COURSE BSc (BIOTECHNOLOGY) III SEM

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ENZYME KINETICS

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ENZYME KINETICS

- ***Kinetics is the study of the rates at which chemical reactions occur.***
- **Rate Equations**
- At constant temperature, elementary reaction rates vary with reactant concentration in a simple manner. 1

$$aA + bB + \text{----------} + zZ \longrightarrow P \qquad (1)$$

- *The rate of this process is proportional to the frequency with which the reacting molecules simultaneously come together, that is, to the products of the concentrations of the reactants.*
- This is expressed by the following **rate equation**

$$\text{Rate} = k[A]^a[B]^b\text{---------}[Z]^z \qquad (2)$$

- where *k* is a proportionality constant known as a **rate constant.**
- The **order** of a reaction is defined as (*a b* p *z*), the sum of the exponents in the rate equation.
- *the order corresponds to the **molecularity** of the reaction, the number of molecules that*

Rates of Reactions

We can experimentally determine the order of a reaction by measuring [A] or [P] as a function of time; that is,

1) $A \rightarrow P$

$$v = -\frac{d[A]}{dt} = \frac{d[P]}{dt}$$

$$v = -\frac{d[A]}{dt} = k[A]$$

2) $A + B \rightarrow P$

$$v = -\frac{d[A]}{dt} = -\frac{d[B]}{dt} = k[A][B]$$

The Michaelis–Menten Equation

- The overall Enzymatic reaction is composed of two elementary reactions in which the substrate forms a complex with the enzyme that subsequently decomposes to products and enzyme:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} P + E \qquad (1)$$

- Here E, S, ES, and P symbolize the enzyme, substrate, **enzyme–substrate complex,** and products, respectively
- *When the substrate concentration becomes high enough to entirely convert the enzyme to the ES form, the second step of the reaction becomes rate limiting and the overall reaction rate becomes insensitive to further increases in substrate concentration.*
- The general expression for the **velocity** (rate) of this reaction Is

$$v = \frac{d[P]}{dt} = k_2[ES] \qquad (2)$$

The Michaelis–Menten Equation

- The overall rate of production of ES is the difference between the rates of the elementary reactions leading to its appearance and those resulting in its disappearance:

$$\frac{d[\mathrm{ES}]}{dt} = k_1[\mathrm{E}][\mathrm{S}] - k_{-1}[\mathrm{ES}] - k_2[\mathrm{ES}] \qquad (3)$$

- This equation cannot be explicitly integrated, however, without simplifying assumptions.
- Two possibilities are

1. **Assumption of equilibrium:** In 1913, Leonor Michaelis and Maud Menten, building on earlier work by Victor Henri, assumed that $k_{-1} >> k_2$, so that the first step of the reaction achieves equilibrium.

$$K_S = \frac{k_{-1}}{k_1} = \frac{[\mathrm{E}][\mathrm{S}]}{[\mathrm{ES}]} \qquad (4)$$

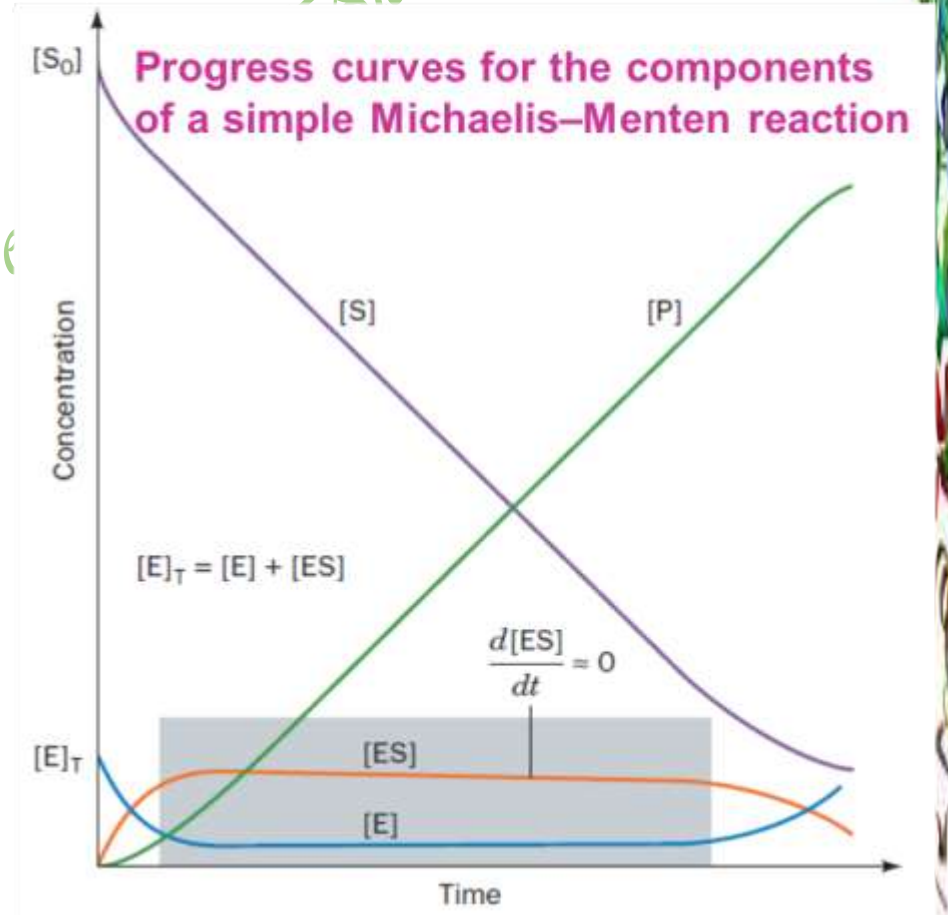
- Here K_S is the dissociation constant of the first step in the enzymatic reaction.
- The noncovalently bound enzyme–substrate complex ES is known as the **Michaelis complex.**

The Michaelis–Menten Equation

2. Assumption of steady state: first proposed in 1925 by George E. Briggs and John B.S. Haldane.

Figure 1 illustrates the progress curves of the various participants in the preceding reaction model under the physiologically common condition that substrate is in great excess over enzyme.

- With the exception of the initial stage of the reaction, the so-called **transient phase,** which is usually over within milli seconds of mixing the enzyme and substrate, [ES] remains approximately constant until the substrate is nearly exhausted.



The Michaelis–Menten Equation

- Hence, the rate of synthesis of ES must equal its rate of consumption over most of the course of the reaction; that is, [ES] maintains a **steady state.** One can therefore assume with a reasonable degree of accuracy that [ES] is constant, that is,

$$\frac{d[\mathrm{ES}]}{dt} = 0 \qquad (5)$$

- This so-called **steady-state assumption** was In order to be of use, kinetic expressions for overall reactions must be formulated in terms of experimentally measurable quantities.
- The quantities [ES] and [E] are not, in general, directly measurable but the total enzyme concentration is usually readily determined.

$$[\mathrm{E}]_\mathrm{T} = [\mathrm{E}] + [\mathrm{ES}] \qquad (6)$$

- The rate equation for our enzymatic reaction is then derived as follows.

The Michaelis–Menten Equation

*V*0 is determined by the breakdown of ES to form product, which is determined by [ES]:

$$V0 = k_2[ES] \quad (1)$$

The term [Et], representing the total enzyme concentration (the sum of free and substrate-bound enzyme). **Free or unbound enzyme [E] can then be represented by [Et] - [ES].**

$$[E]=[Et]-[ES] \quad (2)$$

[S] is ordinarily far greater than [Et], the amount of substrate bound by the enzyme at any given time is negligible compared with the total [S].

Step 1 The rates of formation and breakdown of ES are determined by the steps governed by the rate constants k_1 (formation) and $k_{-1} + k2$ (breakdown to reactants and products, respectively), according to the expressions

$$\text{Rate of ES formation} = k_1([E][S] \text{ OR } k_1([Et]-[ES])[S] \quad (3)$$

$$\text{Rate of ES breakdown} = k_{-1}[ES] + k2[ES] \quad (4)$$

The Michaelis–Menten Equation

Step 2 According to **steady-state assumption**. The expressions in Equations

$$k_1([Et] - [ES])[S] = k_{-1}[ES] + k_2[ES] \quad 5$$

Step 3 In a series of algebraic steps, we now solve Equation for [ES]. First, the left side is multiplied out and the right side simplified to give

$$k_1[Et][S] - k_1[ES][S] = (k_{-1} + k_2)[ES] \quad 6$$

$$(k_{-1} + k_2)[ES] + k_1[ES][S] = k_1[Et][S] \quad 7$$

Dividing both side with k_1

$$(k_{-1} + k_2)/ k_1[ES] + \cancel{k_1}/\cancel{k_1}\ [ES][S] = \cancel{k_1}/\cancel{k_1}\ [Et][S] \quad 8$$

$$(k_{-1} + k_2)/ k_1[ES] + [ES][S] = [Et][S] \quad 9$$

$$[ES]\{(k_{-1} + k_2)/ k_1 + [S]\} = [Et][S] \quad 10$$

$$[ES] = [Et][S]/\{(k_{-1} + k_2)/ k_1 + [S]\} \quad 11$$

The term $(k_{-1} + k_2)/k_1$ is defined as the Michaelis constant, *K*m.

The Michaelis–Menten Equation

OR

$$[ES](k_{-1} + k_2 + k_1[S]) = k_1[E]_T[S] \quad (8)$$

OR

$$(ES) = k_1 (E)_T (S) / k_{-1} + k_2 + k_1(S) \quad (9)$$

OR

$$(ES) = \cancel{k_1} (E)_T (S) / \cancel{k_1}\{(k_{-1} + k_2) / k_1 + (S)\} \quad (10)$$

K_M, which is known as the **Michaelis constant,** is defined as

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

Hence:

$$[ES] = \frac{[E]_T [S]}{K_M + [S]} \quad (11)$$

The Michaelis–Menten Equation

The **initial velocity** can then be expressed in terms of the experimentally measurable quantities $[E]_T$ and [S]:

v/k2 = V/k2 [S]/Km + [S]

$$v_o = \left(\frac{d[P]}{dt}\right)_{t=t_s} = k_2[ES] = \frac{k_2[E]_T[S]}{K_M + [S]}$$ (12)

Since velocity (v) is = k_2 x [ES] and substituting for [ES] in equation 11

The **maximal velocity** of a reaction, *V*max, occurs at high substrate concentrations when the enzyme is **saturated,** that is, when it is entirely in the ES form:

$$V_{max} = k_2 \times [E_T]$$ (13)

and substituting for $[E_T]$ in equation 11

Therefore, combining Eqs. [12] and [13], we obtain

V = Vm[S]/[S] + [S]

$$v_o = \frac{V_{max}[S]}{K_M + [S]}$$ (14)

V = Vm [S]/2[S]

The Michaelis–Menten Equation

*The **Michaelis–Menten equation,** is the basic equation of enzyme kinetics.* It describes a rectangular hyperbola such as is plotted in Fig

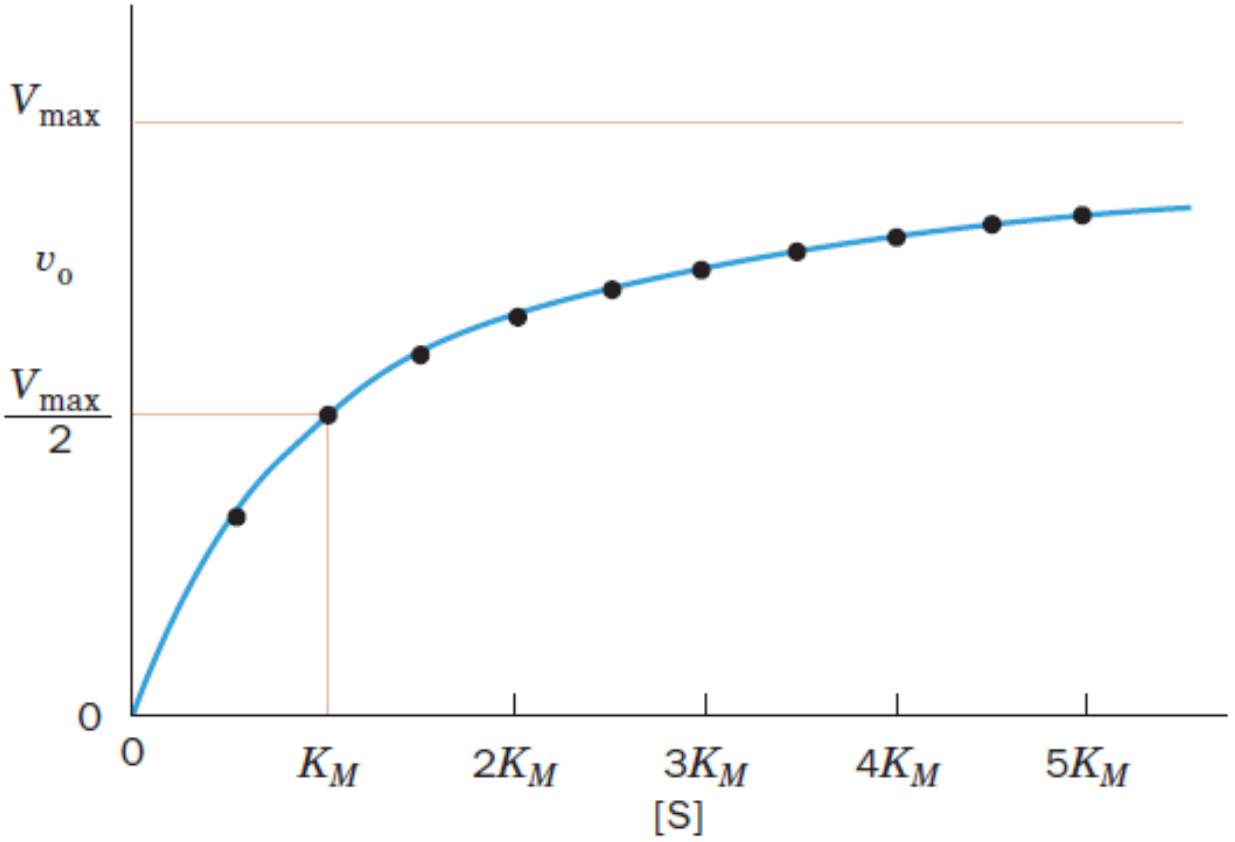


Figure: **Plot of the initial velocity *v*o of a simple Michaelis–Menten reaction versus the substrate concentration [S].**

Significance of the Michaelis Constant

- ***K_M is the substrate concentration at which the reaction velocity is half-maximal.***
- Therefore, if an enzyme has a small value of K_M, it achieves maximal catalytic efficiency at low substrate concentrations.
- The magnitude of K_M varies widely with the identity of the enzyme and the nature of the substrate.
- It is also a function of temperature and pH.
- The Michaelis constant can be expressed as

V = Vm[S]/[S] + [S]

V = Vm [S]/2[S]

$$K_M = \frac{k_{-1}}{k_1} + \frac{k_2}{k_1} = K_S + \frac{k_2}{k_1}$$

- Since K_S is the dissociation constant of the Michaelis complex, as K_S decreases, the enzyme's affinity for substrate increases.
- K_M is therefore also a measure of the affinity of the enzyme for its substrate

k_{cat}/K_M Is a Measure of Catalytic Efficiency

We may define the catalytic constant of an enzyme as

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

- The rate limiting constant could be k_2 OR k_3 OR simply written as k_{cat}
- This quantity is also known as the **turnover number** of an enzyme because it is the number of reaction processes (turnovers) that each active site catalyzes per unit time.
- **k_{cat} may be a function of several rate constants.**
- When [S] <<< K_M, very little ES is formed. Consequently, $[E] \approx [E]_T$, so that Eq. reduces to a second order rate equation.

$$v_o \approx \left(\frac{k_2}{K_M}\right)[E]_T[S] \approx \left(\frac{k_{cat}}{K_M}\right)[E][S]$$

- k_{cat}/K_M is the apparent second-order rate constant of the enzymatic reaction;
- ***The quantity k_{cat}/K_M is therefore a measure of an enzyme's catalytic efficiency.***

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EP \overset{k_3}{\rightleftharpoons} E + P$$

Lineweaver–Burk or double reciprocal plot

- The Michaelis-Menten equation can be algebraically transformed into equations that are more useful in plotting experimental data. One common transformation is derived simply by taking the reciprocal of both sides of the Michaelis-Menten equation:

$$v_0 = \frac{V_{max}[s]}{K_m + [s]} \quad (1)$$

- Separating the components of the numerator on the right side of the equation gives which simplifies to

$$\frac{1}{v} = \frac{K_m + [s]}{V_{max}[s]} \quad (2)$$

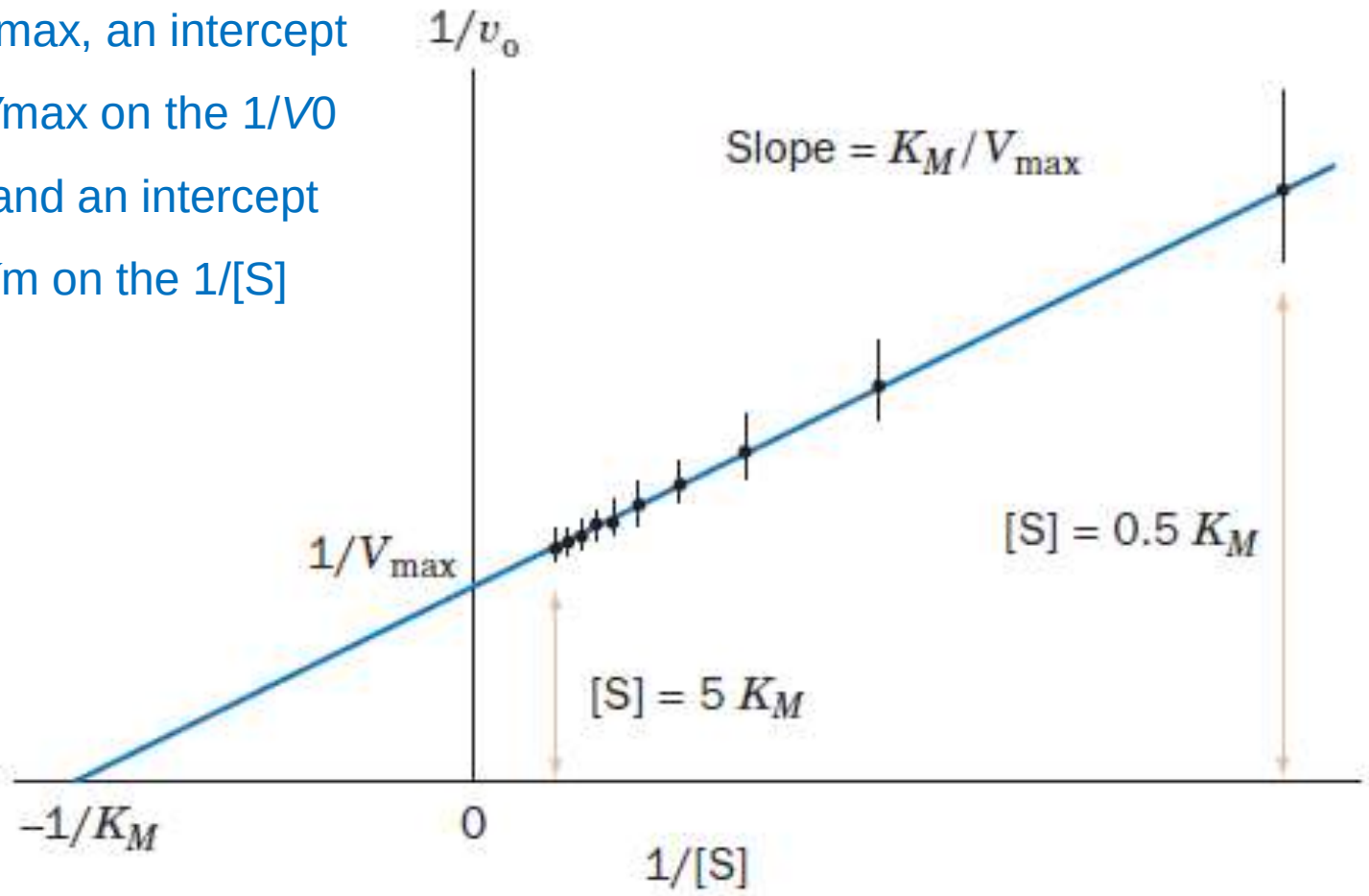
$$\frac{1}{v} = \frac{K_m}{V_{max}}\frac{1}{[s]} + \frac{1}{V_{max}} \quad (3)$$

y = m x + c

- This form of the Michaelis-Menten equation is called the **Lineweaver-Burk equation**.
- For enzymes obeying the Michaelis-Menten relationship, a plot of $1/V_0$ versus 1/[S] (the "double reciprocal" of the V_0 versus [S] plot we have been using to this point) yields a straight line .

A double-reciprocal (Lineweaver–Burk) plot

This line has a slope of *K*m/*V*max, an intercept of 1/*V*max on the 1/*V*0 axis, and an intercept of 1/*K*m on the 1/[S] axis.



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