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Kinetics of enzymatic reactions

Kinetics

- Biochemical Kinetics: the science that studies rates of chemical reactions
- An example is the reaction ($A \rightarrow P$), The velocity, v , or rate, of the reaction $A \rightarrow P$ is the amount of P formed or the amount of A consumed per unit time, t . That is,

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

Reaction Rate Law

- The rate is a term of change over time
- The rate will be proportional to the conc. of the reactants
- It is the mathematical relationship between reaction rate and concentration of reactant(s)
- For the reaction ($A + B \rightarrow P$), the rate law is

$$\text{Rate} = \frac{-\Delta[A]}{\Delta t} = \frac{-\Delta[B]}{\Delta t} = \frac{\Delta[P]}{\Delta t} \quad v = \frac{-d[A]}{dt} = k[A]$$

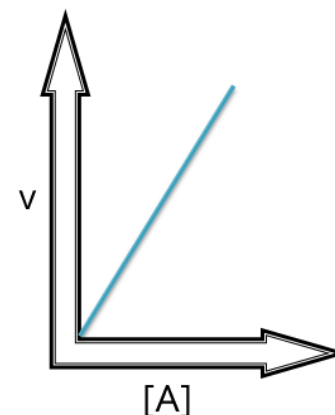
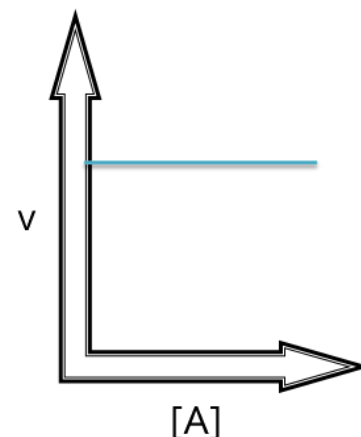
- From this expression, the rate is proportional to the concentration of A, and k is the rate constant

The order of the reaction & the rate constant (k)

- A multistep reaction can go no faster than the slowest step

$$v = k(A)^{n_1}(B)^{n_2}(C)^{n_3}$$

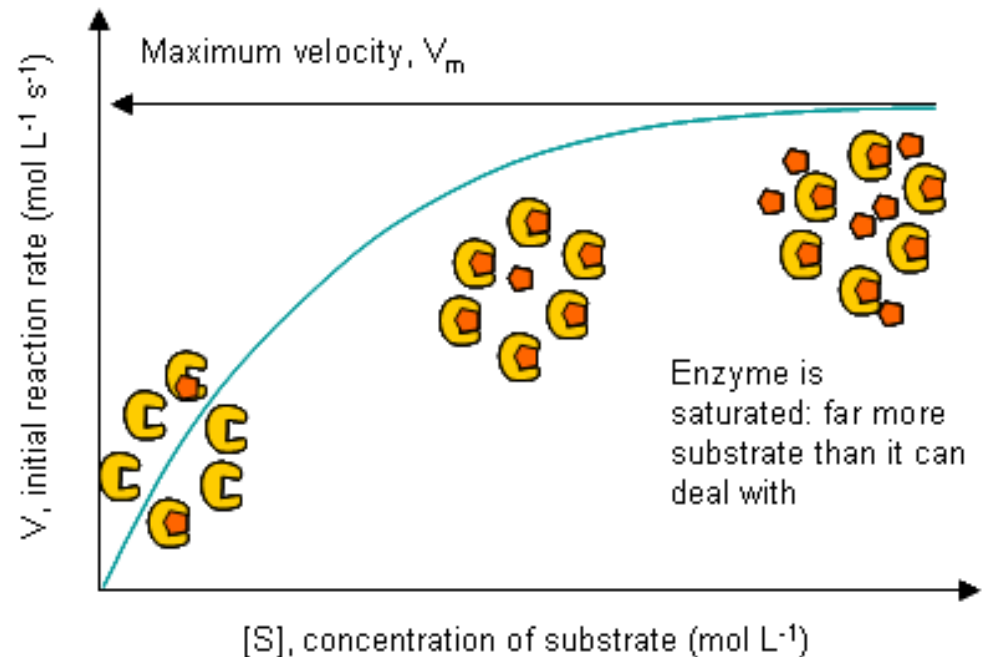
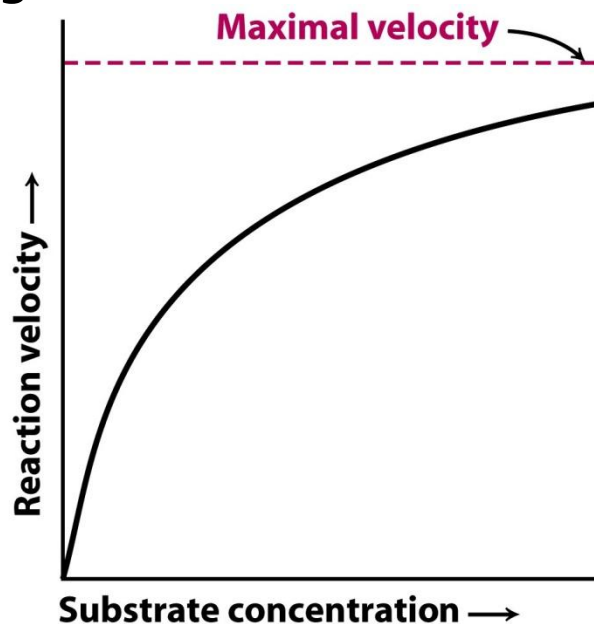
- k is the rate constant: the higher the activation energy (energy barrier), the smaller the value of k
- $(n_1+n_2+n_3)$ is the overall order of the reaction
- Dimensions of k



Overall order	$V=$	Dimensions of k
Zero	k	$(\text{conc.})(\text{time})^{-1}$
First	$k(A)$	$(\text{time})^{-1}$

Enzyme kinetics

- Enzymatic reactions may either have a simple behavior or complex (allosteric) behavior
- Simple behavior of enzymes: as the concentration of the substrate rises, the velocity rises until it reaches a limit
- Thus; enzyme-catalyzed reactions have hyperbolic (saturation) plots

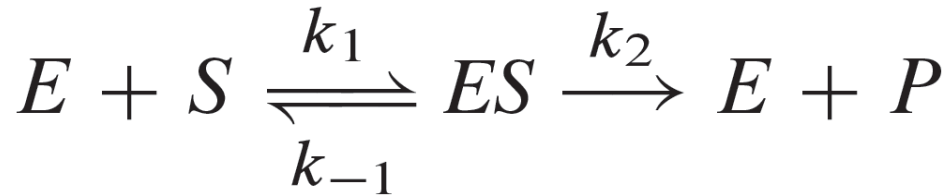
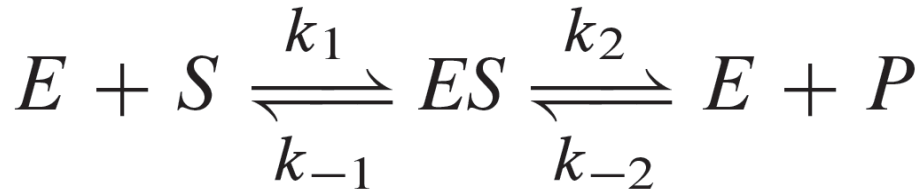


Enzyme kinetics

- The maximal rate, V_{\max} , is achieved when the catalytic sites on the enzyme are saturated with substrate
- V_{\max} reveals the turnover number of an enzyme
 - The number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate
- At V_{\max} , the reaction is in zero-order rate since the substrate has no influence on the rate of the reaction

Expression of enzyme kinetic reactions

"Steady State Assumption"



$$v = k_2 ES$$

$$\frac{dES}{dt} = k_1 E \cdot S - k_{-1} ES - k_2 ES$$

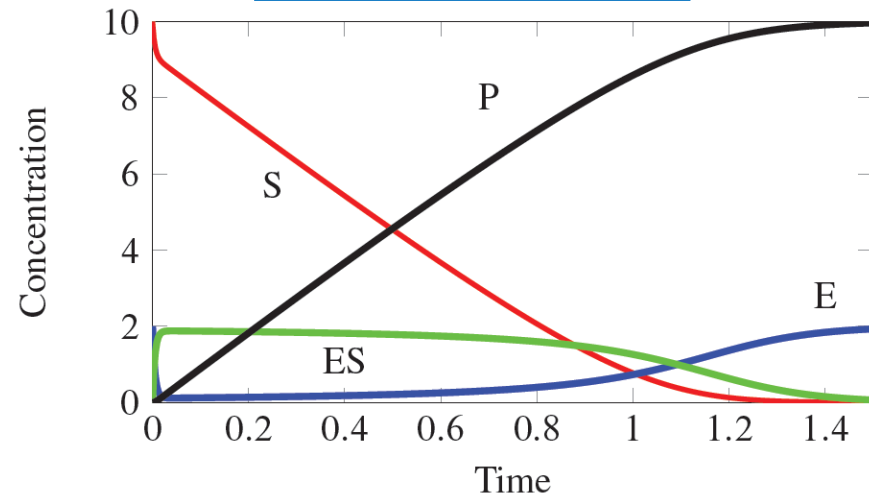
$$0 = k_1 E \cdot S - k_{-1} ES - k_2 ES$$

$$E_t = E + ES$$

$$ES = \frac{E_t \cdot S}{(k_{-1} + k_2)/k_1 + S}$$

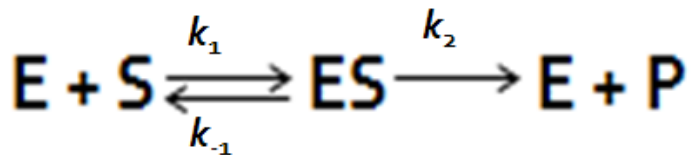
$$v = \frac{E_t k_2 S}{(k_{-1} + k_2)/k_1 + S}$$

$$v = \frac{V_{max} S}{K_m + S}$$



The Michaelis constant (K_m)

- For a reaction:



$$\text{STEADY STATE APPROXIMATION}$$
$$\frac{d[ES]}{dt} = k_1 [E] [S] - k_{-1} [ES] - k_2 [ES] = 0 \text{ (approx.)}$$

$$\frac{[E] [S]}{[ES]} = \frac{k_{-1} + k_2}{k_1} = K_M \quad \text{Equation 1}$$

- K_m , called the Michaelis constant is

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

- In other words, K_m is related to the rate of dissociation of substrate from the enzyme to the enzyme-substrate complex
- K_m describes the affinity of enzyme for the substrate

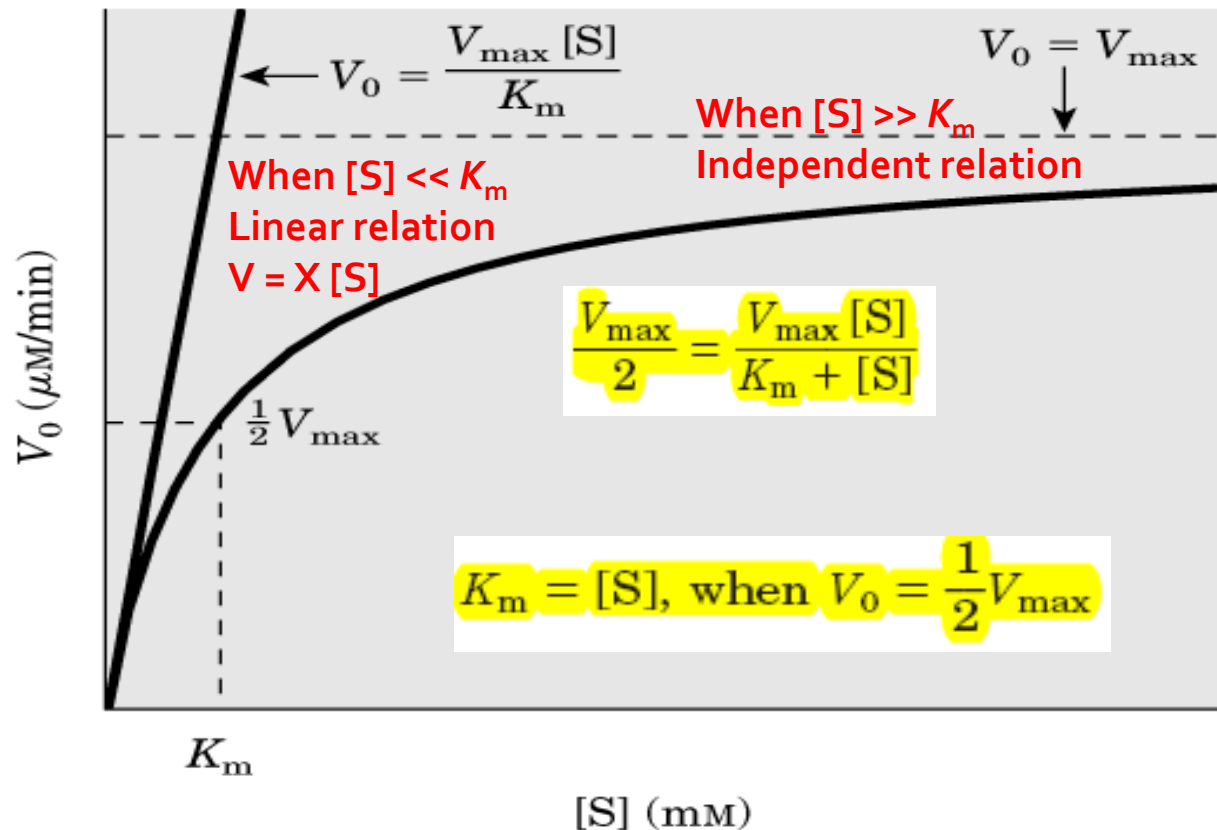
Expression of enzyme kinetic reactions

Michaelis-Menten equation

- A quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_o) & substrate concentration $[S]$
 - ✓ The rate constant (K_m) and maximal velocity (V_{max})

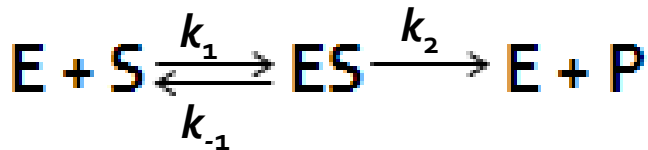
$$V_o = V_{max} \frac{[S]}{[S] + K_M}$$

The substrate concentration at which V_o is half maximal is K_m



V_{\max} & k_{cat}

- For the enzymatic reaction



- The maximal rate, V_{\max} , is equal to the product of k_2 , also known as k_{cat} , and the total concentration of enzyme

$$V_{\max} = k_2 [E]_T$$

- k_{cat} , the turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, or when fully saturated

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

- In other words, the maximal rate, V_{\max} , reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known

Turnover Numbers (k_{cat}) of Some Enzymes

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β -Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

The Michaelis constant (K_m)

$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$

- The lower the K_m of an enzyme towards its substrate, the higher the affinity
- When more than one substrate is involved? Each will have a unique K_m & V_{\max}
- In multi-substrate reaction, the sequence of substrate binding & product release affect the rate equation
- K_m values have a wide range. Mostly between (10^{-1} & 10^{-7} M)

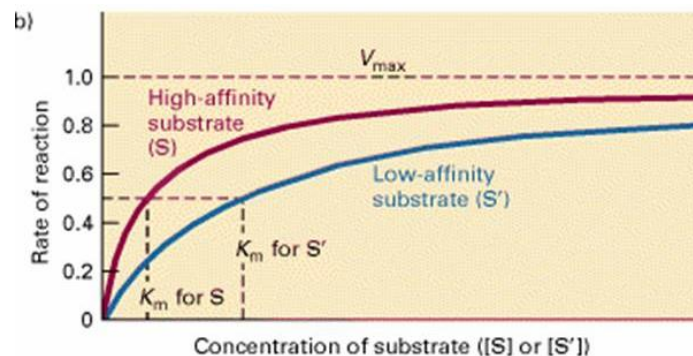
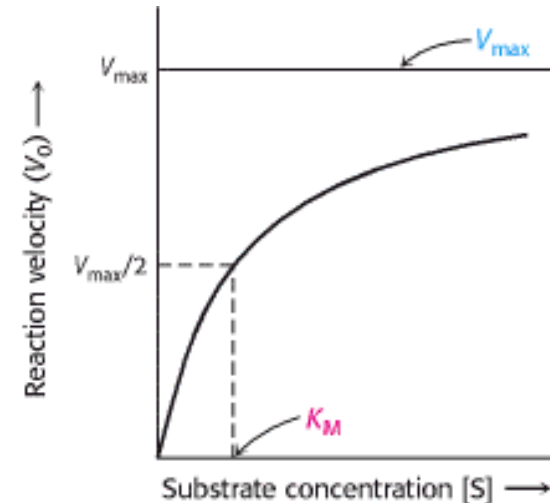


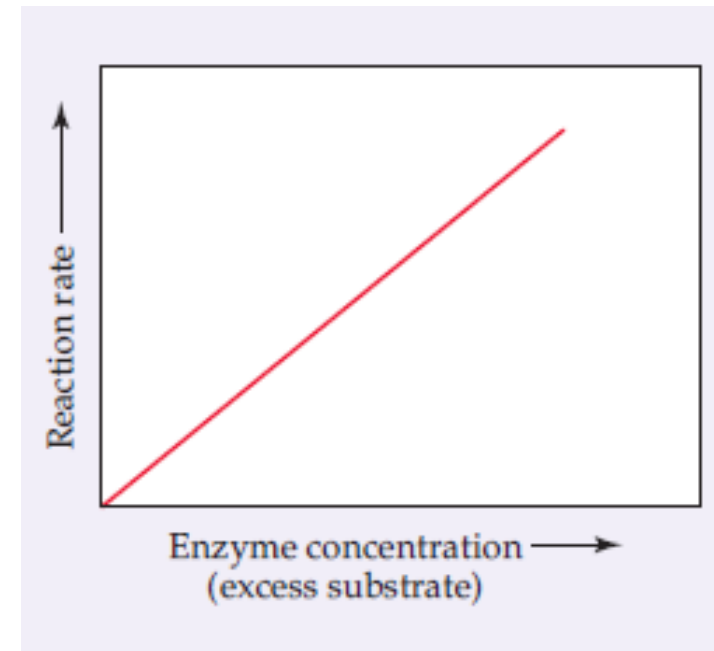
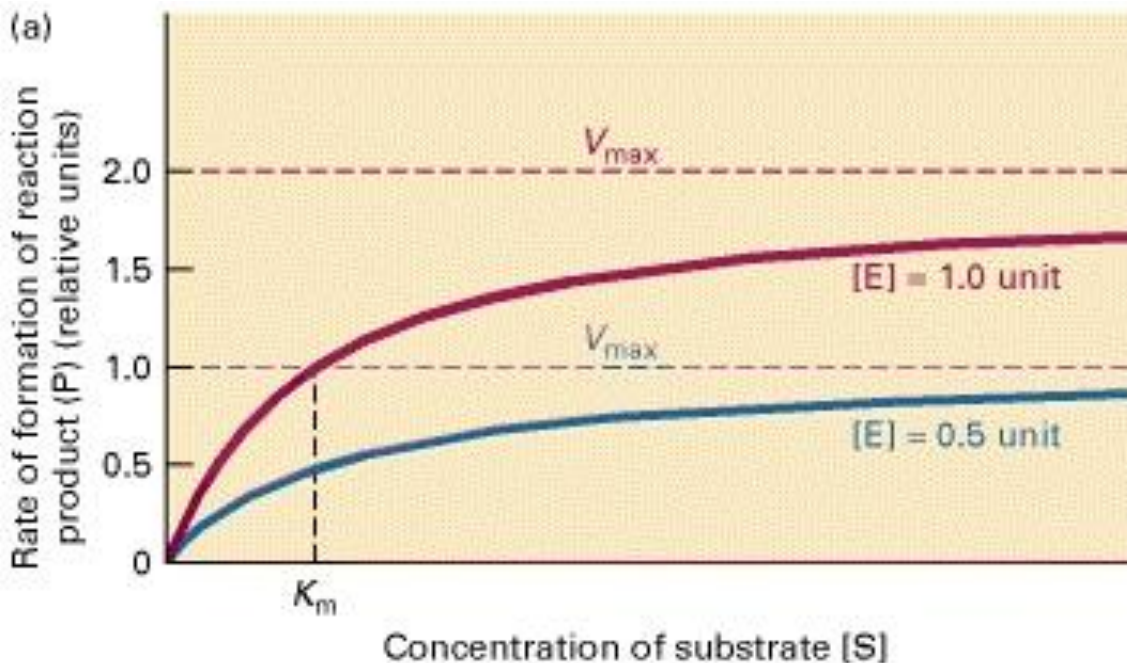
table 8-6

K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Catalase	H ₂ O ₂	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO ₃ ⁻	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

K_M & K_D [E], K_M & V_{\max}

- K_D : dissociation constant, The actual measure of the affinity
 - $K_D = (k_{-1}/k_1)$
- $$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P$$
- When you increase the enzyme concentration, what will happen to V_{\max} & K_m ?



Example

- a 10^{-6} M solution of carbonic anhydrase catalyzes the formation of 0.6 M H_2CO_3 per second when it is fully saturated with substrate
 - ✓ Hence, k_{cat} is $6 \times 10^5 \text{ s}^{-1}$
 - ✓ 10^4 min^{-1}
- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is $1.7 \mu\text{s}$ for carbonic anhydrase
- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 - $10^4 \text{ (s}^{-1}\text{)}$

Fractional Saturation: Physiological $[S]/K_M$

Specificity & Efficiency

- Most enzymes are not normally fully saturated
- Under physiological conditions, the $[S]/K_M$ ratio is typically between 0.01 and 1.0

$$V = \frac{V_{\max} [S]}{K_M + [S]} = \frac{k_{\text{cat}} [E_T][S]}{K_M + [S]}$$

- Specificity constant (k_{cat}/K_M): determines the relative rate of the reaction at low $[S]$

$$V = (k_{\text{cat}}/K_M) [E][S]$$

- k_{cat}/K_M ($\text{M}^{-1} \text{min}^{-1}$) is indicative of:
 - ✓ Enzyme's substrate specificity: the higher the ratio, the higher the specificity
 - ✓ Enzyme's catalytic efficiency: the higher the ratio, the more efficient the enzyme

Table 6.2

Turnover Numbers and K_M for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M **
Catalase	Conversion of H_2O_2 to H_2O and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

k_{cat} values vary over a range of nearly 3000
 K_M values vary over a range of nearly 300
 k_{cat}/K_M , the range is only 4

Reaction rate (v); Enzyme activity; Specific activity; Turnover number

- Reaction rate; measures the concentration of substrate consumed (or product produced) per unit time ($\text{mol}/\{\text{L}\cdot\text{s}\}$ or M/s)
- Enzyme activity; measures the number of moles of substrate consumed (or product produced) per unit time (mol/s)
 - ✓ Enzyme activity = rate of reaction \times reaction volume
- Specific activity; measures moles of substrate converted per unit time per unit mass of enzyme ($\text{mol}/\{\text{s}\cdot\text{g}\}$)
 - ✓ Specific activity = enzyme activity / actual mass of enzyme
 - ✓ This is useful in determining enzyme purity after purification
- Turnover number; measures moles of substrate converted per unit time per moles of enzyme (min^{-1} or s^{-1})
 - ✓ Turnover number = specific activity \times molecular weight of enzyme

Sample calculations:

A solution contains initially $25.0 \times 10^{-4} \text{ mol L}^{-1}$ of peptide substrate and $1.50 \text{ }\mu\text{g}$ chymotrypsin, in 2.5 mL volume. After 10 minutes, $18.6 \times 10^{-4} \text{ mol L}^{-1}$ of peptide substrate remain. Molar mass of chymotrypsin is $25,000 \text{ g mol}^{-1}$.

peptide substrate consumed $= 6.4 \times 10^{-4} \text{ mol L}^{-1}$ in 10 minutes

Rate of reaction $= 6.4 \times 10^{-5} \text{ mol L}^{-1} \text{ min}^{-1}$

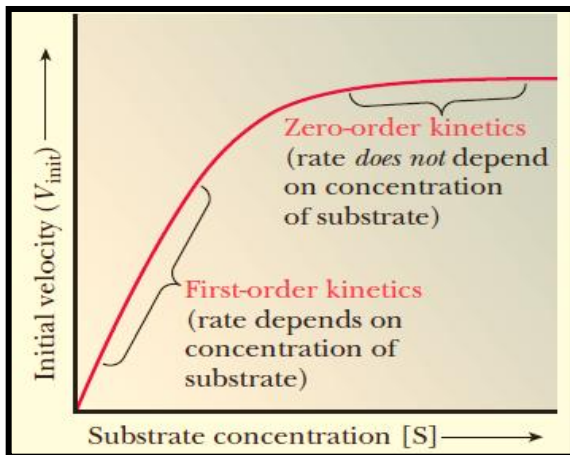
Enzyme activity $= 6.4 \times 10^{-5} \text{ mol L}^{-1} \text{ min}^{-1} \times 2.5 \times 10^{-3} \text{ L}$
(rate \times volume) $= 1.6 \times 10^{-7} \text{ mol min}^{-1}$

Specific activity $= 1.6 \times 10^{-7} \text{ mol min}^{-1} / 1.50 \text{ }\mu\text{g}$
(activity / mass) $= 1.1 \times 10^{-7} \text{ mol }\mu\text{g}^{-1} \text{ min}^{-1}$

Turnover number $= 1.1 \times 10^{-7} \text{ mol }\mu\text{g}^{-1} \text{ min}^{-1} \times 25,000 \text{ g mol}^{-1} \times 10^6 \text{ }\mu\text{g g}^{-1}$
(sp. act. \times molar mass) $= 2.7 \times 10^3 \text{ min}^{-1} = 45 \text{ s}^{-1}$

Disadvantage of Michaelis-Menten equation & Lineweaver-Burk or double-reciprocal plot

- Determining the K_m from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach V_{max}
- This prevents the calculation of both V_{max} & K_m
- Lineweaver-Burk plot: A plot of $1/v_o$ versus $1/[S]$ (double-reciprocal plot), yields a straight line with an y-intercept of $1/V_{max}$ and a slope of K_M/V_{max}
- The intercept on the x-axis is $-1/K_M$



$$v = \frac{V_{max} \cdot [S]}{[S] + K_m}$$

ص = أس + ب

$$\frac{1}{v} = \left[\frac{K_m(1)}{V_{max}[S]} + \frac{1}{V_{max}} \right]$$

