a system, a somewhat more complex one, which arises in a class of practical enzyme reactions using the more general formulation, since there \( e_0/n_0 \) is not small but \( K_m \) is sufficiently large that \( \varepsilon \) as defined in (6.20) is small. Another practical reaction in which it is the large Michaelis constant \( K_m \) which makes \( 0 < \varepsilon \ll 1 \) was studied by Frenzen and Maiti (1988), who used the same type of analysis we discuss in the case study in Section 6.4.

Let us consider then the system (6.13). Suppose we simply look for a regular Taylor expansion solution to \( u \) and \( v \) in the form

\[ u(t; \varepsilon) = \sum_{n=0}^{\infty} \varepsilon^n u_n(t), \quad v(t; \varepsilon) = \sum_{n=0}^{\infty} \varepsilon^n v_n(t), \]

which, on substituting into (6.13) and equating powers of \( \varepsilon \), gives a sequence of differential equations for the \( u_n(t) \) and \( v_n(t) \). In other words we assume that \( u(t; \varepsilon) \) and
$v(t; \varepsilon)$ are analytic functions of $t$ as $\varepsilon \to 0$. The O(1) equations are

$$
\frac{du}{dt} = -u_0 + (u_0 + K - \lambda)u_0,
0 = u_0 - (u_0 + K)v_0,
$$
$$
u_0(0) = 1, \quad v_0(0) = 0.
$$
(6.25)

We can already see a difficulty with this approach since the second equation is simply algebraic and does not satisfy the initial condition; in fact if $v_0 = 1, \ y_0 = 1/(1 + K) \neq 0$. If we solve (6.25)

$$
u_0 = \frac{u_0}{u_0 + K} \quad \Rightarrow \quad \frac{du}{dt} = -u_0 + (u_0 + K - \lambda)u_0 \quad \frac{u_0}{u_0 + K} = -\lambda u_0 \quad \frac{u_0}{u_0 + K}
$$

and so

$$
u_0(t) + K \ln u_0(t) = A - \lambda t,
$$

which is the same $u_0(6.11)$. If we require $u_0(0) = 1$ then $A = 1$. Thus we have a solution $u_0(t)$, given implicitly, and the corresponding $u_0(t)$.

$$
u_0(t) + K \ln u_0(t) = 1 - \lambda t, \quad u_0(t) = \frac{u_0(t)}{u_0(t) + K},
$$

(6.26)

which is the same as the solution (6.9). However, this solution is not a uniformly valid approximate solution for all $\tau \geq 0$ since $v_0(0) \neq 0$. This is not surprising since (6.25) involves only one derivative; it was obtained on setting $\varepsilon = 0$ in (6.13). The system of equations (6.25) has only one constant of integration from the O(1)-equation so it is not surprising that we cannot satisfy initial conditions on both $u_0$ and $v_0$.

The fact that a small parameter $0 < \varepsilon \ll 1$ multiplies a derivative in (6.13) indicates that it is a singular perturbation problem. One class of such problems is immediately recognized if, on setting $\varepsilon = 0$, the order of the system of differential equations is reduced; such a reduced system cannot in general satisfy all the initial conditions. Singular perturbation techniques are very important and powerful methods for determining asymptotic solutions of such systems of equations for small $\varepsilon$. Asymptotic solutions are usually more accurately approximate approximations to the exact solutions. A practical and elementary discussion of some of the key techniques is given in Murray's (1984) book on asymptotic analysis. In the following, the philosophy and a-nula technique of the singular perturbation method is described in detail and the asymptotic solution to (6.13) for $0 < \varepsilon \ll 1$ is derived. The main reason for doing this is to indicate when we can neglect the $\varepsilon$-terms in practical situations.

Since the solution (6.26), specifically $u_0(t)$, does not satisfy the initial conditions (and inclusion of higher-order terms in $\varepsilon$ cannot remedy the problem) we must conclude that at least one of the solutions $u(t; \varepsilon)$ and $v(t; \varepsilon)$ is not an analytic function of $\varepsilon$ as $\varepsilon \to 0$. By assuming $u(t; \varepsilon) = O(\varepsilon)$ (6.25) tacitly assumed $u(t; \varepsilon)$ to be analytic, (6.24) also requires analyticity of course. Since the initial conditions $u(0) = 0$ could not be satisfied because we neglected $\varepsilon t$ we must therefore retain the term in our analysis, at least near $t = 0$. So, a more appropriate timescale near $t = 0$ is
\[ \sigma = \tau / \varepsilon \] rather than \( \tau \); this makes \( d\varepsilon / d\tau = d\varepsilon / d\sigma \). The effect of the transformation \( \sigma = \tau / \varepsilon \) is to magnify the neighbourhood of \( \tau = 0 \) and let us look at this region more closely since, for a fixed \( 0 < \tau < 1 \), we have \( \sigma \gg 1 \) as \( \varepsilon \to 0 \). This is, a very small neighbourhood near \( \tau = 0 \) corresponds to a very large domain in \( \sigma \). We now use this to analyse (6.13) near \( \tau = 0 \), after which we shall get the solution away from \( \tau = 0 \) and finally show how to get a uniformly valid solution for all \( \tau \geq 0 \).

With the transformations

\[ \sigma = \tau / \varepsilon, \quad u(\tau; \varepsilon) = U(\tau; \sigma) \quad \varepsilon(\tau; \varepsilon) = \epsilon V(\tau; \sigma) \]  

(6.27)

the equations in (6.13) become

\[ \frac{dU}{d\sigma} = -\varepsilon U + \varepsilon(U + K - \lambda) V, \quad \frac{dV}{d\sigma} = U - (U + K) V, \]

(6.28)

If we now set \( \varepsilon = 0 \) to get the \( O(1) \) system is a regular perturbation solution

\[ U(\tau; \sigma) = \sum_{n=0}^{\infty} \varepsilon^n U_n(\sigma), \quad V(\tau; \sigma) = \sum_{n=0}^{\infty} \varepsilon^n V_n(\sigma), \]

(6.29)

we get

\[ \frac{dU_0}{d\sigma} = 0, \quad \frac{dV_0}{d\sigma} = U_0 - (U_0 + K) V_0, \]

(6.30)

\[ U_0(0) = 1, \quad V_0(0) = 0 \]

which is not of lower order than the original system (6.28). The solution of (6.30) is

\[ U_0(\sigma) = \frac{1}{1 + K}, \quad V_0(\sigma) = \frac{1}{1 + K} (1 - \exp[-(1 + K)\sigma]). \]

(6.31)

The last solution cannot be expected to hold for all \( \tau \geq 0 \), since if it did it would mean that \( d\varepsilon / d\tau = d\varepsilon / d\sigma \) is \( O(1) \) for all \( \tau \). The part of the solution given by (6.31) is the singular or inner solution for \( \varepsilon \) and \( u \) and is valid for \( 0 \leq \tau \ll 1 \), while (6.26) is the nonsingular or outer solution valid for all \( \tau \) in the immediate neighbourhood of \( \tau = 0 \). If we now let \( \varepsilon \to 0 \) we have for a fixed \( 0 < \tau < 1 \), however small, \( \sigma \to \infty \). Thus in the limit of \( \varepsilon \to 0 \) we expect the solution (6.26) as \( \tau \to 0 \) to be equal to the solution (6.31) as \( \sigma \to \infty \); that is, the singular solution as \( \sigma \to \infty \) matches the nonsingular solution as \( \tau \to 0 \). This is the essence of matching in singular perturbation theory. From (6.31) and (6.26) we see in fact that

\[ \lim_{\sigma \to \infty} [U_0(\sigma), V_0(\sigma)] = \lim_{\varepsilon \to 0} [u(\varepsilon), v(\varepsilon)]. \]

(6.32)

Figure 6.3 illustrates the solution \( u(\tau) \) and \( v(\tau) \), together with the dimensionless enzyme concentration \( e/e_0 \) given by the dimensionless form of (6.6); namely, \( e/e_0 = \)
1 - \nu(t). The thin \( G(t) \) layer near \( \tau = 0 \) is sometimes called the boundary layer and is the \( \tau \)-domain where there are very rapid changes in the solution. Here, from (6.31),

\[
\frac{dV}{dt} \bigg|_{t=0} = \varepsilon^{-1} \frac{dV}{d\sigma} \bigg|_{\sigma=0} = \varepsilon^{-1} \gg 1.
\]

Of course from the original system (6.13) we can see this from the second equation and the boundary conditions.

To proceed in a systematic singular perturbation way, we first look for the outer solution of the full system (6.13) in the form of a regular series expansion (6.24). The sequence of equations is then

\[
O(1): \quad \frac{d\nu_0}{d\tau} = -\nu_0 + (\omega + K - \lambda)\nu_0, \quad 0 = \nu_0 - (\omega + K)\nu_0, \\
O(\varepsilon): \quad \frac{d\nu_1}{d\tau} = \nu_1 (\nu_0 \sim 1) + (\omega + K - \lambda)\nu_1,
\]

which are valid for \( \tau > 0 \). The solutions involve undetermined constants of integration, one at each order, which have to be determined by matching these solutions as \( \tau \to 0 \) with the singular solutions \( \nu_0, \sigma \to \infty \).

The sequence of equations for the singular part of the solution, valid for \( 0 \leq \tau \ll 1 \), is given on substituting (6.29) into (6.28) and equating powers of \( \varepsilon \); namely,

\[
O(1): \quad \frac{dU_0}{d\sigma} = 0 \quad \frac{dV_0}{d\sigma} = U_0 - (U_0 + K)\nu_0, \\
O(\varepsilon): \quad \frac{dU_1}{d\sigma} = -U_1 + (V_0 + K - \lambda)\nu_0, \quad \frac{dV_1}{d\sigma} = (1 - \nu_1)U_1 - (V_0 + K)V_1,
\]

and so on. The solutions of these must satisfy the initial conditions at \( \sigma = 0 \); that is, \( \tau = 0 \).
1 = U(0; \epsilon) = \sum_{n=0}^{\infty} \epsilon^n U_n(0) \Rightarrow U_0(0) = 1, \quad U_{n>0}(0) = 0.
0 = V(0; \epsilon) = \sum_{n=0}^{\infty} \epsilon^n V_n(0) \Rightarrow V_{n>0}(0) = 0.  \tag{6.34}

In this case the singular solutions of (6.33) are determined completely. This is not generally the case in singular perturbation problems (see, for example, Murray 1964). Matching of the inner and outer solutions requires choosing the undetermined constants of integration in the solutions of (6.32) so that to all orders of \epsilon,
\lim_{\epsilon \to 0} \{U(\sigma; \epsilon), V(\sigma; \epsilon, \tau; \epsilon)\} = \lim_{\epsilon \to 0} \{u(\tau; \epsilon), v(\tau; \epsilon)\}. \tag{6.35}

Formally from (6.32), but as we had before,

$$u_0(\tau) + K \ln u_0(\tau) = A - \lambda \tau, \quad u_0(\tau) = \frac{u_0(\tau)}{u_0(\tau) + K},$$

where A is the constant of integration we must determine by matching. The solution of the first of (6.33) with (6.34) has, of course, been given before in (6.31). We get it now by applying the limiting process (6.35) to (6.31) and the last equations

\begin{align*}
\lim_{\epsilon \to 0} \frac{v_0(\sigma)}{1 + \epsilon} &= \lim_{\epsilon \to 0} \frac{u_0(\tau)}{1 + \epsilon} \\
= \eta_0(0) &= \frac{1}{1 + K} = \frac{u_0(0)}{u_0(0) + K} \\
\Rightarrow u_0(0) &= 1 \\
A &= 1.
\end{align*}

We thus get the uniformly valid asymptotic solution for 0 < \epsilon \ll 1 to O(1), derived heuristically before and given by (6.26) for \tau = 0 and (6.35) for 0 < \tau \ll 1, although the singular part of the solution is more naturally expressed in terms of 0 < \tau / \epsilon \ll 1.

We can now proceed to calculate \nu_1(\sigma) and \nu_2(\sigma) from (6.33) and \nu_1(\tau) and \nu_2(\tau) from (6.32) and so on to any order in \epsilon; the solutions become progressively more complicated even though all the equations are linear. In this way we get a uniformly valid asymptotic solution for 0 < \epsilon \ll 1 for all \tau \geq 0 of the nonlinear kinetics represented by (6.13). In summary to O(1) for small \epsilon,

\begin{align*}
u(\tau; \epsilon) &= u_0(\tau) + O(\epsilon), \quad u_0(\tau) + K \ln u_0(\tau) = 1 - \lambda \tau, \\
u(\tau; \epsilon) &= V_0(\sigma) + O(\epsilon), \quad V_0(\sigma) = \frac{1}{1 + \frac{1}{1 + K} \exp \left[-(1 + K) \frac{\sigma}{\epsilon}\right]}, \\
0 < \epsilon \ll 1, \quad \epsilon \ll 1, \quad 0 < \epsilon \ll 1. \tag{6.36}
\end{align*}
Since in most biological applications \(0 < \varepsilon \ll 1\), we need only evaluate the \(O(1)\) terms; the \(O(\varepsilon)\) terms’ contributions are negligible.

To complete the analysis of the original kinetics problem (6.3) with (6.4), if we write the dimensionless product and free enzyme concentrations as

\[
z(t) = \frac{p(t)}{u_0}, \quad w(t) = \frac{e(t)}{e_0}
\]

then, using (6.36) for \(a\) and \(v\), (6.5) and (6.6) give

\[
z(t) = \lambda \int_0^t (e'(r)) dr', \quad w(t) = 1 - v(t).
\]

The rapid change in the substrate-enzyme complex \(x'\) takes place in dimensionless times \(t = O(\varepsilon)\) which is very small. The equivalent dimensional time \(t\) is also very short, \(O(1/k_{123})\) in fact, and for many experimental situations is not measurable. Thus in many experiments the singular solution for \(u(x)\) and \(v(x)\) is never observed. The relevant solution is then the \(O(1)\) outer solution \(u_0(t), u_0(t)\) in (6.26), obtained from the micromixing system (6.13) on setting \(\varepsilon = 0\) and satisfying only the initial condition on \(u(t)\), the substrate concentration. In other words we say that the reaction for the complex \(x(t)\) is essentially in a steady state, or mathematically that \(\partial x/\partial t \approx 0\). That is, the \(x\)-reaction is so fast it is more or less in equilibrium all times. This is the usual Michaelis and Menten’s pseudo- or quasi-steady state hypothesis.

The form of (6.13) is generally like

\[
\frac{du}{dt} = f(u, v), \quad \frac{dv}{dt} = \varepsilon^{-1} g(u, v), \quad 0 < \varepsilon \ll 1,
\]

which immediately shows that \(dv/\varepsilon dt \geq 1\) if \(g(u, v)\) is not approximately equal to zero.

So the \(v\)-reaction is very fast compared with the \(u\)-reaction. The \(v\)-reaction reaches a quasi-steady state very quickly; which means that for times \(t = O(1)\) it is essentially at equilibrium and the model mechanism is then approximated by

\[
\frac{du}{dt} = f(u), \quad g(u, v) = 0, \quad u(0) = 1.
\]

If we solve the algebraic equation \(g(u, v) = 0\) to get \(v = h(u)\) then

\[
\frac{du}{dt} = f(u, h(u)),
\]

which is the rate or uptake equation for the substrate concentration. Moch modeling of biological processes hinges on qualitative assumptions for the uptake function \(f(u, h(u))\).

What is of interest biologically is the rate of reaction, or the rate of uptake; that is, \(dv/dt\) when \(v(t)\) has been found. This usually determined experimentally by measuring the dimensional substrate concentration \(x(t)\) at various times, then extrapolating back to \(t = 0\), and the magnitude \(r\) of the initial rate \(dv/dt\mid_0\) calculated. Since the time
measurements are almost always for \( r \gg 1 \), that is, \( r \gg 1/k_1r_0 \), which is usually of the order of seconds, the equivalent analytical rate is given by the non-singular or outer-solution. Thus, from the first of (6.36) the O(1) solution with \( 0 < r < 1 \) for the rate, \( r_0 \) say, is

\[
\frac{d\alpha(t)}{dt} = \lambda \frac{\alpha_0(0) + K_{s\alpha}}{i + K} \quad (6.40)
\]

In dimensional terms, using (6.12), the O(1) rate of reaction \( R_0 \) is

\[
R_0 = \frac{k_2 \alpha_0(0)}{i + K} = \frac{k_1 \alpha_0}{i + K}, \quad K_{s\alpha} = \frac{k_{-1} + k_1}{k_1}, \quad Q = (R_0)_{\text{max}} = k_2 \alpha_0 \quad (6.41)
\]

where \( Q \) is the maximum velocity, or rate, of the reaction and \( K_{s\alpha} \) is the Michaelis constant (6.9). This rate, based on the pseudo-steady state hypothesis, is what is usually wanted from a biological point of view. From (6.13), the exact initial rate for the substrate is \( d\alpha(0)/dt \bigg|_{t=0} = -1 \) while for the complex \( x \) it is \( d\alpha(0)/dt \bigg|_{t=0} = 1/s \).

When the uptake of substrate, or whatever, is described as a Michaelis-Menten uptake, what is understood is a rate of reaction like (6.41) and which is illustrated in Figure 6.2. The rate of reaction, which in fact varies with time, is the magnitude of \( d\alpha(t)/dt \) from the outer solution \( d\alpha(0)/dt \) and written in dimensional form. Thus the (Michaelis-Menten) uptake of \( S \) is governed by the equation

\[
\frac{dx}{dt} = -\frac{Qx}{K_{s\alpha} + s} \quad (6.42)
\]

This is simply the dimensional form of (6.39) (and the same as (6.10)) on carrying out the algebra for \( f(u,v) \) in (6.38), with (6.13) defining them. For \( s \ll K_{s\alpha} \) the uptake is linear in \( s \); the right-hand side of (6.42) is approximately \(-Q/K_{s\alpha}\). The maximum rate \( Q = k_2 \alpha_0 \) from (6.41), depends on the rate constant \( k_1 \) of the product reaction \( SE \rightarrow P + E \), this is called the rate limiting step in the reaction mechanism (6.1).

Useful and important as the quasi-steady state hypothesis is, something is lost by assuming \( ds/dt \) is negligible in (6.13) and by applying experimental results to a theory which cannot satisfy all the initial conditions. What can be determined, using experi-
mental results with a Michaelis–Menten theory, is a curve such as in Figure 6.2, which gives values for the maximum rate $V$ and the Michaelis constant $K_m$. This does not determine all three rate constants $k_1$, $k_2$, and $k_3$, only $k_2$ and a relationship between them all. To determine all of them, measurements for $t = O(t)$ would be required. Usually, however, the rate of uptake from the quasi-steady state hypothesis, that is, a Michaelis–Menten theory, is all that is required.
6.5 Cooperative Phenomena

In the model mechanism (6.1) one enzyme molecule combines with one substrate molecule; that is, the enzyme has one binding site. There are many enzymes which have more than one binding site for substrate molecules. For example, haemoglobin (Hb), the oxygen-carrying protein in red blood cells, has 4 binding sites for oxygen (O₂) molecules. A reaction between an enzyme and a substrate is described as cooperative if a single enzyme molecule, after binding a substrate molecule at one site, can then bind another substrate molecule at another site. Such phenomena are common.

Another important cooperative behaviour is when an enzyme with several binding sites is such that the binding of one substrate molecule at one site can affect the activity of binding other substrate molecules at another site. This indirect interaction between distinct and specific binding sites is called allosteric, or an allosteric effect, and an enzyme exhibiting it, an allosteric enzyme. If a substrate that binds at one site increases the binding activity at another site then the substrate is an activator; if it decreases the
activity it is an inhibitor. The detailed mathematical analysis for the kinetics of such allosteric reactions is given briefly in the book by Murray (1977) and in more detail in the one by Rubinstein (1975). The latter book also gives a graph-theoretic approach to enzyme kinetics.

As an example of a cooperative phenomenon we consider the case where an enzyme has 2 binding sites and calculate in equivalent quasi-steady state approximation and the substrate uptake function. A model for this consists of an enzyme molecule E which binds a substrate molecule S to form a single bound substrate-enzyme complex C1. This complex C1 not only breaks down to form a product P and the enzyme E again; it can also combine with another substrate molecule to form a dual bound substrate–enzyme complex C2. This C2 complex breaks down to form the product P and the single-bound complex C1. A reaction mechanism for this model is then

\[ S + E \xrightarrow{k_1} C_1 \xrightarrow{k_4} E + P, \]

\[ S + C_1 \xrightarrow{k_3} C_2 \xrightarrow{k_2} C_1 + P, \]

where the \( k_i \)'s are the rate constants as indicated.

With lowercase letters denoting concentrations, the mass action law applied to (6.98) gives

\[ \frac{dx}{dt} = -k_1 xe + (k_1 + k_4)c_1 + k_3c_2, \]

\[ \frac{dc_1}{dt} = k_1xe - (k_4 + k_2 + k_3)c_1 + (k_1 + k_4)c_2, \]

\[ \frac{dc_2}{dt} = k_3c_1 - (k_2 + k_4)c_2, \]

\[ \frac{dp}{dt} = -k_3 xc_1 + k_2c_2. \]  

Appropriate initial conditions are

\[ x(0) = x_0, \quad e(0) = e_0, \quad c_1(0) = c_2(0) = p(0) = 0. \]  

(6.100)

The conservation of the enzyme is obtained by adding the 2nd, 3rd and 4th equations in (6.99) and using the initial conditions; it is

\[ \frac{dc_1}{dt} + \frac{dc_2}{dt} + \frac{dp}{dt} = 0 \implies e + c_1 + c_2 = e_0. \]  

(6.101)

The equation for the product \( p(t) \) is again uncoupled and given, by integration, once \( c_1 \) and \( c_2 \) have been found. Thus, using (6.101), the resulting system we have to solve is
\[ \frac{ds}{dt} = -k_1y + (k_1 + k_2 + k_3)x + k_2x \]
\[ \frac{dc}{dt} = k_4y - (k_1 + k_2 + k_3)x + k_1x \]
\[ \frac{dt}{dt} = k_3x - (k_1 + k_2)x \]

(6.102)

with initial conditions (6.100).

As always, we nondimensionalise the system. As we saw above, there are several ways we can do this. If \( n_0/n_0 < 1 \), we write

\[ x = \frac{x}{n_0}, \quad y = \frac{y}{n_0}, \quad t = \frac{t}{n_0}, \quad u = \frac{u}{n_0}, \quad v_1 = \frac{v_1}{n_0} \]

(6.103)

and (6.102) becomes

\[ \frac{dx}{dt} = -u + (u - a_1u + a_2v_1 + (a_4 + u)v_2 = f(u, v_1, v_2). \]

(6.104)

\[ \frac{dv}{dt} = -u - (u + a_2u + a_1 + a_2)v_1 + (a_4 + a_2^2 - a)x = g_1(u, v_1, v_2). \]

(6.105)

\[ \frac{dt}{dt} = a_3u_1 - (a_4 + a_5)v_2 = g_2(u, v_1, v_2). \]

(6.106)

which, with the initial conditions

\[ u(0) = 1, \quad v_1(0) = v_2(0) = 0. \]

(6.107)

represents a well-posed mathematical problem.

This problem, just as the Michaelis-Menten one (6.13) analyzed in Section 6.5, is a singular perturbation one for \( 0 < \varepsilon \ll 1 \). The complete inner and outer solution can be found in a comparable way using the method set out there so we leave it as an exercise. What is of interest here, however, is the form of the uptake function for the substrate concentration \( u \), for times \( \tau \gg \varepsilon \), that is, for times in the experimentally measurable regime. So, we only need the outer, or noningular, solution which is given to \( O(1) \) for \( 0 < \varepsilon \ll 1 \) by (6.104)-(6.107) on setting the \( \varepsilon \)-terms to zero. This gives

\[ \frac{du}{dt} = f(u, v_1, v_2). \]

(6.104)

\[ g_1(u, v_1, v_2) = 0, \quad g_2(u, v_1, v_2) = 0. \]

The last two equations are algebraic, which on solving for \( v_1 \) and \( v_2 \) give

\[ v_2 = \frac{a_1u + a_3}{a_2u + a_3}, \quad v_1 = \frac{u}{a_1 + a_2 + a_3u^2(a_4 + a_5)^{-1}}. \]
Substituting these into \( f(u, v_1(u), v_2(u)) \) we get the uptake equation, or rate equation, for \( u \) as

\[
\frac{du}{dt} = f(u, v_1(u), v_2(u)) = -u \frac{a_1 + a_2 + u + a_3 u (a_4 + a_5)^{-1}}{a_1 + a_2 + u + a_3^2 (a_4 + a_5)^{-1}}
\]

(6.108)

The dimensionless velocity of the reaction is thus \( r(u) \). In dimensional terms, using (6.103), the Michaelis–Menten velocity of the reaction for \( 0 < e_9/e_9' < 1 \), denoted by \( R_0(u) \), say, is, from (6.108),

\[
R_0(u) = \frac{dS}{dt} \mid_{u=0} = e_9 e_9' \left( \frac{k_2 K_m + k_1 n}{K_m K_n + K_n' n_0 + \frac{e_9}{2}} \right)
\]

(6.109)

where \( K_m \) and \( K_m' \) are the Michaelis constants for the mechanism (6.98), equivalent to the Michaelis constant in (6.41).

The rate of the reaction \( R_0(u) \) is illustrated in Figure 6.5. If some of the parameters are zero there is a point of inflexion: for example, if \( k_2 = 0 \) it is clear from (6.109) since then for \( u_0 \) small, \( R_0 \propto u_0^2 \). A good example of such a cooperative behaviour is the binding of oxygen by haemoglobin; the experimental measurements give an uptake curve very like the lower curve in Figure 6.5. Myoglobin (Mb), a protein in abundance in red muscle fibres, on the other hand has only one oxygen binding site and its uptake is of the Michaelis–Menten form also shown in Figure 6.5 for comparison.

When a cooperative phenomenon in an enzymatic reaction is suspected, a Hill plot is often made. The underlying assumption is that the reaction velocity or uptake function is of the form

\[
R_0(S_0) = \frac{Q_0}{K_m + S_0^n},
\]

(6.110)

where \( n > 0 \) is not usually an integer; this is often called a Hill equation. Solving the

\[
\text{Figure 6.5. Rate of reaction, or substrate uptake, as a function of substrate concentration} \ n_0 \text{ for the cooperative reaction} \ (6.98). \text{Note the inflexion in the cooperative uptake curve when} \ k_2 = 0.
\]
last equation for \( k_n^0 \) we have
\[
s_0^n = \frac{R_o K_m}{Q - R_o} \Rightarrow n \ln n = \ln K_m + \ln \frac{R_o}{Q - R_o}
\]
A Hill plot is the graph of \( \ln(\frac{R_o}{Q - R_o}) \) against \( \ln s_0 \), the slope of which gives \( n \), and is a constant if the Hill equation is a valid description for the uptake kinetics. If \( n < 1, n = 1 \) or \( n > 1 \) we say that there is negative, zero or positive cooperativity respectively. Although the Hill equation may be a reasonable quantitative form to describe a reaction's velocity in a Michaelis–Menten sense, the detailed reactions which give rise to it are not too realistic: essentially it is (6.1) but now instead of \( E + S = E + S \) we require \( E + nS \) combining to form the complex in one step. This is somewhat unlikely if \( n \) is not an integer although it could be a stoichiometric form. If \( n \) is an integer and \( n \geq 2 \), the reaction is then trimolecular or higher. Such reactions do not occur except possibly through what is in effect a telescoping together of several reactions, because intermediary reactions are very fast.
Even with such drawbacks as regards the implied reaction mechanisms, empirical rate forms like the Hill equation are extremely useful in modelling. After all, what we want from a model is some understanding of the underlying dynamics and mechanisms governing the phenomena. A very positive first step is to find a biologically reasonable model which qualitatively describes the behaviour. Detailed refinements or amendments come later.

### 6.6 Autocatalysis, Activation and Inhibition

Many biological systems have feedback controls built into them. These are very important and we must know how to model them. In the next chapter on biological oscillators, we shall describe one area where they are essential. A review of theoretical models and the dynamics of metabolic feedback control systems is given by Tyson and Othmer (1978). Here we describe some of the more important types of feedback control. Basically feedback is when the product of one step in a reaction sequence has an effect on other reaction steps in the sequence. The effect is generally nonlinear and may be to activate or inhibit these reactions. The next chapter gives some specific examples with actual reaction mechanisms.

**Autocatalysis** is the process whereby a chemical is involved in its own production. A very simple pedagogical example is
\[
A + X \xrightarrow{k_1} 2X,
\]
where a molecule of \( X \) combines with one of \( A \) to form two molecules of \( X \). If \( A \) is maintained at a constant concentration \( a \), the Law of Mass Action applied to this reaction gives the rate of reaction as
\[
\frac{dx}{dt} = k_2 ax - k_{-1} x^2 \Rightarrow x(t) \rightarrow x_f = \frac{k_2 a}{k_{-1}},
\]
(6.111)