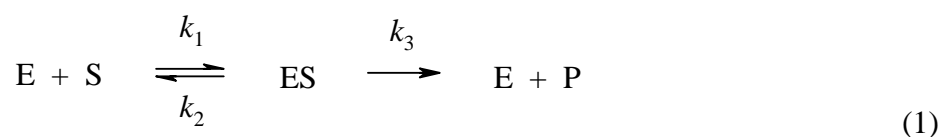


Derivation of the Michaelis-Menten Equation

The Michaelis-Menten equation is an important equation in biochemistry and as such it is imperative that you understand the derivation of this equation. By understanding the derivation, you will have insight into the assumptions that went into this model, and therefore you will have a better appreciation for the proper use of this equation as well as the limitations of this model. In the following sections you will see two different derivations of the Michaelis-Menten equation. When one is learning a subject for the first time, it often helps to have the same or similar information presented from alternative perspectives. One way might be clearer to you whereas the other way might be clearer to someone else. That is ok! You should familiarize yourself with both approaches, and then settle on the one that you prefer. The first derivation was adapted from “An Introduction to Enzyme Kinetics” by Addison Ault (*J. Chem. Ed.* **1974**, 51, 381 – 386).

First Derivation. We start with the kinetic mechanism shown in equation (eq) 1:



In eq 1, E is enzyme, S is substrate, ES is the enzyme-substrate complex, and P is product. This equation includes the assumption that during the early stages of the reaction so little product is formed that the reverse reaction (product combining with enzyme and re-forming substrate) can be ignored (hence the unidirectional arrow under k_3). Another assumption is that the concentration of substrate is much greater than that of total enzyme ($[\text{S}] \gg [\text{E}_t]$), so it can essentially be treated as a constant.

From General Chemistry we can equate the rate of this process ($k_3[ES]$) to the change in product concentration as a function of time ($d[P]/dt$), or, equivalently, we can designate the rate with an italicized v (v) as follows in eq 2:

$$\frac{d[P]}{dt} = v = k_3[ES] \quad (2)$$

Because the concentration of the enzyme•substrate complex ($[ES]$) cannot be measured experimentally, we need an alternative expression for this term. Because the enzyme that we add to the reaction will either be unbound (E) or bound (ES) we can express the fraction of bound enzyme as follows:

$$\frac{[ES]}{[E_t]} = \frac{[ES]}{[ES] + [E]} \quad (3)$$

In eq 3 E_t is the concentration of total enzyme, and the other variables are as defined above. If we multiply both sides of eq 3 by E_t we arrive at eq 4:

$$[ES] = \frac{[E_t][ES]}{[ES] + [E]} \quad (4)$$

If we multiply the numerator and denominator of the right-hand side of eq 4 by $1/[ES]$, we are, in effect, multiplying by one and we do not change the value of this expression.

When we do this we obtain eq 5:

$$[ES] = \frac{[E_t]}{1 + \frac{[E]}{[ES]}} \quad (5)$$

We have almost achieved our goal of isolating $[ES]$. Next, we need to come up with an alternative expression for the ratio $[E]/[ES]$. We do this by recalling that a major assumption in enzyme kinetics is the steady-state assumption. Basically, it says the rate of change of $[ES]$ as a function of time is zero: $d[ES]/dt = 0$. Another way to express the

steady-state assumption is that the rate of formation of ES equals the rate of breakdown of ES. We can express this latter statement mathematically as in eq 6:

$$k_1[E][S] = k_2[ES] + k_3[ES] = (k_2 + k_3)[ES] \quad (6)$$

The left-hand side of eq 6 expresses the rate of formation of ES (according to eq 1), and the right-hand side expresses the two ways that ES can break down (also according to eq 1). We can rearrange eq 6 to isolate the ratio $[E]/[ES]$. When we do we get eq 7:

$$\frac{[E]}{[ES]} = \frac{(k_2 + k_3)}{k_1[S]} \quad (7)$$

We now define a new constant, the Michaelis constant (K_m), as follows in eq 8:

$$K_m = \frac{(k_2 + k_3)}{k_1} \quad (8)$$

If we substitute K_m back into eq 7 we obtain eq 9:

$$\frac{[E]}{[ES]} = \frac{K_m}{[S]} \quad (9)$$

We now substitute the ratio $K_m/[S]$ from eq 9 in place of the ratio $[E]/[ES]$ in eq 5 and we obtain eq 10:

$$[ES] = \frac{[E_t]}{1 + \frac{K_m}{[S]}} \quad (10)$$

If we multiply the numerator and denominator of the right-hand side of eq 10 by $[S]$, we are, in effect, multiplying by one and we do not change the value of this expression.

When we do this we obtain eq 11:

$$[ES] = \frac{[E_t][S]}{[S] + K_m} = \frac{[E_t][S]}{K_m + [S]} \quad (11)$$

Now we have achieved our goal of isolating [ES] and we can substitute this alternative expression of [ES] into eq 2 and obtain eq 12:

$$v = \frac{k_3[E_t][S]}{K_m + [S]} \quad (12)$$

Next, we imagine what happens to eq 12 when $[S] \gg K_m$ as follows in eq 13:

$$v \approx \frac{k_3[E_t][S]}{[S]} = k_3[E_t] = k_{cat}[E_t] \quad (13)$$

The constant k_{cat} in the right-hand most term of eq 13 is used to signify that k_3 is considered the catalytic constant. Under such conditions, when [S] is said to be saturating, the enzyme is functioning as fast as it can and we define $k_3[E_t]$ (or $k_{cat}[E_t]$) to be equal to V_{max} , the maximum velocity that can be obtained. Therefore, eq 12 can be rewritten into the familiar form of the Michaelis-Menten equation (eq 14):

$$v = \frac{V_{max}[S]}{K_m + [S]} \quad (14)$$

Next, we imagine what happens when $K_m \gg [S]$ as follows in eq 15:

$$v \approx \frac{V_{max}[S]}{K_m} = k'[S] \quad (15)$$

Since $k' = V_{max}/K_m$ in eq 15, we refer to V_{max}/K_m as an apparent (or pseudo) first order rate constant. Another way to look at a similar, related concept is to rewrite eq 14 as follows:

$$v = \frac{k_{cat}[E_t][S]}{K_m + [S]} \quad (16)$$

Since we are imagining the case where $K_m \gg [S]$ we neglect [S] in the denominator and include the assumption that $[E_t] \approx [E]$ since at very low [S] relatively little [ES] should form:

$$v \approx \frac{k_{\text{cat}}[E][S]}{K_m} \approx k''[E][S] \quad (17)$$

Once again, since $k'' = k_{\text{cat}}/K_m$ in eq 17, we refer to k_{cat}/K_m as an apparent second order rate constant. Because k_{cat}/K_m is a measure of the rate of the reaction divided by the term that reflects the steady-state affinity of the enzyme for the substrate, it is considered an indicator of the catalytic efficiency of the enzyme and sometimes is called the specificity constant. It also is more relevant to the physiological situation because in cells, $[S]$ generally is equal to or less than K_m . Is there an upper limit to the value that k_{cat}/K_m can approach? Yes, there is and the following shows how we can determine this limit. To illustrate this limit we first need to rewrite k_{cat}/K_m as follows:

$$\frac{k_{\text{cat}}}{K_m} = \frac{k_3}{\frac{k_2 + k_3}{k_1}} \quad (18)$$

Next, we imagine the case where $k_3 \gg k_2$:

$$\frac{k_{\text{cat}}}{K_m} \approx \frac{k_3}{\frac{k_3}{k_1}} \approx k_1 \quad (19)$$

So we see that k_{cat}/K_m can approach k_1 as a limiting value, and k_1 is the second-order rate constant for the productive collision of enzyme and substrate and as such it is limited by diffusion to about $10^8 - 10^9 \text{ M}^{-1} \text{ s}^{-1}$. Thus, if we see an enzyme that has a k_{cat}/K_m value in the neighborhood of $10^8 - 10^9 \text{ M}^{-1} \text{ s}^{-1}$ we say that the enzyme has attained “catalytic perfection”. You will see later in the class that a number of enzymes that catalyze “near-equilibrium” reactions in metabolic pathways are catalytically perfect.

Next, we return to eq 16 and consider what happens when $v = \frac{1}{2} V_{\text{max}}$:

$$\frac{V_{\max}}{2} = \frac{V_{\max} [S]}{K_m + [S]} \quad (20)$$

When we simplify eq 20 we find that $K_m = [S]$ (under the above conditions; i.e., $v = \frac{1}{2} V_{\max}$). So, in other words, K_m is formally defined as a collection of rate constants (eq. 8), but it is also equal to the substrate concentration that gives half-maximal velocity of the enzyme-catalyzed reaction.

Before we discuss the second derivation, we will consider what happens when we take the reciprocal of both sides of eq 14. When we do this we obtain eq 21:

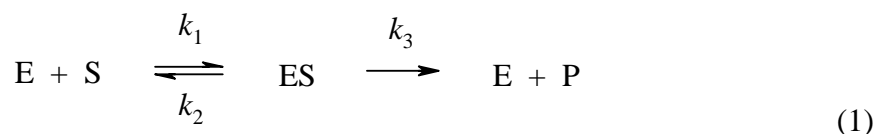
$$\frac{1}{v} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}} \quad (21)$$

Eq 21 is in the form of an equation for a straight line (i.e., $y = mx + b$, with $y = 1/v$; $m = K_m/V_{\max}$; $x = 1/[S]$; and $b = 1/V_{\max}$). When experimental data are plotted using this transformation the resulting plots are called double-reciprocal plots or Lineweaver-Burk plots in honor of the researchers who pioneered this method.

The authors of many textbooks extol the virtues of using Lineweaver-Burk plots to obtain estimates of V_{\max} and K_m . I disagree strongly with this practice because initial velocity data determined at low substrate concentrations (where there is inherently more uncertainty since $[S] \leq K_m$) end up being the points in a Lineweaver-Burk plot that have too much sway in determining the best-fit line through the data (see for example, “Disadvantages of Double Reciprocal Plots” by R. Bruce Martin, *J. Chem. Ed.* **1997**, 74, 1238 – 1240). In fact, Lineweaver and Burk recognized this problem and in their famous paper (*J. Am. Chem. Soc.* **1934**, 56, 658 – 666) they had consulted with a statistician to determine the proper weighting factors for the data points. To repeat: eq 21 is *not* a useful form of the equation for obtaining estimates of V_{\max} and K_m . Instead, one should use a

modern software program that allows for iterative fitting of the experimental data to estimate these parameters. (This type of fitting is called nonlinear least-squares fitting.) Eq 21 is useful for plotting data that have been obtained in the presence of increasing concentrations of an inhibitor. Such plots allow a researcher to “diagnose” the type of inhibition that is occurring. We will discuss this later on in class.

Second Derivation. For the second approach, we consider equations 1, 2, and 6 (repeated for your convenience):



$$\frac{d[\text{P}]}{dt} = v = k_3[\text{ES}] \quad (2)$$

$$k_1[\text{E}][\text{S}] = k_2[\text{ES}] + k_3[\text{ES}] = (k_2 + k_3)[\text{ES}] \quad (6)$$

Once again, because we cannot experimentally measure [ES], we seek an alternative expression for this parameter so that when we obtain this alternative expression we can multiply it by k_3 in eq 2 and thus obtain our desired rate equation. To proceed, we will divide the right-hand-most and left-hand-most expressions in eq 6 by $(k_2 + k_3)$. Doing so will isolate [ES]. While we are at it, we will collect rate constants and define K_m as we did above in eq 8:

$$[\text{ES}] = \frac{k_1[\text{E}][\text{S}]}{(k_2 + k_3)} = \frac{[\text{E}][\text{S}]}{\frac{(k_2 + k_3)}{k_1}} = \frac{[\text{E}][\text{S}]}{K_m} \quad (22)$$

It looks like we succeeded in isolating [ES], but notice that on the right-hand side of eq 22 we have free enzyme (i.e., [E]). Just as we are unable to experimentally measure [ES], we also are unable to experimentally measure [E]. All we can say definitively is

that free enzyme ($[E]$) is equal to total enzyme ($[E_t]$) minus the form of the enzyme that is bound to substrate ($[ES]$):

$$[E] = [E_t] - [ES] \quad (23)$$

Substituting the right-hand side of eq 23 in place of $[E]$ in eq 22 leads to eq 24:

$$[ES] = \frac{([E_t] - [ES])[S]}{K_m} \quad (24)$$

Multiplying both sides of eq 24 by K_m and multiplying $[E_t]$ and $[ES]$ by $[S]$ in the numerator of the right-hand side of this equation leads to eq 25:

$$K_m [ES] = [E_t][S] - [ES][S] \quad (25)$$

Adding $[ES][S]$ to both sides of eq 25 and then factoring out $[ES]$ leads to eq 26:

$$[ES](K_m + [S]) = [E_t][S] \quad (26)$$

Finally, dividing both sides of eq 26 by $(K_m + [S])$ effectively isolates $[ES]$ and yields eq 27:

$$[ES] = \frac{[E_t][S]}{(K_m + [S])} \quad (27)$$

Eq 27 is the same expression that we obtained in eq 11. Our work is done. (Well, it's done once we substitute the right-hand side of eq 27 into eq 2 and then substitute V_{\max} for $k_3[E_t]$ as we did above when we obtained eq 14; these last few steps yield the sought after Michaelis-Menten equation.)