ENZYME KINETICS

Introduction

§1. As you read these lines thousands of chemical reactions are taking place in your body, in exquisite balance (most of the time). They keep you alive, allow you to sit, concentrate, and remember how to solve the differential equations of chemical kinetics. Most of these reactions are catalyzed by biological macromolecules called enzymes.

The simplest catalytic reaction can be written as

\[ \text{Cat} + \text{R} \rightleftharpoons \text{Cat} + \text{P} \]

where Cat represents the catalyst, R the reactants, and P the products. The catalyst binds chemically to the reactants and the products but it is neither produced nor consumed in the net reaction. Because Cat appears in both the initial and final state in equal amounts, its presence does not affect chemical equilibrium. This means that the catalyst cannot change the maximum yield of a reaction: it only changes the rate of getting there. It changes the rate constant, but does not modify the equilibrium constant.

Over 1500 enzymes have already been discovered and you can image that there is considerable variation among them. But there are some common features. Most enzymes are proteins, which are polymers formed by amino acids. The protein
is folded to form a fairly compact structure that has a cleft in it. The chemical reaction takes place on an active site inside the cleft. The reactants diffuse in the cleft, bind to the active site, and undergo a reaction to form a product that leaves the cleft.

A man-made hydrogenation catalyst may turn acetylene into ethylene, but it will also produce ethane. It is very difficult to find a catalyst that produces ethylene only. The ability of a catalyst to perform one, and only one, reaction is called selectivity. A selective catalyst does not waste reactant (e.g. acetylene) to make an unwanted compound (e.g. ethane), nor does it force us to spend money to separate the desired product from the one that is not required.

Enzymes are extremely selective catalysts. Not only do they catalyze one type of reaction only (e.g. hydrogenation), but they will perform it with only one specific molecule (e.g. ethylene but not propene). The selectivity of some enzymes is so high that they are capable of discriminating between left- and right-handed isomers. It is believed that selectivity is controlled mainly by the size, shape, and chemical nature of the cleft.

The rate of an enzymatic reaction depends on enzyme and reactant concentration, pH, and ionic strength. It can be strongly inhibited or activated by the presence of specific substances (called effectors). It depends strongly on temperature, through the usual Arrhenius dependence of the rate constant and through additional factors: the protein is destroyed by high temperature, while low temperature slows down the protein motion needed for performing the reaction.

The protein is not just an inert scaffold for the cleft and the active site. It sometimes changes during the reaction. There are examples in which changing one amino acid in the protein, at a remote location from the active site, causes substantial changes in catalytic activity. This demonstrates that in some cases the overall structure of the protein and its ability to change shape are important. Finally, it is believed that the shape and size of the cleft are flexible and change in time, and these can affect the yield and selectivity.

An enzyme is a very complex system whose function is still insufficiently understood. Nevertheless, run-of-the-mill phenomenological chemical kinetics can be used to provide a quantitative description of the reaction rate. This illustrates the power and weakness of this approach. It describes quantitatively the evolution of the concentrations, but all the interesting details are buried in the value of the rate constants. We can determine these constants, by fitting the data, but we do not understand what their value tells us about the dynamics of the system during the reaction.
The Michaelis–Menten Mechanism: Exact Numerical Solution

§2. The Mechanism. For the purpose of illustration I consider here the Michaelis–Menten mechanism, which is the simplest possible mechanism of enzymatic catalytic activity. This is described by two reactions:

\[ E + R \xrightarrow{k_1} C \]

and

\[ C \xrightarrow{k_2} P + E \]

E is the enzyme, R is the reactant, and C is the complex made when the reactant binds to the active center. The letters above or below the arrows specify the rate constants of the forward and backward reactions.

Biologists call the reactant the “substrate.” For a catalytic chemist, the substrate is the oxide on which the metallic clusters (the catalyst) are deposited. I use here the name “reactant,” which is more in keeping with the customs of physical chemists.

The first reaction (Eq. 9.1) is the formation of a complex between the reactant and the active center. In the second one (Eq. 9.2) the enzyme–reactant complex C is turned into the product, which leaves the cleft and returns the enzyme to its state prior to the reaction. This mechanism assumes that the product does not linger in the cleft, so we need not consider the existence of a complex between the active site and the product.

The first reaction is reversible: a reactant may form the complex C, and then detach itself from the active site and leave the cleft without reacting.

The rates of various processes, invoked by this mechanism, are controlled by the rate constants \( k_1 \), \( k_{-1} \), and \( k_2 \). \( k_1 \) controls the rate of active-site occupation to form C; \( k_{-1} \), that of active-site “liberation” to form E and R; and \( k_2 \), that of conversion of the complex C into the product P. Changes in pH, ionic strength, temperature, binding site, or protein cause changes in the magnitude of these constants.

Many enzymatic reactions follow a more complicated mechanism.

§3. The Rate Equations. Enzymes are very complicated and the task of describing the kinetics of the reactions they catalyze is intimidating. Nevertheless, we take the plunge and assume that they are described by the same rate laws as any other
reaction. Therefore, the rate of change of enzyme concentration is

\[ \frac{dE(t)}{dt} = -k_1 E(t)R(t) + k_{-1} C(t) + k_2 C(t) \]  

(9.3)

Here \( E(t) \), \( R(t) \), and \( C(t) \) are the concentrations (usually in mol/liter of solvent), at time \( t \), of the enzyme, the reactant, and the enzyme–reactant complex, respectively. In this equation, \(-k_1 E(t)R(t)\) is the rate of enzyme “consumption” by the formation of a reactant–active site complex \( C \); \( k_2 C(t) \) is the rate of enzyme regeneration because of product formation and release from the cleft; and \( k_{-1} C(t) \) is the rate of enzyme regeneration when the reactant leaves the active site.

The concentration of the complex \( C(t) \) changes with the rate

\[ \frac{dC(t)}{dt} = k_1 E(t)R(t) - k_{-1} C(t) - k_2 C(t) \]  

(9.4)

Here \( k_1 E(t)R(t) \) is the rate of complex formation by the combination of the enzyme with the reactant; \( k_{-1} C(t) \) is the rate of complex disappearance because the reactant leaves the cleft; and \( k_2 C(t) \) is the rate of complex “destruction” when the product is formed and leaves the cleft.

The enzyme concentration and that of the complex satisfy

\[ E(t) + C(t) = E(0) \]  

(9.5)

You can think of this equation as a mass balance for the amount of protein: initially all the protein was in the enzyme; at any time \( t \), some of it is in the enzyme and some in the complex. Because of Eq. 9.5, the rate of evolution of \( E(t) \) is the negative of the rate of evolution of \( C(t) \). For this reason, Eq. 9.4 is the same equation as Eq. 9.3 (from Eq. 9.5, \( \frac{dE(t)}{dt} = -\frac{dC(t)}{dt} \)).

The rate of product formation is

\[ \frac{dP(t)}{dt} = k_2 C(t) \]  

(9.6)

and that of reactant consumption is

\[ \frac{dR(t)}{dt} = -k_1 E(t)R(t) + k_{-1} C(t) \]  

(9.7)

Given the complexity of an enzymatic reaction, one might worry that this theory is too simple. We settle such concerns by solving the equations and testing whether
they fit the data. In a large number of cases they do. When they don’t, a few simple and reasonable modifications in the mechanism — but not in the methodology — bring the theory into agreement with the facts.

§4. The Extents of Reaction. Once this description is accepted, we move from the intimidating complexity of biology into the simple and comforting domain of chemical kinetics. To solve these equations, we follow the methodology explained in Chapter 7, where we studied coupled reactions. We define two extents of reaction $\eta_1$ and $\eta_2$ (two, because we have two reactions) and derive the mass conservation relations. Then we rewrite the rate equations in terms of $\eta_1$ and $\eta_2$, pick two independent rate equations, and solve them. This gives us the time dependence of $\eta_1$ and $\eta_2$, which is then used to find how the concentrations vary in time. This equation should look very familiar: biology or no biology, chemical kinetics is “the same darn thing over and over again.”

Defining the extents of reaction requires mostly an ability to follow the rules learned in Chapter 7. For the first reaction we have

\[ d\eta_1 = \frac{dE_1}{-1} = \frac{dR}{-1} = \frac{dC_1}{1} \]  \hspace{1cm} (9.8)

where $dE_1$ and $dC_1$ are the change in enzyme concentration and complex concentration caused by the first reaction. $dR$ is the total change in reactant concentration ($R$ is only involved in the first reaction). These changes are, as usual, divided by the stoichiometric coefficients of the compounds in the first reaction (negative for reactants and positive for products).

For the second reaction, we have

\[ d\eta_2 = \frac{dC_2}{-1} = \frac{dP}{1} = \frac{dE_2}{1} \]  \hspace{1cm} (9.9)

with obvious meaning for $dC_2$, $dP$, and $dE_2$.

§5. Mass Conservation. Following the standard procedure, we integrate Eqs 9.8 and 9.9 to find the mass conservation rules. The net change of enzyme concentration is

\[ dE = dE_1 + dE_2 = -d\eta_1 + d\eta_2 \]  \hspace{1cm} (9.10)
Integration with the conditions $\eta_1(0) = 0$ and $\eta_2(0) = 0$, gives

$$E(t) = E(0) - \eta_1(t) + \eta_2(t)$$  \hspace{1cm} (9.11)

Similarly, we obtain

$$C(t) = C(0) - \eta_1(t) + \eta_2(t) = \eta_2(t) - \eta_1(t)$$  \hspace{1cm} (9.12)

In most experiments the initial concentration $C(0)$ of the enzyme–reactant complex is zero.

Repeating this procedure leads to the mass conservation relations for the other reaction participants:

$$R(t) = R(0) - \eta_1(t)$$  \hspace{1cm} (9.13)

$$P(t) = P(0) + \eta_2(t) = \eta_2(t)$$  \hspace{1cm} (9.14)

§6. The Rate Equations for $\eta_1(t)$ and $\eta_2(t)$. We have two unknown quantities $\eta_1(t)$ and $\eta_2(t)$ and we need only two rate equations to calculate them. I pick Eqs 9.6 and 9.7, because they are simpler. Inserting Eqs 9.12 and 9.14 in Eq. 9.6 gives

$$\frac{d\eta_2(t)}{dt} = k_2 C(0) + \eta_1(t) - \eta_2(t)$$  \hspace{1cm} (9.15)

Using Eqs 9.11–9.13 in Eq. 9.7 leads to

$$\frac{d\eta_1(t)}{dt} = k_1 [E(0) + \eta_2(t) - \eta_1(t)] [R(0) - \eta_1(t)] - k_{-1} [\eta_1(t) - \eta_2(t)]$$  \hspace{1cm} (9.16)

All that is left to do is to solve these equations for $\eta_1(t)$ and $\eta_2(t)$ and then use $\eta_1(t)$ and $\eta_2(t)$ to calculate the evolution of the concentrations from the mass conservation equations (Eqs 9.11–9.14).

If someone has already determined $k_1$, $k_{-1}$, and $k_2$ for a reaction that we are interested in, we can integrate Eqs 9.15 and 9.16 and use the results to calculate the evolution of the concentrations for any initial reaction conditions (i.e. initial concentrations).

If the evolution of one concentration has been measured, we can vary $k_1$, $k_{-1}$, and $k_2$ in our calculations until they fit the data. The procedure for doing this is the same as in the preceding chapters.
§7. The Solution of the Rate Equations. As far as I know, we do not have sufficient human or artificial intelligence to solve Eqs 9.15 and 9.16 analytically. We have difficulties because of the terms $\eta_1(t)^2$ and $\eta_1(t)\eta_2(t)$, which make Eq. 9.16 nonlinear. Meeting such equations in your work should cause fear and excitement: they are hard to solve but, as a reward for your hard work, they often have very interesting solutions. Alas, ours are hard to solve but the solutions lack charisma. Indeed, we can anticipate that $R$ decays in time and $P$ increases, and this behavior is unlikely to take a chemist’s breath away.

Prior to the computer age, not being able to obtain an analytic solution was as tragic as the life of a kineticist would get, short of an explosion in the laboratory. Nowadays, many computer programs that solve such equations numerically are available. For example, Mathematica has the function NDSolve, which does a good job.

In Fig. 9.1, I show the extents of the two reactions for the case when $k_1 = 1.2$, $k_{-1} = 0.6$, $k_2 = 2.8$, $R(0) = 1$, $E(0) = 1$. The calculation is performed in Workbook K9.1. The initial enzyme concentration ($E(0) = 1$) is much higher than that used in practice, but it is typical of a non-enzymatic bimolecular reaction. I am using such a high concentration to contrast it with the realistic case, when $E(0)$ is much smaller than $R(0)$. As you will see later, the smallness of $E(0)$ is the reason for the success of the steady-state approximation, which is widely used in practice.

Fig. 9.1 shows that the extents of reaction are positive and grow in time. No surprise here. The second reaction is irreversible, and this means that the two reactions go on until all reactant is converted to product. Since both reactions evolve from left to right, $\eta_1$ and $\eta_2$ must be positive (see Chapter 1) and will grow until all reactant is used up. At that time, $\eta_1$ and $\eta_2$ will equal $R(0)$ (in Fig. 9.1 $R(0) = 1$).

![Figure 9.1](image)

**Figure 9.1** The extents of reaction $\eta_1(t)$ and $\eta_2(t)$ for $E(0) = 1$, $R(0) = 1$, $k_1 = 1.2$, $k_{-1} = 0.6$, $k_2 = 2.8$. 
This conclusion is dictated by common sense. But since common sense is not very common, you might feel more inclined to accept it if we derive it mathematically. Insights based on experience provide wonderful shortcuts, but they are sometimes shortcuts to disaster; mathematical proofs are more tedious, but safer.

Since \( R(t) \to 0 \) as \( t \to \infty \) (all reactant is used up), Eq. 9.13 tells us that \( \eta_1 \to R(0) \). Furthermore, since \( P(t) \to R(0) \) as \( t \to \infty \) (all reactant is converted to product), Eq. 9.14 tells us that \( \eta_2(t) \to R(0) \). This proves our intuitive argument. Note though, that at the basis of this mathematical argument stands the intuitive assertion that, because Reaction 9.2 is irreversible, all reactant is used up to form product. It is impossible to do physics or chemistry on purely mathematical grounds: certain empirical, intuitive conditions anchor mathematics to reality.

How the concentrations (of the enzyme, complex, reactant, and product) evolve is shown in Fig. 9.2. They were calculated in Workbook K9.1 by using Eqs 9.11–9.14. The reactant concentration decreases steadily and that of the product increases. The enzyme concentration has a minimum at early time.

It is easy to understand where these results come from. At early times, \( E(t) \) and \( R(t) \) are largest and \( C(t) \) is very small. As a result, the rate \( k_1 E(t) R(t) \) of enzyme “consumption” is much larger than the rates \( k_{-1} C(t) \) and \( k_2 C(t) \) of enzyme recovery. This makes \( E(t) \) and \( R(t) \) decay and \( C(t) \) increase, as seen in the graph. As \( C(t) \) becomes larger and \( R(t) \) becomes smaller, the rate of enzyme consumption (which is \( k_1 R(t) E(t) \)) decreases and that of enzyme recovery (which is \( -k_{-1} C(t) + k_2 C(t) \)) increases. In time the recovery rate becomes larger than the decay rate, and \( E(t) \) will

\[ \text{Figure 9.2} \] The concentrations \( E(t) \), \( C(t) \), \( R(t) \), and \( P(t) \) for \( E(0) = 1 \), \( R(0) = 1 \), \( k_1 = 1.2 \), \( k_{-1} = 0.6 \), \( k_2 = 2.8 \).
start to grow. A function that decays early and grows later must have a minimum. Since \( E(t) \) can only grow at the expense of \( C(t) \) (see Eq. 9.5), when \( E(t) \) starts growing \( C(t) \) begins to decay; when \( E(t) \) has a minimum, \( C(t) \) has a maximum. These extrema occur at the same time.

This argument indicates that the presence of a minimum in \( E(t) \) and a maximum in \( C(t) \) is a general feature of this kind of “consecutive” reaction mechanism (see a very similar behavior in Chapter 7); its presence does not depend on the values of \( k_1, k_{-1}, k_2, E(0), \) or \( R(0) \); only the time when the extrema are reached does. The steady-state approximation (see Chapter 7), which is widely used in practice (see next section), gives formulae for \( C(t) \) and \( E(t) \) that do not have extrema. This is an error of the approximation. As you will see soon, this error is harmless if the conditions are such that the extrema occur at very early times.

**Exercise 9.1**

Use the rate equations to prove mathematically that \( E(t) \) has a minimum and \( C(t) \) has a maximum. Find the time when these extrema occur and determine expressions for \( E(t) \) and \( C(t) \) at the extrema.

**Exercise 9.2**

In Cell 5 of Workbook K9.1, I have written a function that will plot the concentrations for any values of \( R(0), E(0), k_1, k_{-1}, \) and \( k_2 \). Use it to examine the following cases:

(a) \( k_1 = 0.6, k_{-1} = 1.2, k_2 = 0.3 \)

(b) \( k_1 = 2, k_{-1} = 0.3, k_2 = 8 \)

In both cases, take \( E(0) = R(0) = 1 \). Before you do the calculations, try to guess how the curves for (a) and (b) will differ from those in Fig. 9.2.

**The Michaelis–Menten Mechanism: the Steady-state Approximation**

§8. **Introduction.** Before computers were widely available, kineticists made approximations that allowed them to solve the rate equations. One of the most popular methods was the steady-state approximation, which we have discussed in Chapter 7. In this section, I apply the steady-state approximation to the Michaelis–Menten mechanism and examine its accuracy and limitations.
§9. *The Steady-state Approximation.* The steady-state approximation stipulates that after a certain time

\[ \frac{dC(t)}{dt} \approx 0 \]  
(9.17)

The intermediate complex C reaches a steady state and \( C(t) \) no longer changes in time. With this assumption, Eq. 9.4 becomes

\[ \frac{dC(t)}{dt} = k_1 E(t) R(t) - (k_{-1} + k_2) C(t) \approx 0 \]  
(9.18)

Some art is involved in picking the concentration that will reach a steady state: in general, we assume that a reaction intermediate, such as C, has this property. You will not make this assumption for R or P. Since the reaction \( C \to P + E \) is irreversible, it is not hard to see that the reactant concentration will decay to zero and that the product concentration will grow until it becomes equal to the initial concentration of the reactant. These two concentrations cannot possibly reach a steady state during the reaction. Since the rates of change of \( E(t) \) and \( C(t) \) are equal and of opposite sign, \( E(t) \) reaches a steady state if \( C(t) \) does.

**Exercise 9.3**

Just for fun, examine what would happen if you assume that R or E or P reaches a steady state. Would you obtain conflicting results?

Before moving on, I point out again that the assumption \( \frac{dC(t)}{dt} = 0 \) is a great simplification: it replaces the differential equation Eq. 9.4 with a simple algebraic equation Eq. 9.18. It also decouples the equation for \( R(t) \) from the other equations.

§10. *Inventory.* We should pause now for an inventory of the equations we have and for mapping where we are going. There are four concentrations \( R(t), C(t), E(t), \) and \( P(t) \), and we need equations for all of them. Besides the algebraic equation (Eq. 9.18), we have the differential equations:

\[ \frac{dR(t)}{dt} = -k_1 E(t) R(t) + k_{-1} C(t) \]  
(9.19)

\[ \frac{dP(t)}{dt} = k_2 C(t) \]  
(9.20)
and

\[ \frac{dE(t)}{dt} = -k_1 E(t) R(t) + k_1 C(t) + k_2 C(t) \] (9.21)

These equations are coupled (e.g. Eq. 9.19 contains the derivative of \( R(t) \) and also \( E(t) \) and \( C(t) \)). You will see shortly that the steady-state approximation decouples \( R(t) \) from the other variables.

§11. The Differential Equation for \( R(t) \). Let us start with Eq. 9.19 for \( R(t) \). I will simplify it by using Eq. 9.18 and mass conservation in the form

\[ E(t) = E(0) + \eta_2(t) - \eta_1(t) = E(0) - C(t) \] (9.22)

(this is obtained by combining Eqs 9.11 and 9.12). I solve Eqs 9.18 and 9.22 to express \( E(t) \) and \( C(t) \) as functions of \( R(t) \). The result is (see Workbook K9.1, Cell 1):

\[ E(t) = \frac{E(0)K_m}{K_m + R(t)} \] (9.23)

and

\[ C(t) = \frac{E(0)R(t)}{K_m + R(t)} \] (9.24)

Here

\[ K_m \equiv \frac{k_1}{k_1 + k_2} \] (9.25)

is the Michaelis–Menten constant.

Before moving on, I note that Eq. 9.24 is in conflict with Eq. 9.18, which says that \( C(t) \) is a constant (since \( dC(t)/dt = 0 \)), and Eq. 9.24 says that it changes in time. This kind of internal contradiction is typical of the steady-state approximation and it does not do too much damage to the theory. Its effect is to make the steady-state approximation valid at longer times only (for a discussion of this see Chapter 7 §16–§18 and §19 of the present chapter).

I can now use Eqs 9.24 and 9.23, giving \( E(t) \) and \( C(t) \) in terms of \( R(t) \), into the differential equation Eq. 9.19. I obtain (use also Eq. 9.25)

\[ \frac{dR(t)}{dt} = -\frac{E(0)k_2 R(t)}{K_m + R(t)} \] (9.26)
This is the differential equation for the evolution of $R(t)$ and it is decoupled from the other concentrations. I will solve this equation later.

§12. The Differential Equation for the Evolution of $P(t)$. The evolution of $P(t)$ is described by the differential equation Eq. 9.20, in which the change of $P(t)$ depends on $C(t)$. Since I decided that I will not determine $C(t)$ experimentally, I will use Eq. 9.24 to eliminate $C(t)$ from Eq. 9.20. The result is

$$\frac{dP(t)}{dt} = k_2 E(0) \frac{R(t)}{K_m + R(t)} \quad (9.27)$$

In this equation the evolution of $C(t)$ is coupled to that of $R(t)$.

If you compare Eq. 9.26 with Eq. 9.27 you can easily see that

$$\frac{dP(t)}{dt} = -\frac{dR(t)}{dt} \quad (9.28)$$

This equation tells us that the rate of reactant consumption is equal and opposite in sign to the rate of product formation. This is true only because we have made the steady-state assumption, according to which the amount of complex is constant.

Exercise 9.4

Use mass balance to show: (a) that the equation Eq. 9.28 is incorrect, in general; and (b) that Eq. 9.28 is correct if $C(t)$ is constant (i.e. if the steady-state approximation is correct).

The main results of the steady state approximation, for the Michaelis–Menten mechanism, are Eqs 9.18, 9.26, and 9.27. In principle, I will have to solve the differential equation Eq. 9.26, to obtain an expression for $R(t)$. Then I can use Eq. 9.28 to calculate the evolution of $P(t)$. If I want to know how $E(t)$ and $C(t)$ evolve, I will use Eqs 9.23 and 9.24. I will implement this in §15–§18 on p. 155–156. Before doing that I will show you a trick used by experimentalists to determine $K_m$ and $k_2$ from the data, without solving the differential equations.

Practical Use of the Steady-state Approximation to Determine $K_m$ and $k_2 E(0)$

§13. Introduction. Before computers became widely available, kineticists had a sturdy and legitimate aversion to differential equations, and developed a number
of very ingenious methods to avoid solving them. These methods are instructive and are also useful today. For that reason I discuss the basic ideas here. The discussion is schematic and incomplete: practical applications have to take into account various complications that appear when data are taken. To learn how this is really implemented you should read a book on enzyme kinetics (see Further Reading on p. 161).

§14. How to Use Eq. 9.27 Without Solving it. In general, to use the differential equations, Eqs 9.26 and 9.27, one has to solve them and then determine the rate constants by fitting the data. It is possible, however, to get the rate constants without obtaining an explicit solution of the equations.

To do this you will have to measure \( P(t) \) and \( R(t) \) at a variety of times. Then, you use the data on \( P(t) \) to calculate the derivative \( \frac{dP}{dt} \). I will tell you shortly how you can do that. For now, let us say that you have calculated \( \frac{dP}{dt} \) at time \( t_i \) and you denoted it by \( \frac{dP}{dt_i} \). You also know, since you measured it, the value \( R(t_i) \) of \( R \) at the same time. Using these values in Eq. 9.27 gives

\[
\frac{dP(t_i)}{dt_i} = k_2 E(0) \frac{R(t_i)}{K_m + R(t_i)}
\]  

(9.29)

By writing such a relationship for many values of \( t_i \), many equations can be obtained in which the only unknown quantities are \( k_2 E(0) \) and \( K_m \). One could use a least-squares fitting method to determine the values of these constants from these equations. If you know \( E(0) \) (usually you don’t), you can calculate \( k_2 \) from the value of \( k_2 E(0) \) provided by the fitting. A good fit indicates that the Michaelis–Menten mechanism, with the steady-state approximation, represents well the kinetics of the reaction.

Before we proceed with the implementation of this idea, I note that there are many ways of calculating the derivative \( \frac{dP(t_i)}{dt_i} \). For example, to obtain \( \frac{dP}{dt} \) at a time \( t_i \), you make a plot of \( P(t) \) versus \( t \) and draw a tangent at the time \( t_i \). The slope of the tangent gives you the derivative. Or you can use

\[
\frac{dP}{dt} \approx \frac{P(t_{i+1}) - P(t_{i-1})}{2(t_{i+1} - t_{i-1})}
\]

which gives the derivative correctly if the times \( t_{i+1} \) and \( t_{i-1} \) are sufficiently close to each other.
More accurate methods for calculating the derivative of a function, from a table of its values, are beyond the scope of this introductory book.

Assuming that \( \frac{dP(t)}{dt} \) and \( R(t) \) have been obtained for a variety of times a graphic method can be used to determine \( K_m \) and \( k_2E(0) \). To do this we start by noticing that we can rewrite Eq. 9.27 as:

\[
\frac{1}{\left( \frac{dP}{dt} \right)} = \frac{K_m}{k_2E(0)} \frac{1}{R} + \frac{1}{k_2E(0)}
\]  

(9.30)

This tells us that a plot of \((dP/dt)^{-1}\) versus \(1/R\) is a straight line (solid line in Fig. 9.3), if the Michaelis–Menten mechanism with the steady-state approximation correctly describes the reaction. Biochemists call this a Lineweaver–Burke plot. We can extrapolate this line until it cuts the axes (see the dotted line in Fig. 9.3).

According to Eq. 9.30, the dotted line cuts the vertical axis at \(1/k_2E(0)\) (make \(1/R = 0\) in Eq. 9.30); the horizontal axis is cut when \(dP/dt = 0\) and that intercept is \(-1/K_m\). Thus, from the two intercepts you can calculate \(k_2E(0)\) and \(K_m\).

Neither \(1/R(t) = 0\) nor \(1/(dP/dt) = 0\) is physically possible. The physically meaningful data are shown by the solid line. The dotted line is a mathematical fiction based on Eq. 9.30. This physically absurd extrapolation is a mathematically legitimate way of determining the constants in Eq. 9.30.

**Figure 9.3** If the Michaelis–Menten mechanism is correct, the plot of \(dP/dt\) versus \(1/R\) is a straight line.
Exercise 9.5
Show that, if the mechanism is Michaelis–Menten, a plot of \( R/(dP/dt) \) versus \( R \) is a straight line with slope equal to \( 1/k_2E(0) \), intercept with the vertical axis at \( K_m/k_2E(0) \), and intercept with the horizontal axis at \(-K_m\).

No matter how we perform the analysis, the steady-state approximation only allows us to determine two (i.e. \( k_2 \) and \( K_m \)) of the three constants (\( k_1 \), \( k_{-1} \), and \( k_2 \)) characterizing the mechanism. Because of this, an analysis based on the steady-state approximation is necessarily incomplete.

The Evolution of the Concentrations in the Steady-state Approximation

§15. Introduction. The method discussed in the previous section is sufficient for determining \( k_2E(0) \) and \( K_m \). However, this is not the end of the story. Once we know these constants we would like to use them to calculate the evolution of the concentrations \( R(t) \), \( E(t) \), \( C(t) \), and \( P(t) \) for other initial conditions. To perform such calculations we must solve first the differential equation Eq. 9.26.

§16. The Evolution of \( R(t) \). To solve Eq. 9.26, separate the variables and rewrite it as

\[
dR \frac{K_m + R}{R} = -E(0)k_2dt
\]  

(9.31)

Integrating this expression gives:

\[
\int_{R(0)}^{R(t)} dR \frac{K_m + R}{R} = - \int_0^t E(0)k_2dt
\]  

(9.32)

These integrals are easy to perform (see Workbook K9.3, Cell 1) and the result is

\[
R(t) - R(0) + k_m \ln \left( \frac{R(t)}{R(0)} \right) = -k_2E(0)t
\]  

(9.33)

To obtain an explicit expression for \( R(t) \), I have to solve Eq. 9.33. This innocent-looking equation does not have an algebraic solution. However we can express \( R(t) \).
in terms of a function Pln(x), called Lambert’s function, which Mathematica calls \textbf{ProductLog}. The result is (see Workbook K9.3, Cell 1):

\[ R_s(t) = K_m \text{Pln} \left( \frac{R(0)}{K_m} \exp \left[ \frac{R(0) - E(0)k_2t}{K_m} \right] \right) \]  

(9.34)

The subscript \( s \) reminds me that this equation is valid only when the steady-state approximation is correct.

The function Pln(z) is the solution \( w \) of the equation \( z = we^w \). The function \textbf{ProductLog}[z] in Mathematica calculates the values of Pln(z) for a given value of z. Note the analogy with the ordinary logarithm, \( \ln z \), which is the solution \( w \) of the equation \( z = e^w \).

We can use Eq. 9.34 to determine \( K_m \) and \( E(0)k_2 \) by fitting the data on the evolution of \( R(t) \). These data alone cannot be used to determine \( k_{-1} \) and \( k_1 \).

§17. \textit{The Evolution of P(t) in the Steady-state Approximation.} Now that we know how to calculate \( R(t) \) it is easy to obtain \( P(t) \) by integrating Eq. 9.28. This gives

\[ P_s(t) - P(0) = -(R(t) - R(0)) = R(0) - K_m \text{Pln} \left( \frac{R(0)}{K_m} \exp \left[ \frac{R(0) - E(0)k_2t}{K_m} \right] \right) \]  

(9.35)

The last equality was obtained by using Eq. 9.34. Again, the subscript \( s \) reminds me that \( P_s(t) \) is the concentration of the product when the steady-state approximation is valid.

§18. \textit{The Concentration of the Complex and of the Enzyme in the Steady-state Approximation.} The calculation of \( C(t) \) and \( E(t) \) in the steady-state approximation uses Eqs 9.24 and 9.23. These express \( C(t) \) and \( E(t) \) in terms of \( R(t) \), which, in turn, is given by Eq. 9.34.

\textbf{The Michaelis–Menten Mechanism: How Good is the Steady-state Approximation?}

§19. \textit{Introduction.} Approximations simplify a problem but add new burdens: if we plan to use the results to fit the experiments, we must ensure that the data used in the analysis were taken under conditions for which the errors made by the approximation are acceptable.
In this section I examine, by using two examples, whether the steady-state approximation works well for the Michaelis–Menten mechanism. We will find that this is an excellent approximation when the enzyme concentration is much smaller than that of the reactant. This is the case in many enzyme-catalyzed reactions.

Fig. 9.4 shows a comparison of the numerically exact $C(t)$ with the one obtained from the steady-state approximation. The calculations were made in Cell 2 of Workbook K9.4, for $R(0) = E(0) = 1$, $k_1 = 1.2$, $k_{-1} = 0.6$, and $k_2 = 2.8$. Note that the rate constants are of comparable magnitude and the initial concentration of the enzyme is the same as that of the reactant. In practice the concentration of the enzyme is much lower than that of the reactant, but we examine this case to see how well the steady-state approximation works under these conditions.

There are substantial differences between the exact and the steady-state values of $C(t)$ at short time. This is not a surprise: the initial concentration $C(0)$ is zero, while the steady-state approximation (see Eq. 9.24) gives

$$C(0) = \frac{E(0)R(0)}{K_m + R(0)}$$

Also, the exact $C(t)$ has a maximum while the steady-state $C(t)$ decays monotonically. However, the two calculations agree better as $t$ increases.

**Figure 9.4** The change in concentration of the complex with time in the case when $R(0) = 1$, $E(0) = 1$, $P(0) = 0$, $k_1 = 1.2$, $k_{-1} = 0.6$, and $k_2 = 2.8$. The solid line shows the result of the exact calculation, and the dotted line, that of the steady-state approximation.
Fig. 9.5 shows that some errors exist in the calculation of $P(t)$. The least erroneous is $R(t)$ (see Fig. 9.6).

The results of a calculation in which we take $E(0) = 10^{-3}$, and leave the other parameters unchanged, tells a completely different story. The steady-state approximation is practically exact, except at very short times, when the results for $C(t)$ differ from the exact values (see Cell 4 in Workbook K9.4).
Exercise 9.6
Examine how the steady-state approximation performs when \( R(0) = 1, \ E(0) = 10^{-3}, \ k_1 = 1, \ k_1 = 0.1, \) and \( k_2 = 10^{-3}. \) You can look at Cell 4 of Workbook K9.4, but you will learn more if you do the exercise yourself. Try to explain the result qualitatively.

Exercise 9.7
Analyze the following situation: in parallel with the reactions discussed in this chapter, one also has an equilibrium \( E + I \rightleftharpoons EI, \) where \( I \) is an inhibitor (a molecule that can bind to the pocket of the enzyme blocking the access of the reactant). Perform calculations of the evolution of the concentrations by taking numerical values for the rate constants and the equilibrium constant of the inhibition reaction. Solve the resulting equations numerically and also use the steady-state approximation.