

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,

or

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

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

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

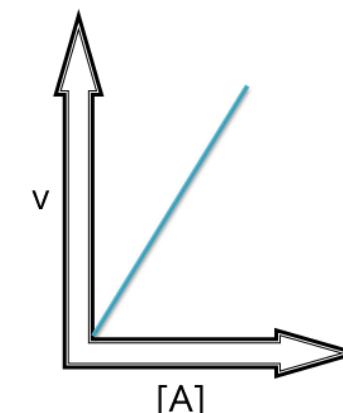
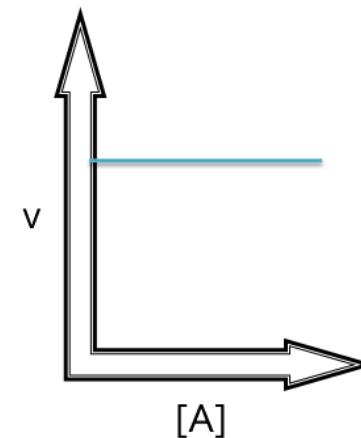
The order of the reaction & the rate constant (k)

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

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

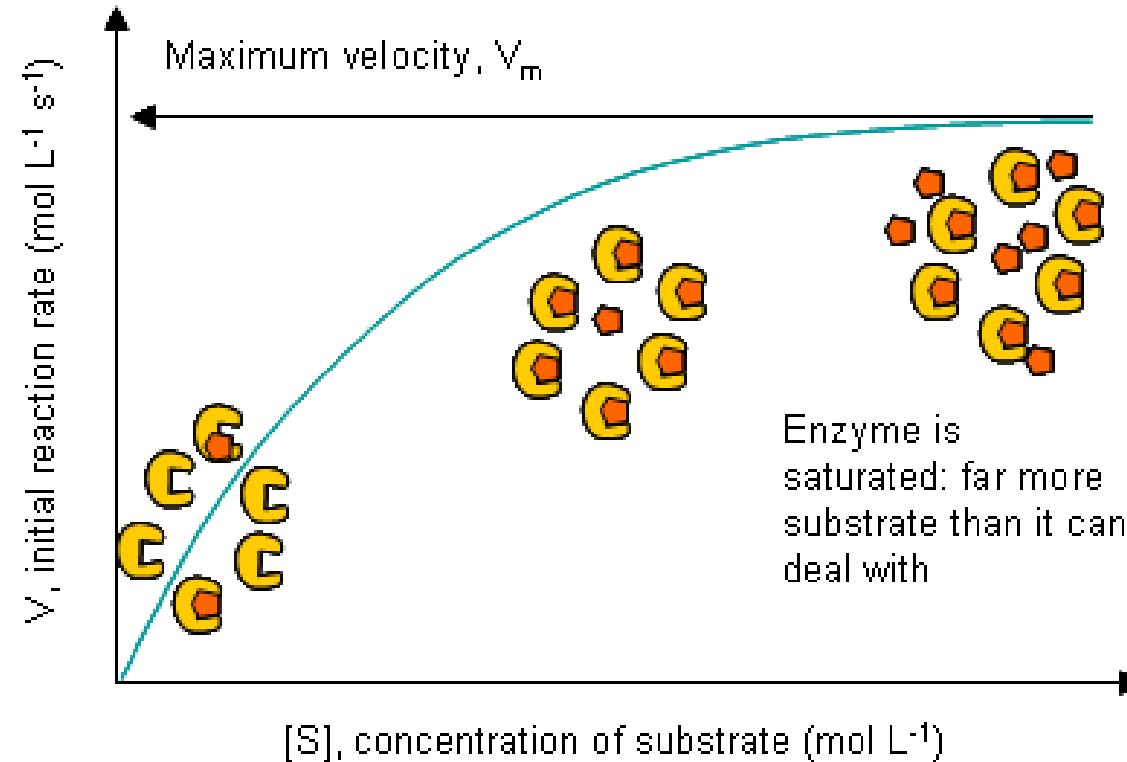
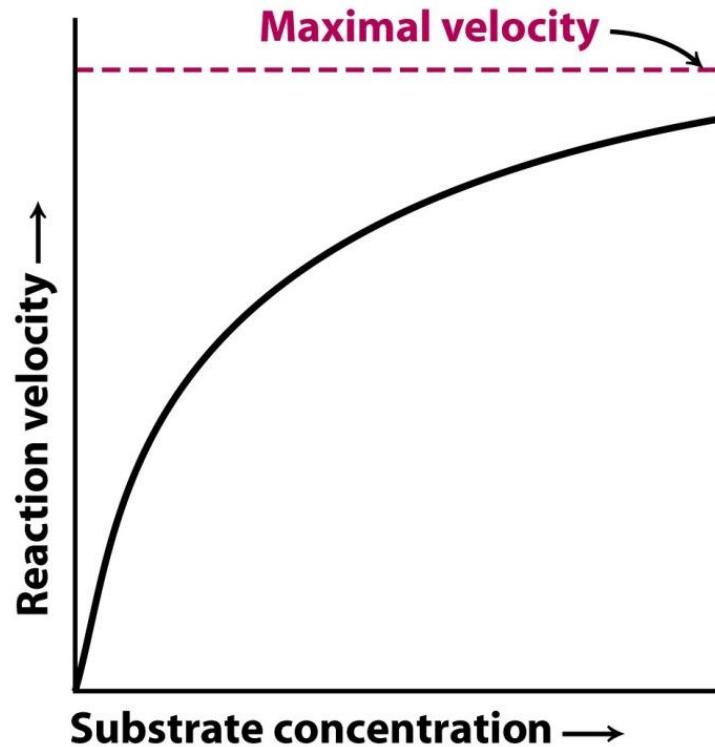
- k is the rate constant: the higher the activation energy (energy barrier), the smaller the value of k
- $(n_1+n_2+n_3)$ is the overall order of the reaction
- Dimensions of k

Overall order	$v =$	Dimentions of k
Zero	k	(conc.)(time) $^{-1}$
First	$k(A)$	(time) $^{-1}$



Enzyme kinetics

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

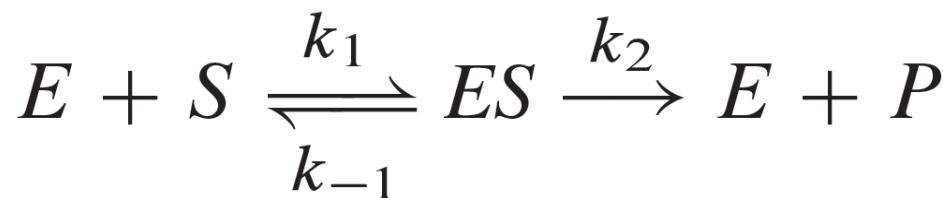
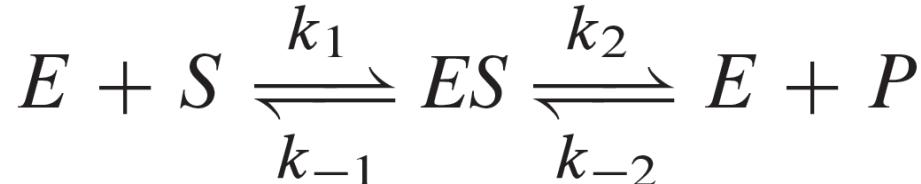


Enzyme kinetics

- 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 of 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”



$$v = k_2 ES$$

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

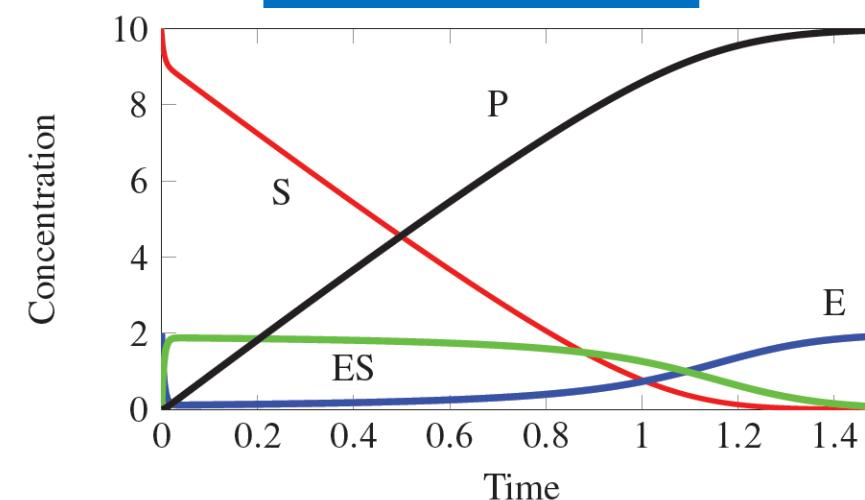
$$0 = k_1 E \cdot S - k_{-1} ES - k_2 ES$$

$$E_t = E + ES$$

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

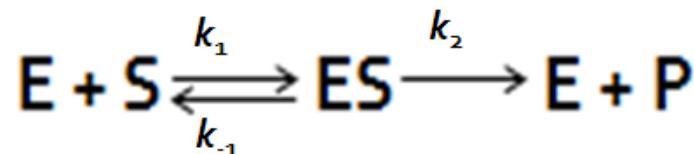
$$v = \frac{E_t k_2 S}{(k_{-1} + k_2)/k_1 + S}$$

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



The Michaelis constant (K_m)

- For a reaction:



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

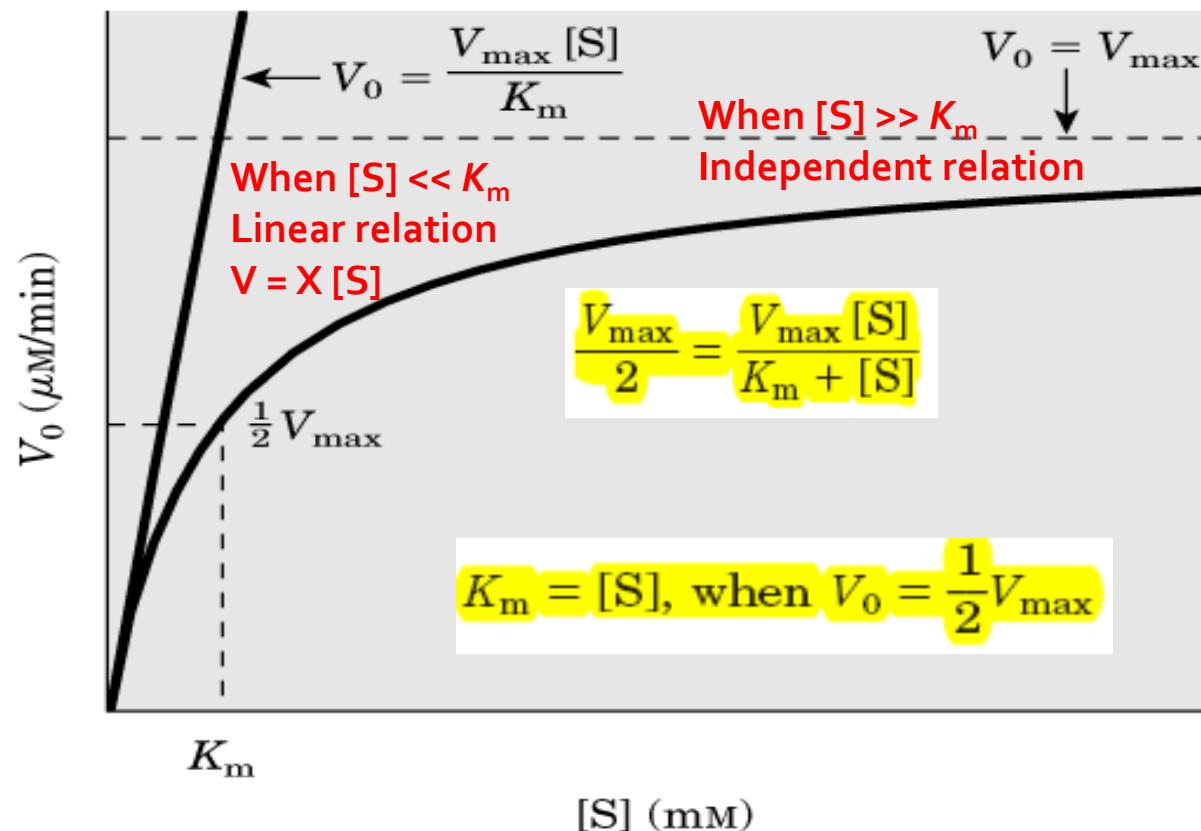
Expression of enzyme kinetic reactions

Michaelis-Menten equation

- A quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_0) & substrate concentration [S]
 - ✓ The rate constant (K_m) and maximal velocity (V_{max})

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

The substrate concentration at which V_0 is half maximal is K_m



The Michaelis constant (K_m)

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

- K_m and affinity
- When more than one substrate is involved? Each will have a unique K_m & V_{\max}
- K_m values have a wide range. Mostly between (10^{-1} & 10^{-7} M)

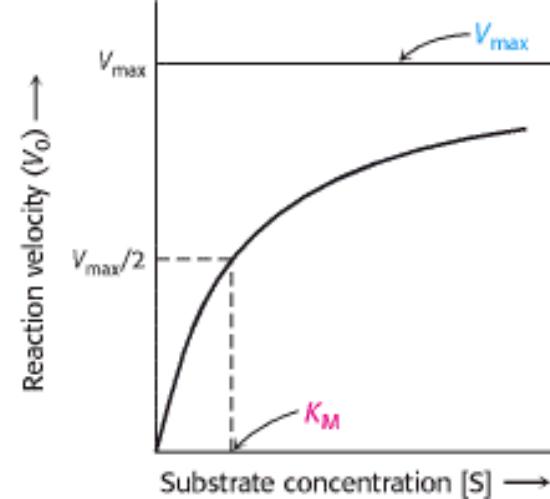
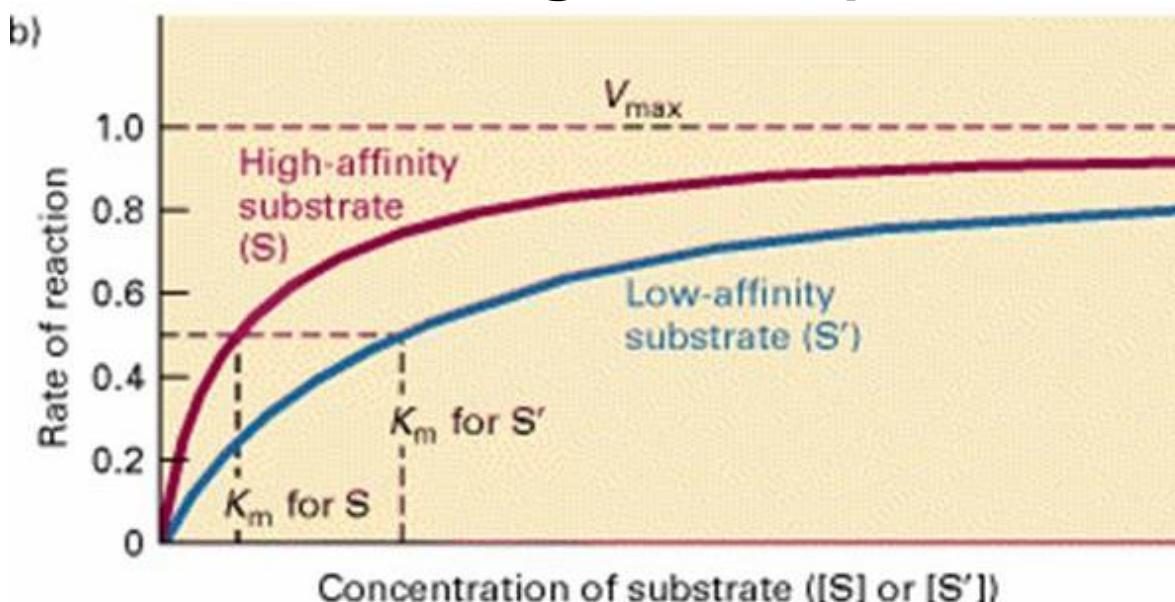


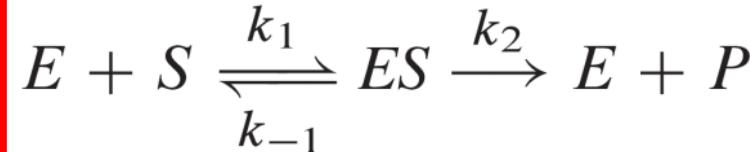
table 8-6

K_m for Some Enzymes and Substrates

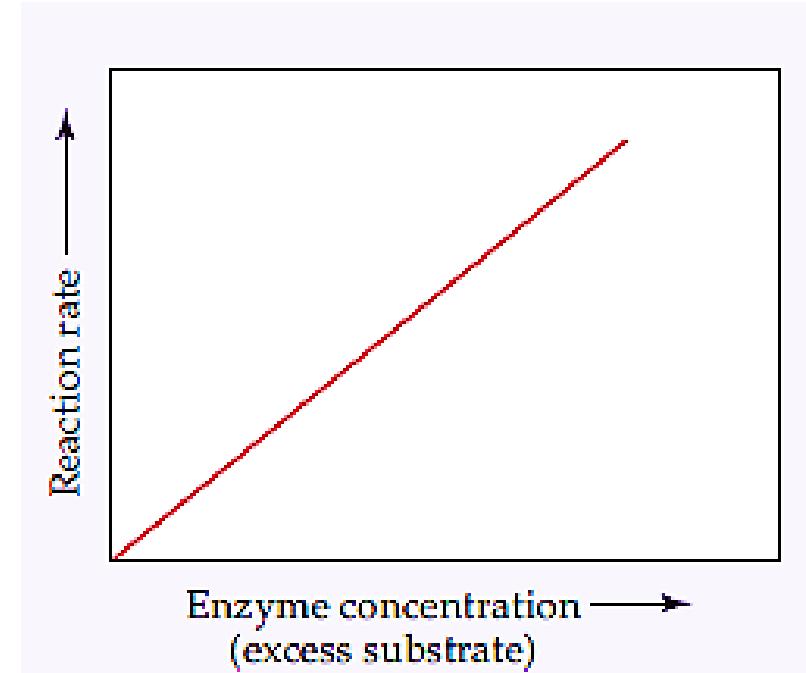
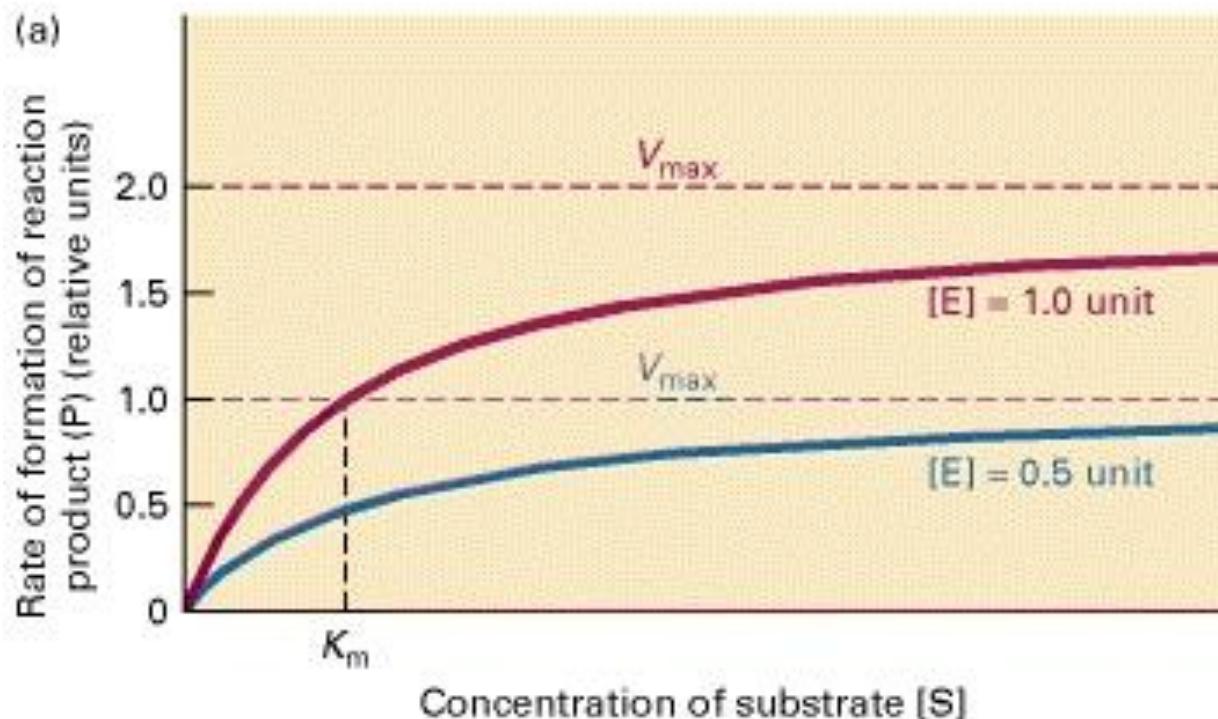
Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosylglycine	108
	N-Benzoyltyrosinamide	2.5
β -Galactosidase	D-Lactose	4.0
	L-Threonine	5.0
Threonine dehydratase		

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

- K_D : dissociation constant, The actual measure of the affinity
- $K_D = (k_{-1}/k_1)$

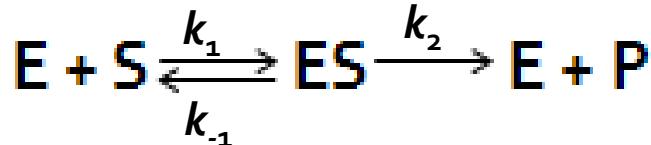


- When you increase enzyme conc., what will happen to V_{max} & K_m ?



V_{max} & k_{cat}

- For the enzymatic reaction



- The maximal rate, V_{max}, is equal to the product of k₂, also known as k_{cat}, and the total concentration of enzyme

$$V_{\text{max}} = k_2 [E]_{\text{T}}$$

- 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

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

- 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 ₂ O 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

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 $6 \times 10^5 \text{ s}^{-1}$
 - ✓ $3.6 \times 10^7 \text{ min}^{-1}$
- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is 1.7 μs for carbonic anhydrase
- The turnover numbers of most enzymes with their physiological substrates fall in the range from 1 to 10^4 per second

Specificity & Efficiency

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

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

- Specificity constant (k_{cat}/K_M): determines the relative rate of the reaction at low [S]
- k_{cat}/K_M ($M^{-1} \text{ min}^{-1}$) 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	$k_{\text{cat}} = \text{Turnover Number}^*$	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	Degradates bacterial cell-wall polysaccharides	0.5	6×10^{-3}

k_{cat} values vary over a wide range

K_M values also vary over a wide range

K_{cat}/K_M , the range is only 4

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

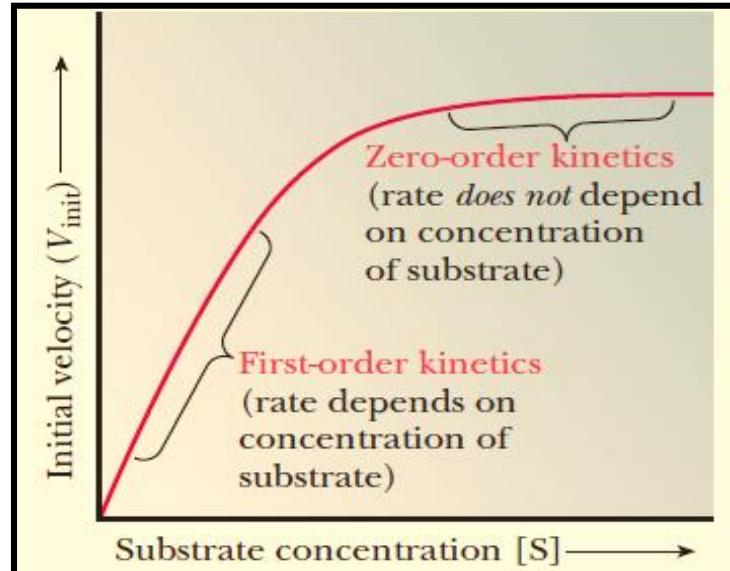
- Reaction rate; measures the concentration of substrate consumed (or product produced) per unit time (mol/{L.s} or M/s)
- Enzyme activity; measures the number of moles of substrate consumed (or product produced) per unit time (mol/s)
 - ✓ Enzyme activity = rate of reaction × reaction volume
- Specific activity; measures moles of substrate converted per unit time per unit mass of enzyme (mol/{s.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 × molecular weight of enzyme

Sample calculations

- A solution contains initially 25×10^{-4} mol L⁻¹ of peptide substrate and 1.5 µg chymotrypsin in 2.5 ml volume. After 10 minutes, 18.6×10^{-4} mol L⁻¹ of peptide substrate remain. Molar mass of chymotrypsin is 25,000 g mol⁻¹.
- How much is the rate of the reaction?
 - (conc./time)
- How much is the enzyme activity?
 - (mol./time)
- How much is the specific activity?
 - (enz. Act. / enz. Mass)
- How much is the turn over number?
 - (sp. Act. X enz. molar mass)

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

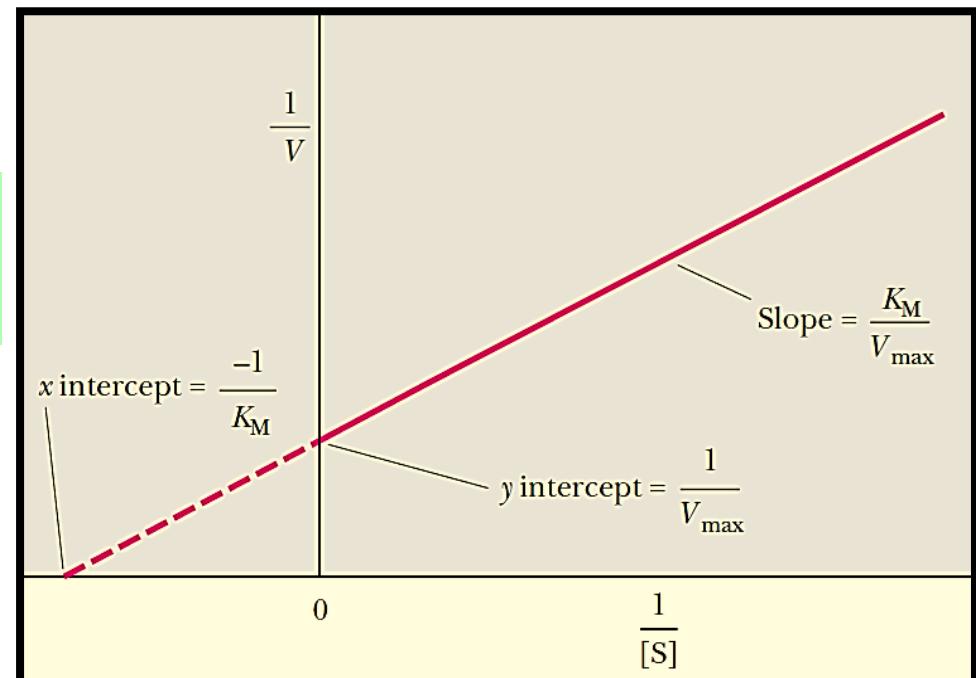
- Determining the K_m from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach V_{max}
- This prevents the calculation of both V_{max} & K_m
- Lineweaver-Burk plot: A plot of $1/v_o$ versus $1/[S]$ (double-reciprocal plot), yields a straight line with an y-intercept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

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



Example

- A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, V_{max} of this enzyme is ... & K_m is ...?

- A. 5000 & 699
- B. 699 & 5000
- C. 621 & 50
- D. 94 & 1
- E. 700 & 8

Substrate Concentration (μM)	Initial velocity ($\mu\text{mol/min}$)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

- You are working on the enzyme “Medicine” which has a molecular weight of 50,000 g/mol. You have used 10 μg of the enzyme in an experiment and the results show that the enzyme converts 9.6 μmol per min at 25 °C. the turn-over number (k_{cat}) for the enzyme is:

- A. 9.6 s^{-1}
- B. 48 s^{-1}
- C. 800 s^{-1}
- D. 960 s^{-1}
- E. 1920 s^{-1}