Enzymes II

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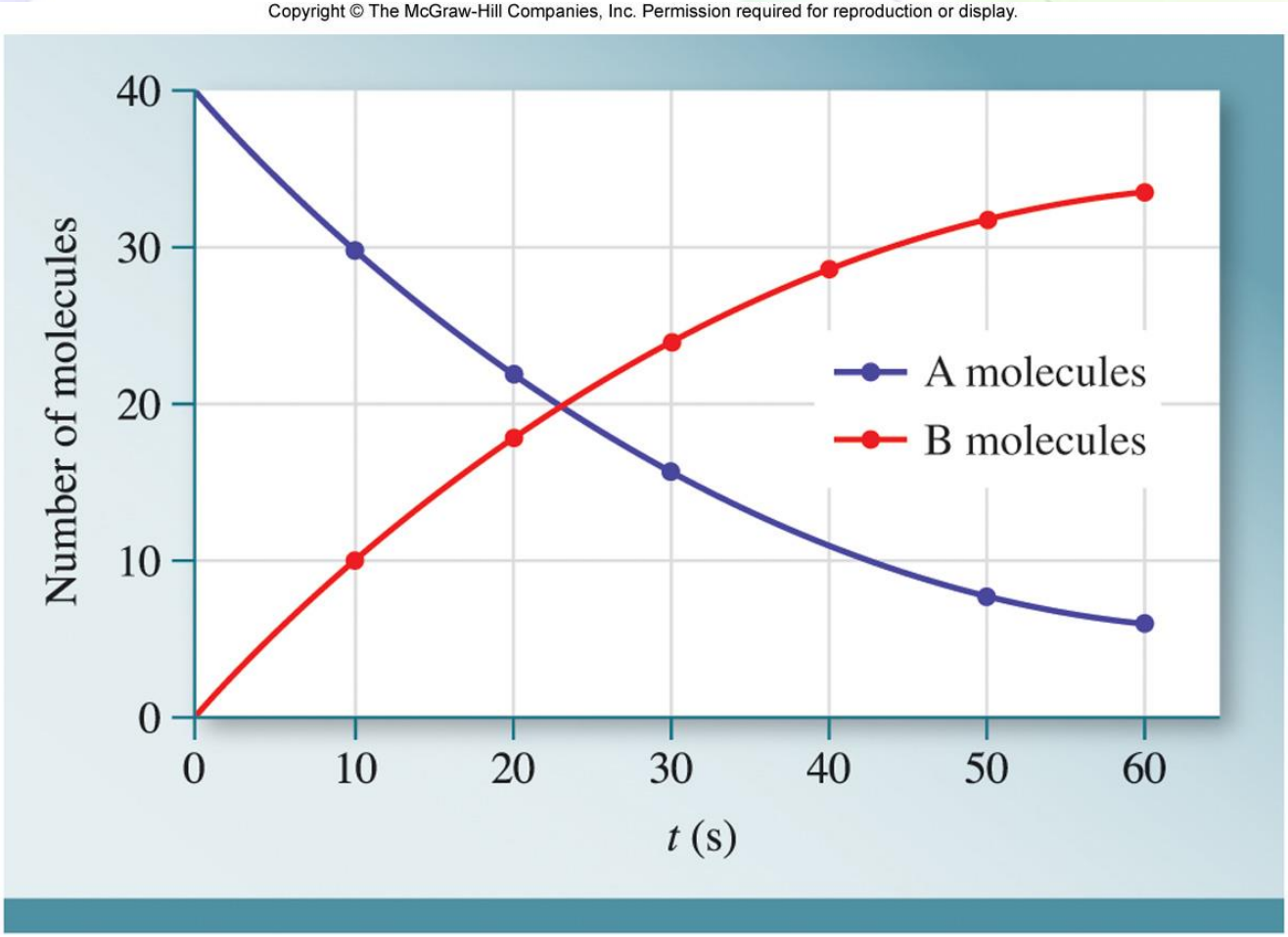
Kinetics

- Kinetics deals with the rates of chemical reactions.
- Chemical kinetics is the study of the rates of chemical reactions.
- For the reaction ($\mathrm{A} \rightarrow \mathrm{B}$), velocity (*v)* or rate of reaction is the amount of B formed or the amount of A consumed per unit time, *t*. That is,

Rate of reaction (velocity or v) = $-\frac{\Delta [\mathrm{A}]}{\Delta t}$ *or* $\frac{\Delta [\mathrm{B}]}{\Delta t}$

It looks like this…



Rate law

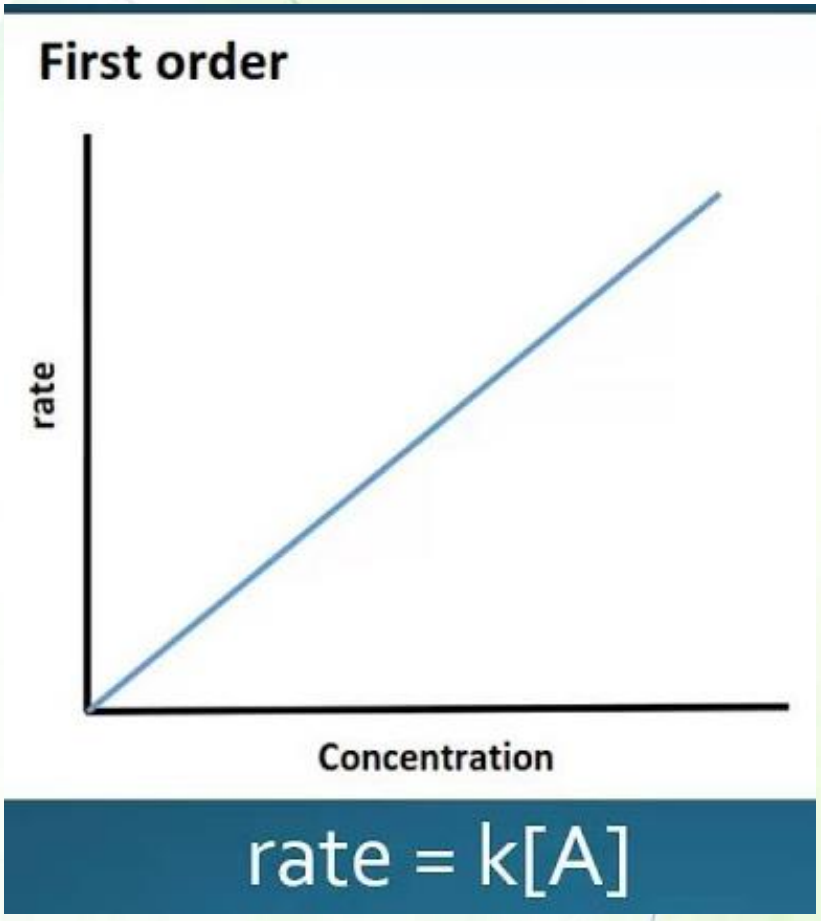
- The relationship between reaction rate and concentration of reactant(s) is known as the rate law, which describes *how concentrations of reactants affect the rate of the reaction during a certain period*.
- For the reaction ($\mathrm{A} \rightarrow \mathrm{B}$), the rate law is

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

- Note: the rate is proportional to the concentration of A, and *k* is the rate constant.
  - *k* has the units of (time)$^{-1}$, usually sec^{-1}.

If (A → B) is a first-order reaction…

- The plot indicates that the rate of a reaction increases linearly with increasing substrate concentration.

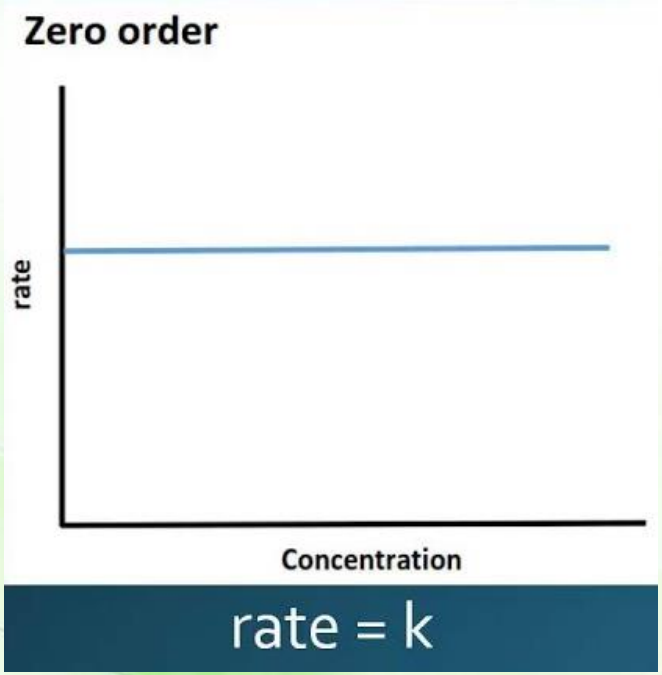


Zero-order reactions

- For a reaction (A $\rightarrow$ B) where

$$\text{rate} = k[A]^0 \quad = k$$

The rate of the reaction is independent of substrates and known as a zero-order reaction.



Enzyme kinetics

- Enzyme kinetics: studying the biological roles of enzymatic reactions.
- Enzyme-catalyzed reactions have hyperbolic plots.

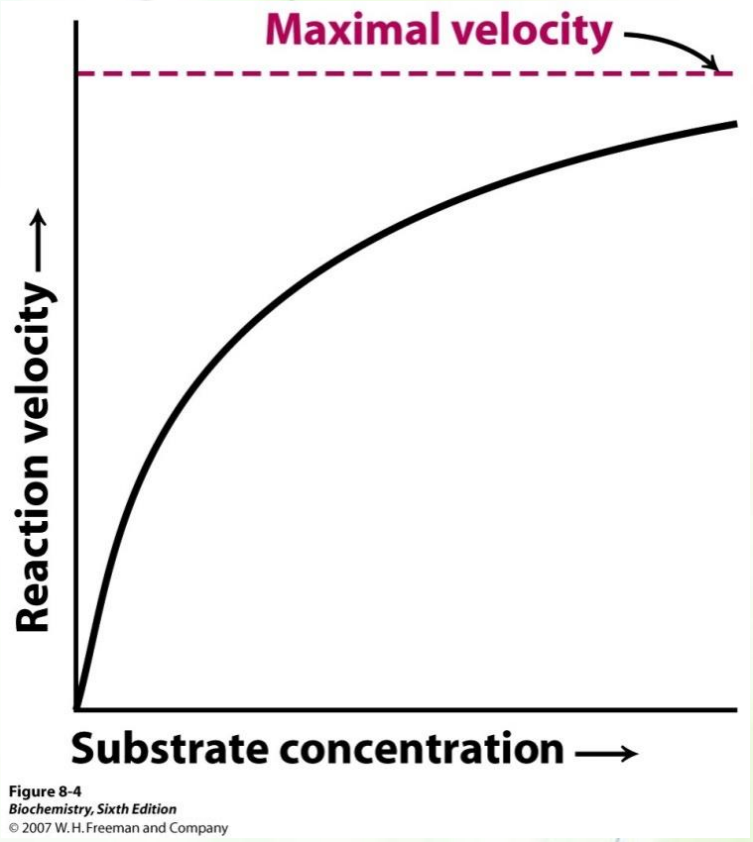
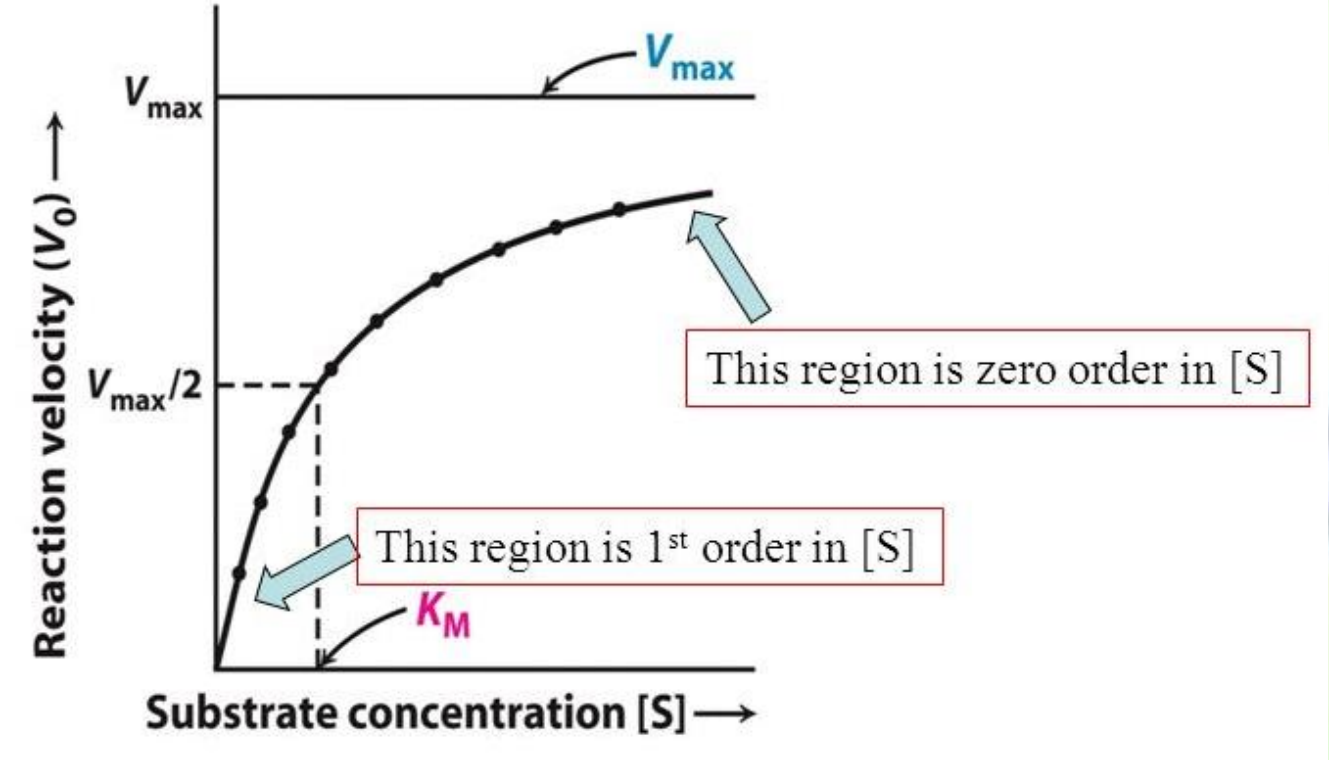


Figure 8-4
Biochemistry, Sixth Edition

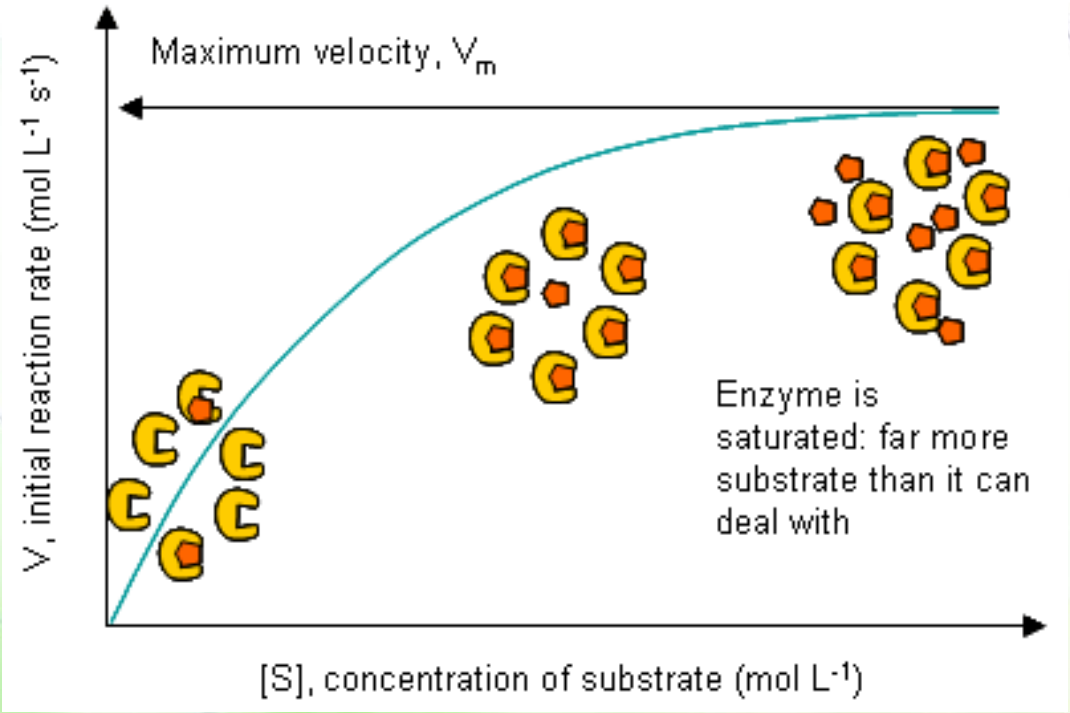
How?

- Initial velocity (V_0) varies with the substrate concentration [S] where the rate of catalysis rises linearly as substrate concentration increases and then begins to level off and approach a maximum at higher substrate concentrations.



Why?

- The hyperbolic plot is known as a saturation plot because the enzyme becomes "saturated" with substrate, i.e. each enzyme molecule has a substrate molecule associated with it.



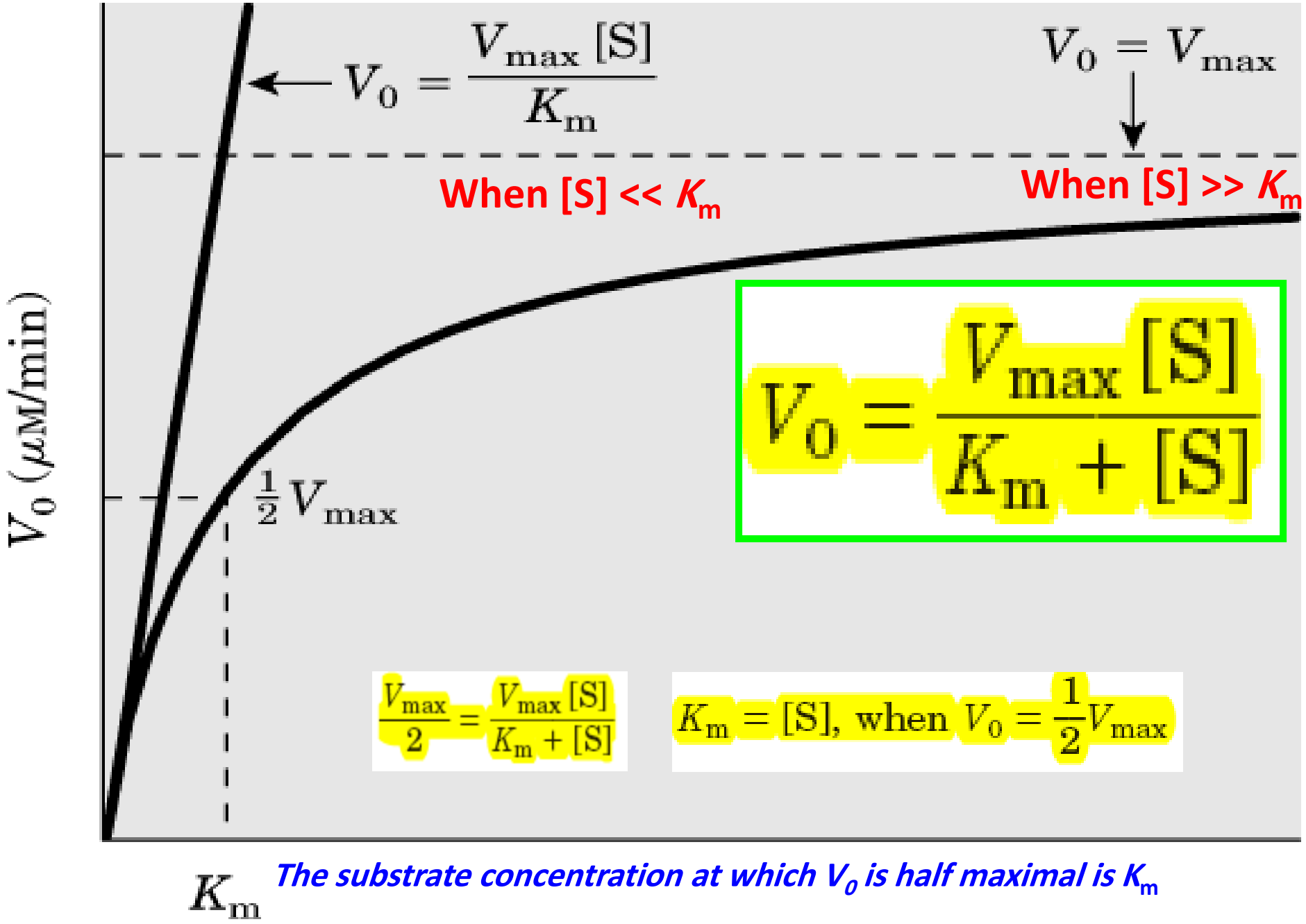
More explanation

- At a fixed concentration of enzyme, V_0 is almost linearly proportional to [S] when [S] is small.
- However, V_0 is nearly independent of [S] when [S] is large
- The maximal rate, Vmax, is achieved when the catalytic sites on the enzyme are saturated with substrate.
- Vmax 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.

The Michaelis-Menten equation

- The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_0), substrate concentration [S], a rate constant (K_M) and maximal velocity (Vmax).

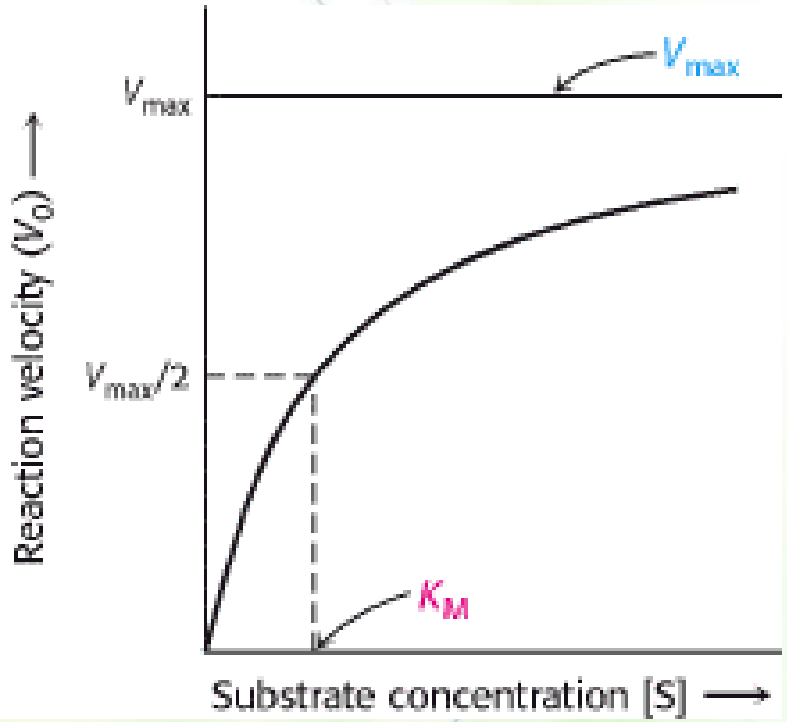
$$V_0 = V_{\max} \frac{[S]}{[S] + K_M}$$


$V_0 = \frac{V_{max}[S]}{K_m}$
$V_0 = V_{max}$
When [S] << K_m
When [S] >> K_m
$V_0 = \frac{V_{max}[S]}{K_m + [S]}$
$\frac{1}{2} V_{max}$
$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$
$K_m = [S]$, when $V_0 = \frac{1}{2} V_{max}$
V_0 (μM/min)
K_m
The substrate concentration at which V_0 is half maximal is K_m
[S] (mM)

The Michaelis constant (K_M)

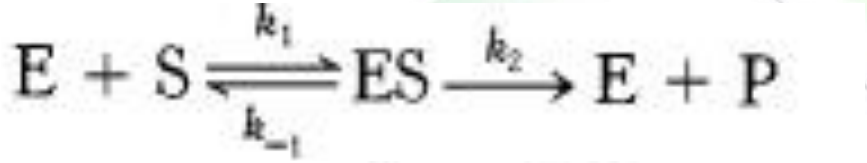
- K_M is the concentration of substrate at which half the active sites are filled.
- When [S] = K_M, then V_0 = Vmax/2
- Therefore, it provides a measure of enzyme affinity towards a substrate.
- The lower the K_M of an enzyme towards a substrate is, the higher its affinity to the same substrate is.

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



The Michaelis constant (K_M)

- For a reaction:



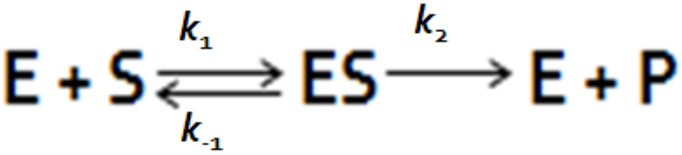
$$K_M = \frac{k_{-1} + k_2}{k_1}$$

($k_{-1} >> k_2$), so $K_M = k_{-1}/k_1$)

- 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, but is not an accurate measure of affinity.

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

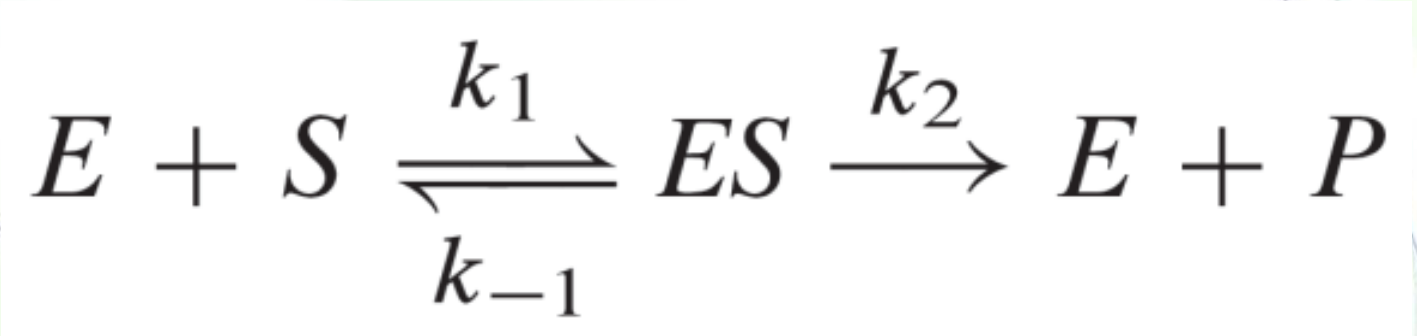
$$K_M = \frac{k_{-1} + k_2}{k_1}$$

- 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

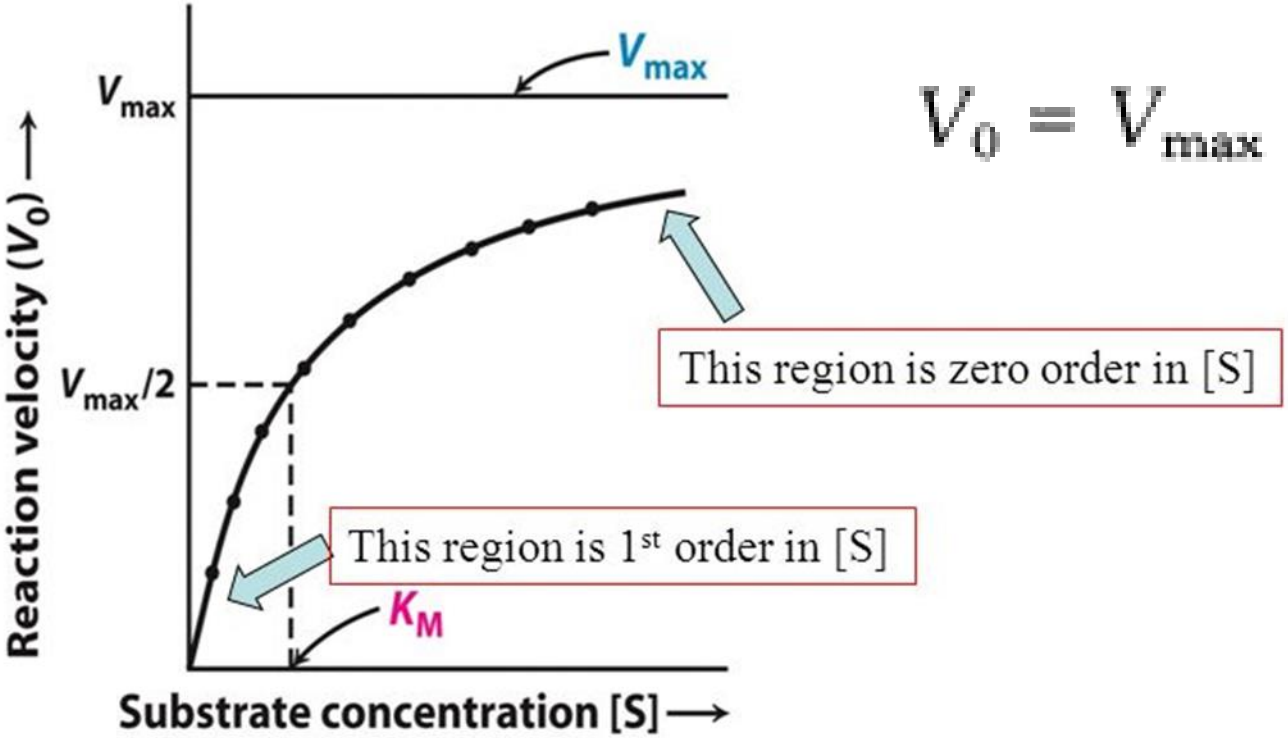
Dissociation constant (K_D)

- K_D (dissociation constant) is the actual measure of the affinity.

$$K_D = (k\text{-}1/k1)$$



- At very low substrate concentration, when [S] is much less than K_M , V_0 = Vmax.[S]/(K_M); that is, the rate is directly proportional to the substrate concentration.
- At high substrate concentration, when [S] is much greater than K_M , V_0 = Vmax; that is, the rate is maximal, independent of substrate concentration.



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

Note

- The K_M values of enzymes range widely (mostly, 10^{-1} and 10^{-7} M).
- Each substrate will have a unique K_M for a given enzymatic process, but Vmax will be the same for the same reaction of more than one substrate.

table 8–6

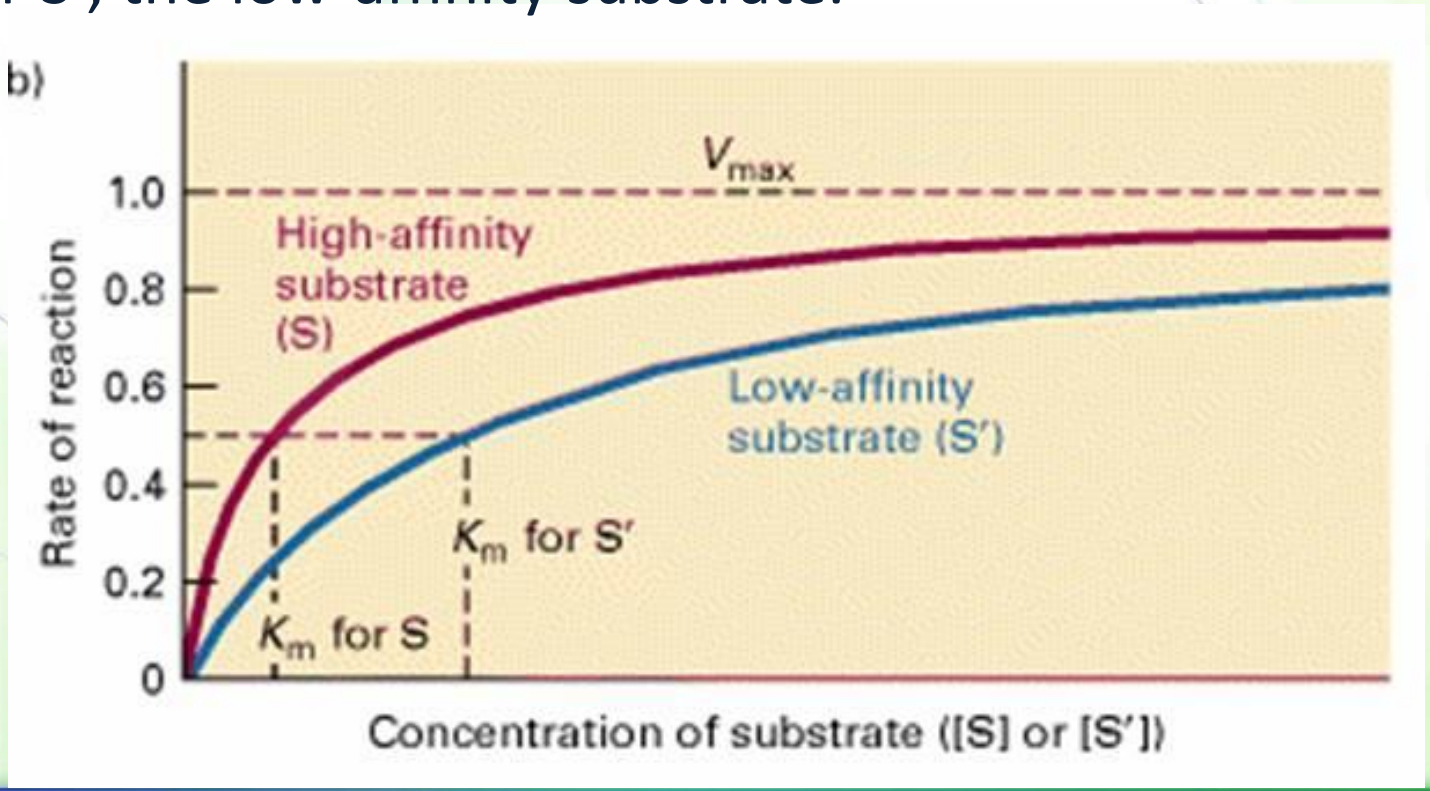
K_m for Some Enzymes and Substrates

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	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Same enzyme, different substrates

Example: Hexokinase – enzyme that phophorylates glucose

- A reactions is catalyzed by an enzyme with substrate S (high affinity) and with substrate S′ (low affinity).
- Vmax is the same with both substrates, but K_M is higher for S′, the low-affinity substrate.



Note

- If an enzyme binds to another substrate generating different product(s), then Vmax will be different.
  - For example, hexokinase phosphorylates glucose and fructose at different Vmax values.

Rates of phosphorylation of glucose and fructose in the brain

Sugar	Properties of brain hexokinase		Sugar concn in brain cell	Calculated rate of phosphorylation In vivo
	Vmax	K_M		
Glucose	17	10^{-5}	10^{-5}	8.5
Fructose	25	10^{-3}	10^{-6}	10^{-2}

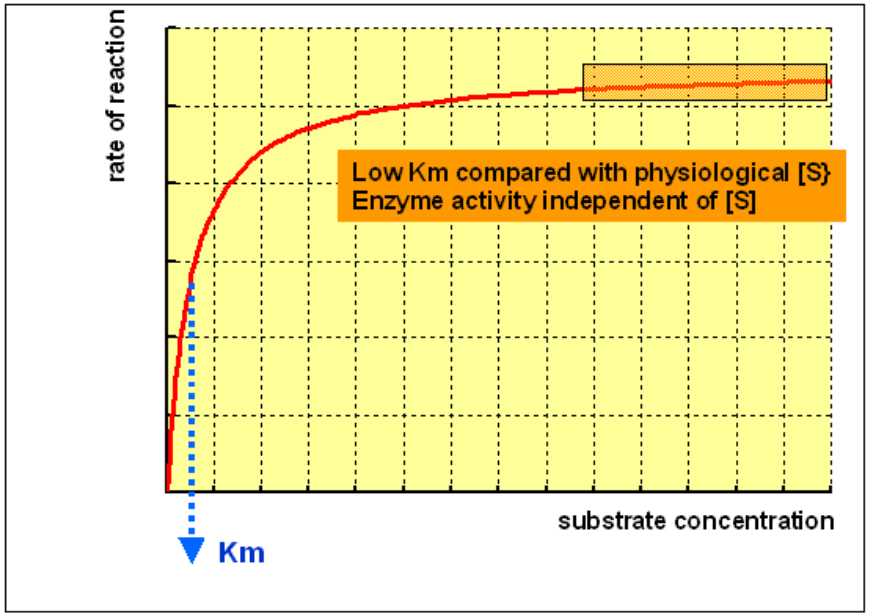
Example

- A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, Vmax of this enzyme is … & K_M is …?
  - 5000 & 699
  - 699 & 5000
  - 621 & 50
  - 94 & 1
  - 700 & 8

Substrate Concentration (μM)	Initial velocity (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

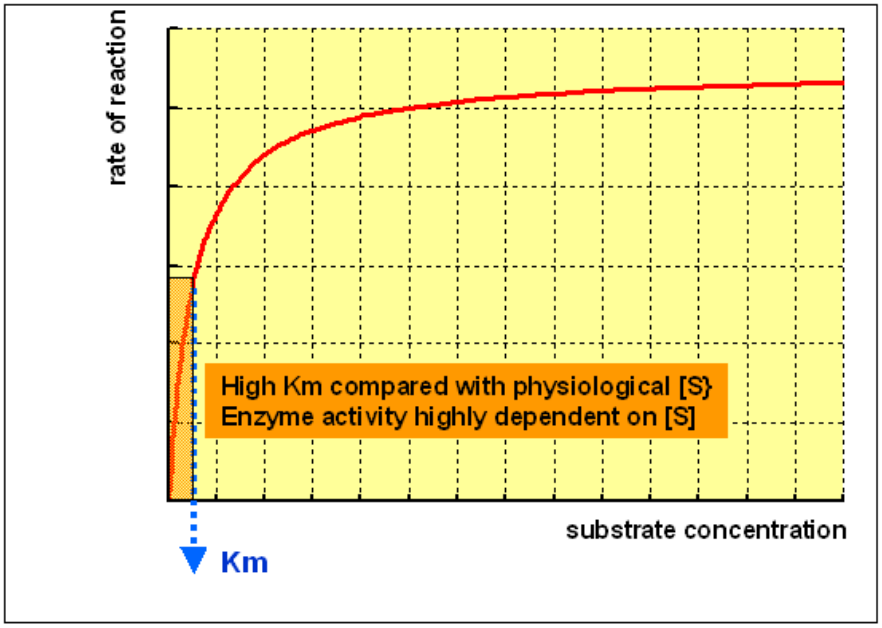
Importance of K_M

If K_M is lower than physiological concentration of S



The enzyme is normally saturated with substrate and will act at a constant rate, regardless of variations in the concentration of substrate.

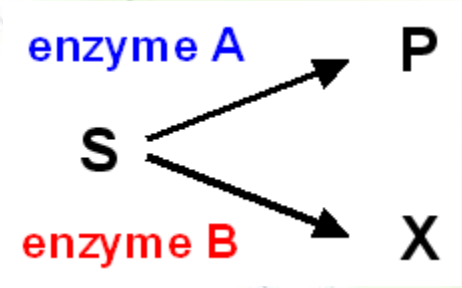
If K_M is lower than physiological concentration of S

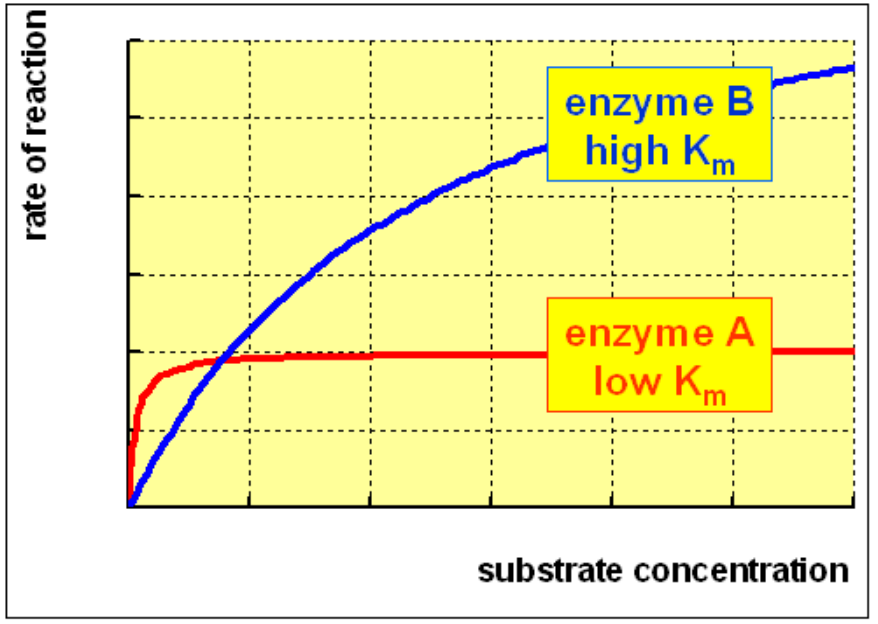


The enzyme is not saturated with substrate and its activity will vary as the concentration of substrate varies and the rate of formation of product will depend on the availability of substrate.

Metabolic pathways

- If two enzymes, in different pathways, compete for the same substrate, then knowing the values of Km and Vmax for both enzymes permits prediction of the metabolic fate of the substrate and the relative amount that will flow through each pathway under various conditions.



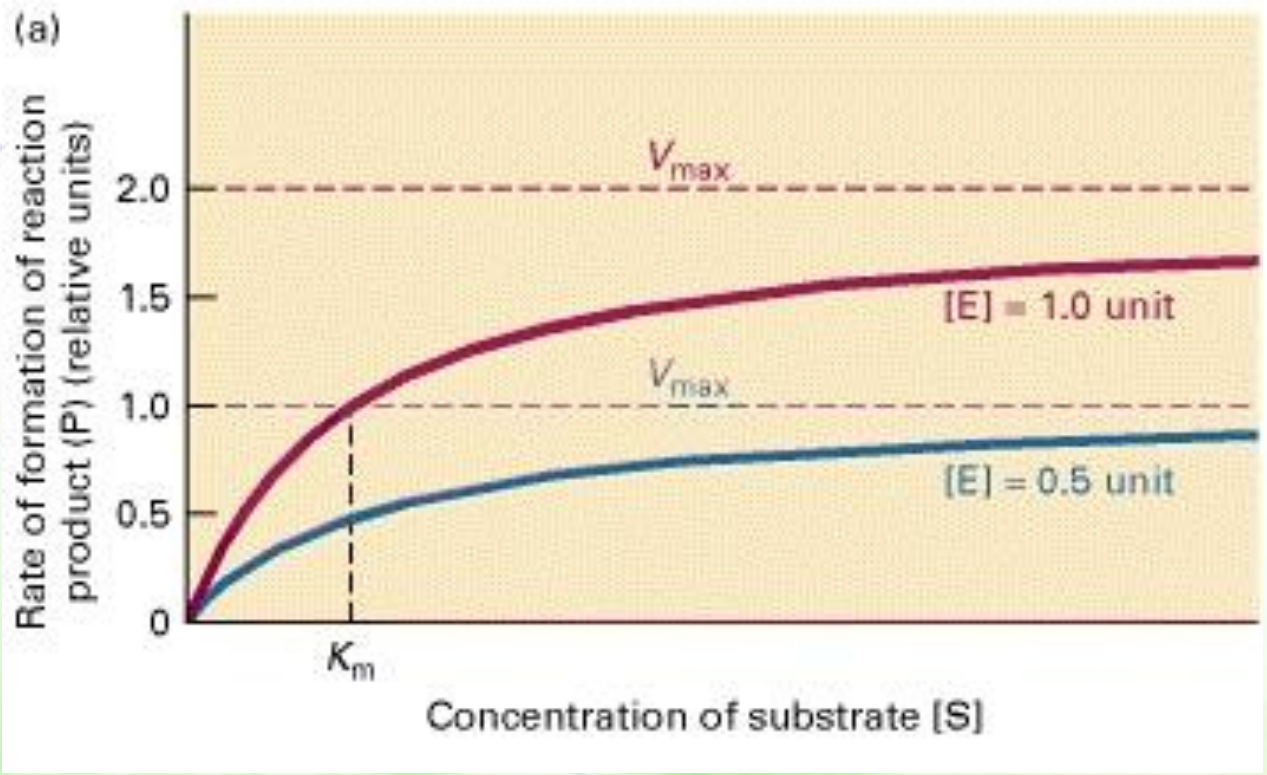


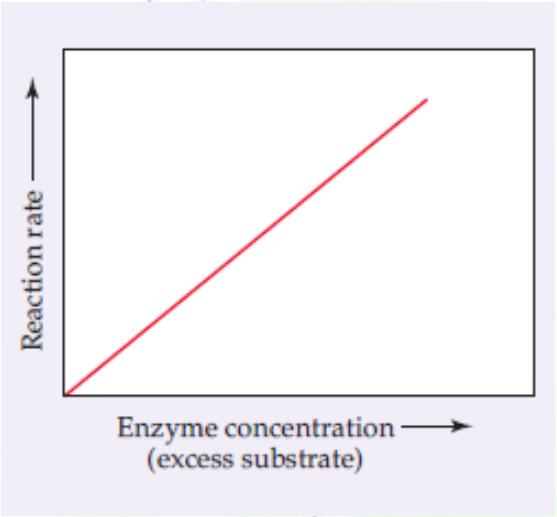
Uses of K_M

- Determine the substrate preferences of an enzyme.
  - If more than one endogenous compound can act as a substrate for an enzyme, the substrate with the lowest Km is probably the preferred physiological substrate.
- Distinguish isozymes, which are different enzymes catalyzing the same reaction.
  - Isozymes often have different affinities for the same substrate.
- Check for abnormalities in an enzyme.

Vmax and enzyme concentration

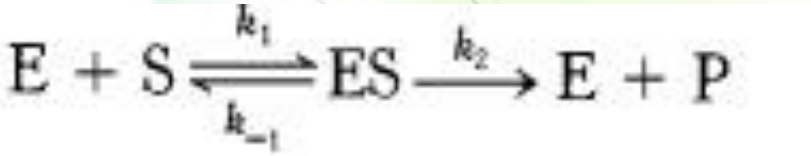
- Doubling the concentration of enzyme causes a proportional increase in the reaction rate, so that the maximal velocity V_{max} is doubled; the K_M , however, is unaltered.





Vmax & kcat

(a measure of enzyme efficiency)

- For the enzymatic reaction 

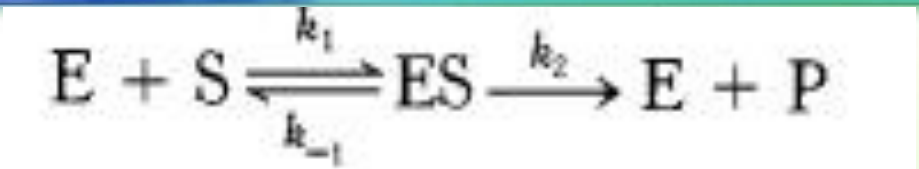
- The maximal rate, V_{max}, is equal to the product of k_2, also known as k_{cat}, and the total concentration of enzyme.

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

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

Kcat



- kcat, 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.
- It describes how quickly an enzyme acts, i.e. how fast the ES complex proceeds to E + P.
- In other words, the maximal rate, Vmax, reveals the turnover number of an enzyme if the total concentration of active sites $[E]_T$ is known.
- kcat is a constant for any given enzyme.

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

Example

- 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 at best converts 9.6 µmol of the substrate per min at 25˚C. The turnover number (kcat) 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}

- MW = 50,000 g/mol
- Weight = 10 µg
- V_{max} = 9.6 µmol of the substrate per min

- Kcat = (9.6/60)/(10 µg /50,000)
  = 800 s^{-1}

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.

Kcat = Vmax/[E] = 0.6 /10^{-6} = 6 x 10^5 / sec

6 x 10^5 x 60 sec/min = 3.6 x 10^7 / min

1 / 3.6 x 10^7 = 2.7 x 10^{-6} min per reaction

- Each catalyzed reaction takes place in a time equal to $1/k_2$, which is 2.7 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.

40,000,000 molecules of H_2O_2 are converted to H_2O and O_2 by ONE catalase molecule within one second

table 8–7

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

Kcat vs. K_M

Table 6.2

Turnover Numbers and Km for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M**
Catalase	Conversion of H_2O_2 to H_20 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 2X10^7

K_M values vary over a range of nearly 4000

Rate of reaction (velocity)

- Rate of reaction is calculated as concentration of substrate disappearing (or concentration of product appearing) per unit time (mol L^{-1} . sec^{-1} or M . sec^{-1}).

Enzyme activity

- In order to measure enzyme activity, we measure the number of moles of substrate disappearing (or products appearing) per unit time ($mol \cdot . sec^{-1}$)

- In other words,

  enzyme activity = rate of reaction $\times$ reaction volume

Specific activity

- Specific activity is usually a measure of enzyme purity and quality in a sample.
- It is described as moles of substrate converted per unit time per unit mass of enzyme (mol . sec^{-1} . g^{-1}).

Specific activity =enzyme activity / mass of enzyme (grams)

- This is useful in determining enzyme purity after purification.
- It is also used when the molar enzyme concentration is not known.
  - (If the moles of enzyme present is unknown, it is impossible to calculate *kcat*.)

Turnover number

- Turnover number (kcat) is related to the specific activity of the enzyme where it is

  Turnover number = specific activity × molecular weight of enzyme

  - It is expressed as moles of substrate converted per unit time (usually per second)/moles of enzyme (min^{-1} or sec^{-1})

- Remember: $k_{cat} = V_{max} / [E]_T$

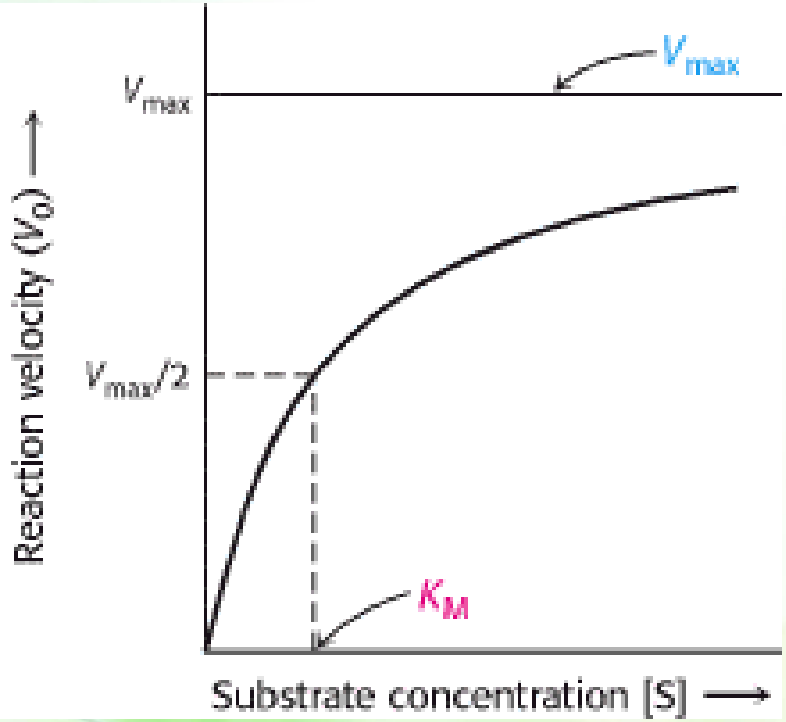
Sample calculations:

A solution contains initially **25.0×10^{-4} mol L^{-1}** of peptide substrate and **1.50 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}.**

peptide substrate consumed	= 6.4 x 10^{-4} mol L^{-1} in 10 minutes
Rate of reaction	= **6.4 x 10^{-5} mol L^{-1} min^{-1}**
Enzyme activity	= 6.4 x 10^{-5} mol L^{-1} min^{-1} x 2.5 x 10^{-3} L
(rate × volume)	= **1.6 x 10^{-7} mol min^{-1}**
Specific activity	= 1.6 x 10^{-7} mol min^{-1} / 1.50 µg
(activity / mass)	= **1.1 x 10^{-7} mol $µg^{-1}$ min^{-1}**
Turnover number	= 1.1 x 10^{-7} mol $µg^{-1}$ min^{-1} x 25,000 g mol^{-1} x 10^{6} µg g^{-1}
(sp. act. × molar mass)	= 2.7 x 10^{3} min^{-1} = **45 s^{-1}**

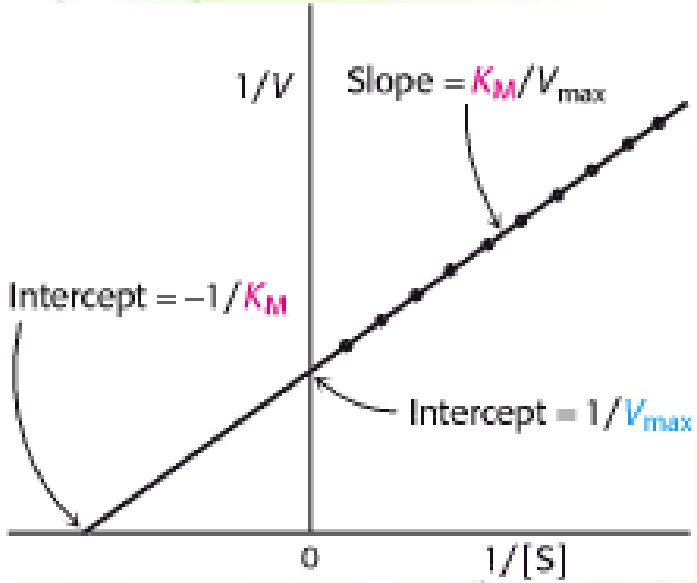
Disadvantage of Michaelis-Menten equation

- Determination of Km from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach Vmax.
- This prevents the calculation of both Vmax and K_M.



The Lineweaver-Burk or double-reciprocal plot

- A plot of 1/ V_0 versus 1/[S], called a Lineweaver-Burk or double-reciprocal plot, yields a straight line with an intercept of 1/Vmax and a slope of K_M /Vmax.
- The intercept on the x-axis is -1/ K_M.

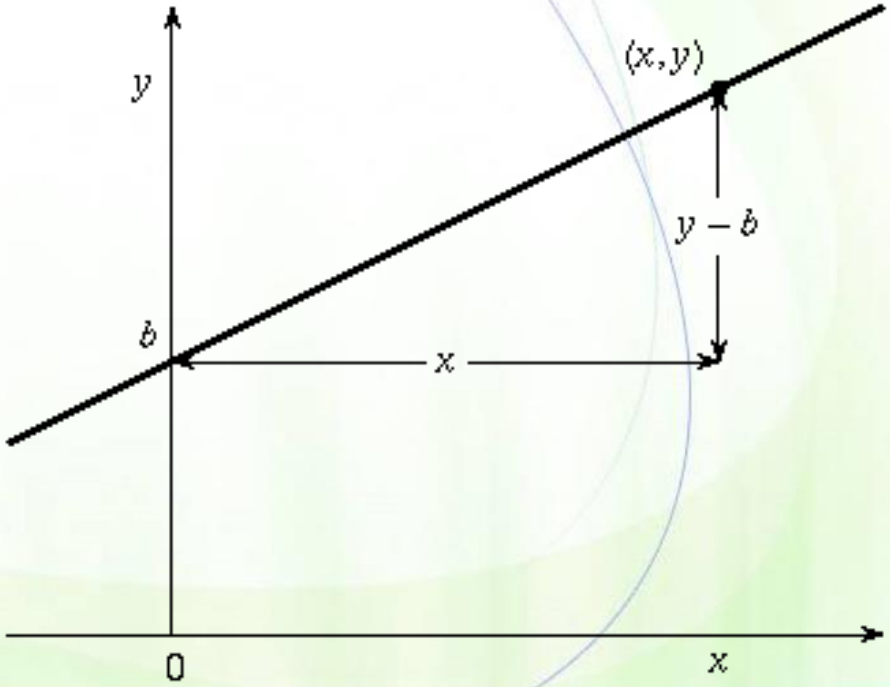


$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

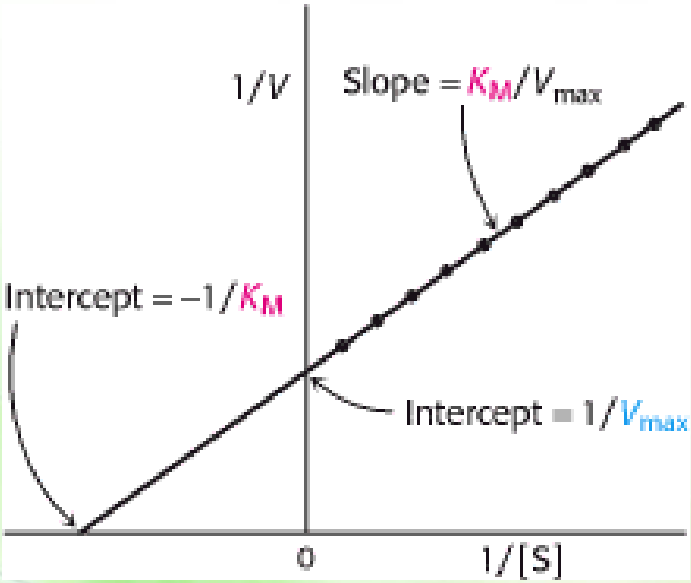
- y is y-axis = 1/ V_0
- x is x-axis = 1/[S]
- m is slope = K_M/ Vmax
- B is 1/Vmax



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

- If x = 0, then y = b (x-axis is 0, then y-intercept = 1/Vmax)



$$\frac{1}{V_0} = \frac{1}{V_{max}} + \frac{K_M}{V_{max}} \cdot \frac{1}{[S]}$$

y= b + mx

If y = 0, then mx = -b (y-axis is 0, then x-intercept = -1/Km)

How?

0 = 1/Vmax + (K_M/Vax) . (1/[S])

-1/Vmax = (K_M/Vax) . (1/[S])

-1 = K_M . (1/[S])

-1/ K_M= 1/[S]