Kinetics of enzymatic reactions

Kinetics

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

$$v = \frac{d[\mathrm{P}]}{dt} \qquad \textbf{or} \qquad v = \frac{-d[\mathrm{A}]}{dt}$$

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 → P), the rate law is**

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

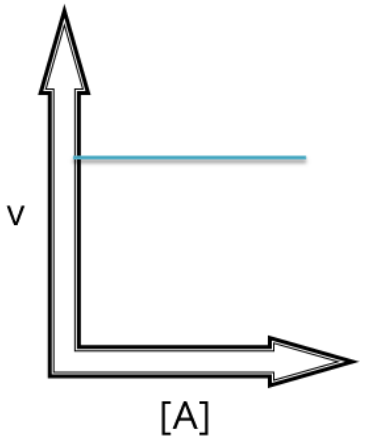
- **From this expression, the rate is proportional to the concentration of A, and *k* is the rate constant**

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

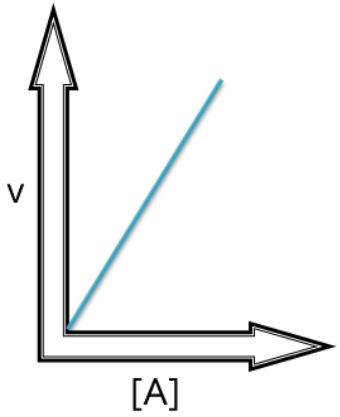
- **A multistep reaction can go no faster than the slowest step**

$$v = k(A)^{n1}(B)^{n2}(C)^{n3}$$

- ***k* is the rate constant: the higher the activation energy (energy barrier), the smaller the value of k**
- **(n1+n2+n3) is the overall order of the reaction**
- **Dimensions of k**

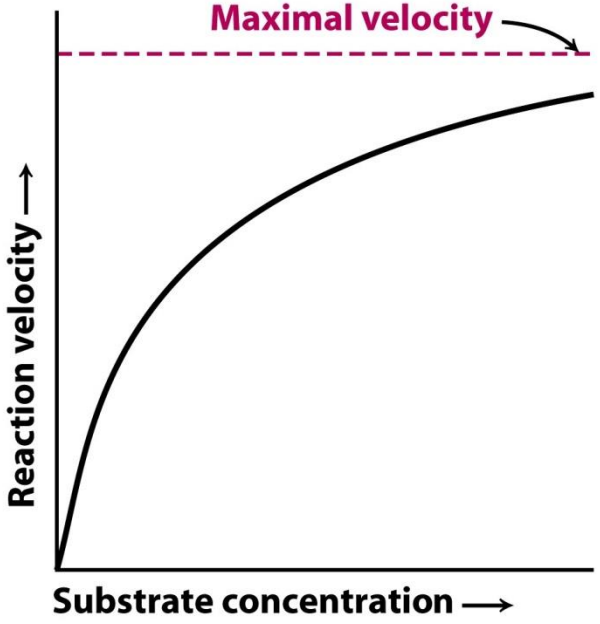


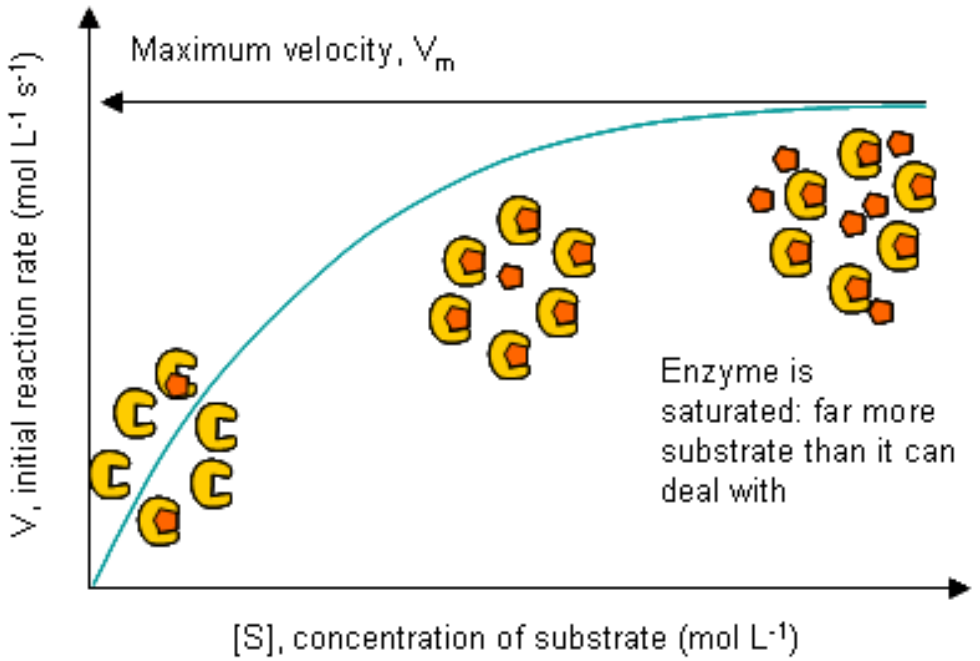
Overall order	V=	Dimentions of k
Zero	*k*	$(conc.)(time)^{-1}$
First	*k*(A)	$(time)^{-1}$



Enzyme kinetics

- **Enzymatic reactions 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 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"

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

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

$$v = k_2\, ES$$

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

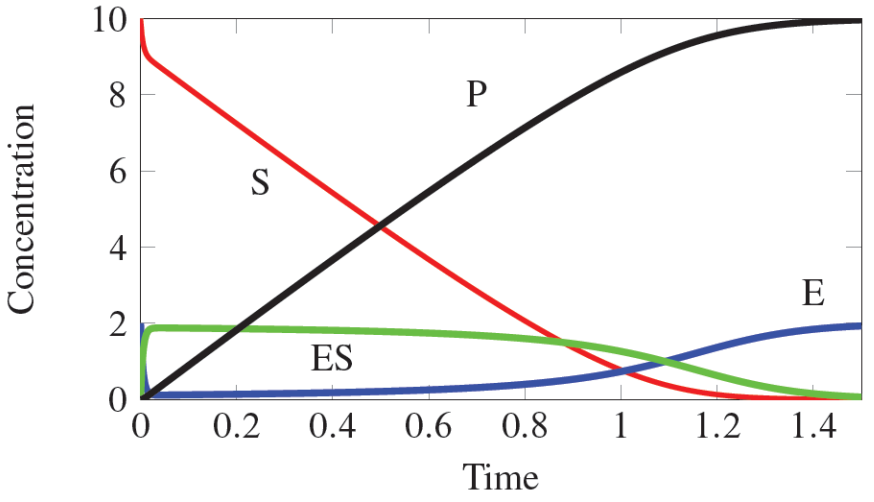
$$0 = k_1\, E\,.\,S - k_{-1}\, ES - k_2\, ES$$

$$E_t = E + ES$$

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

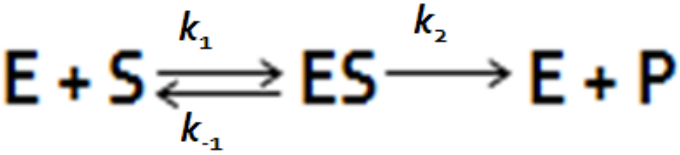
$$v = \frac{E_t\, k_2\, S}{(k_{-1} + k_2)/k_1 + S}$$

$$v = \frac{Vmax\, S}{K_m + S}$$



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 disso from the enzyme to the enzyme-substrate complex**

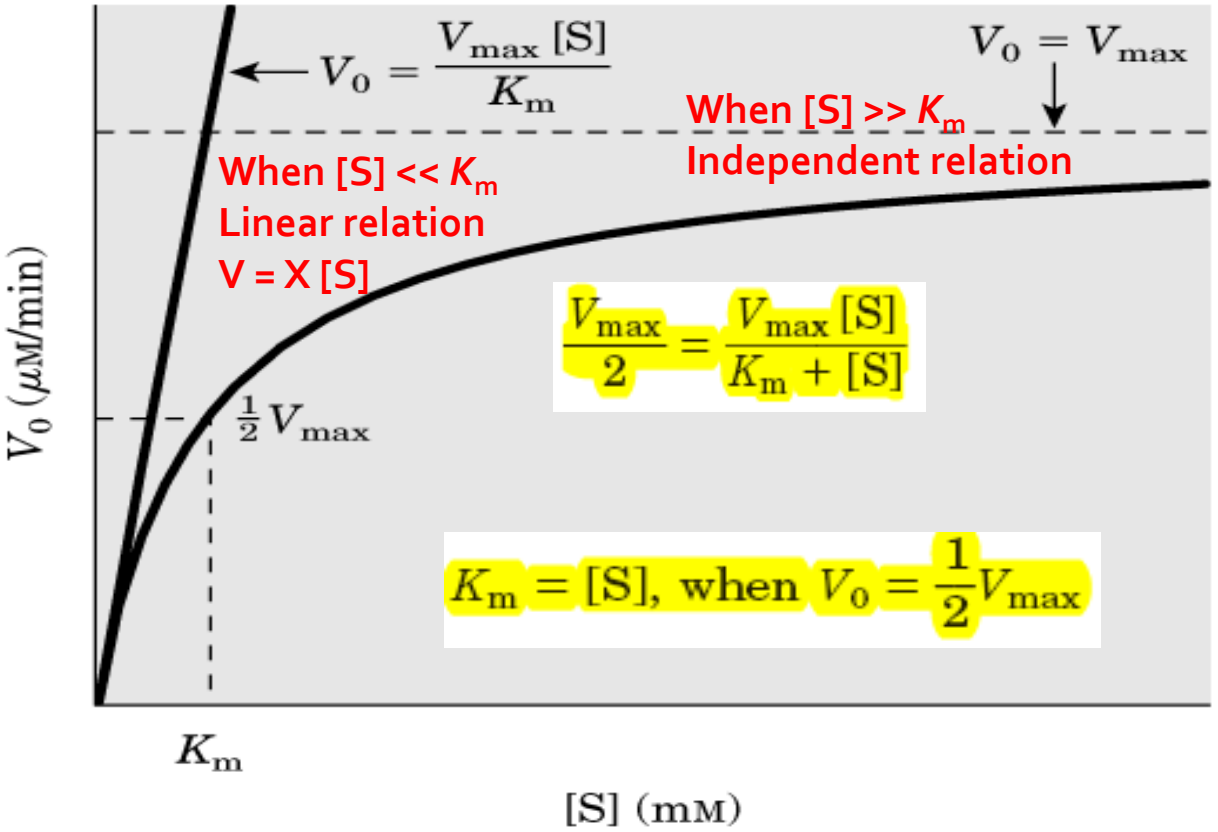
- ***K_m* describes the affinity of enzyme for the substrate**

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})**

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

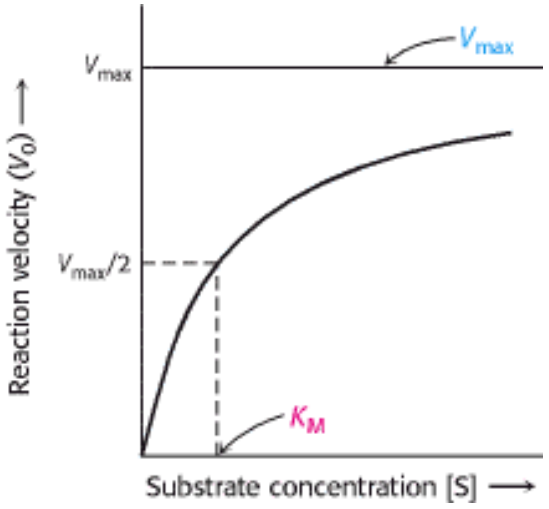
The substrate concentration at which V_o is half maximal is K_m



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}**
- **K_m values have a wide range. Mostly between (10^{-1} & 10^{-7} M)**



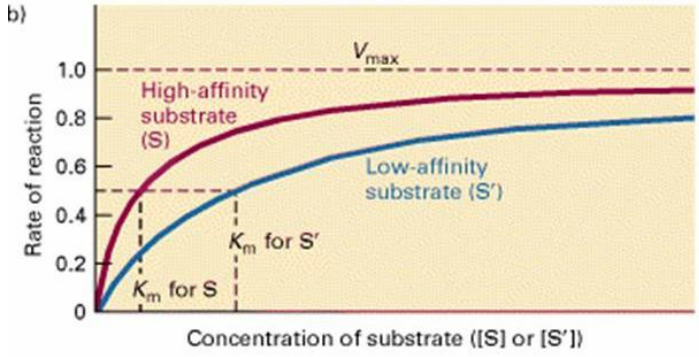


table 8–6

K_m for Some Enzymes and Substrates

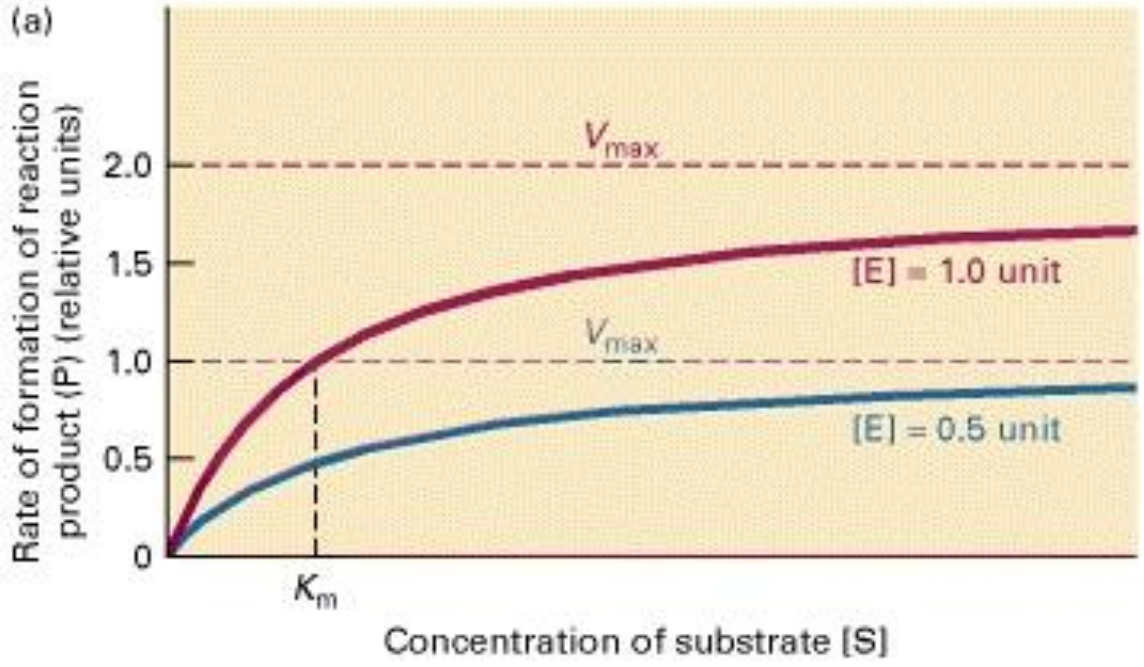
Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

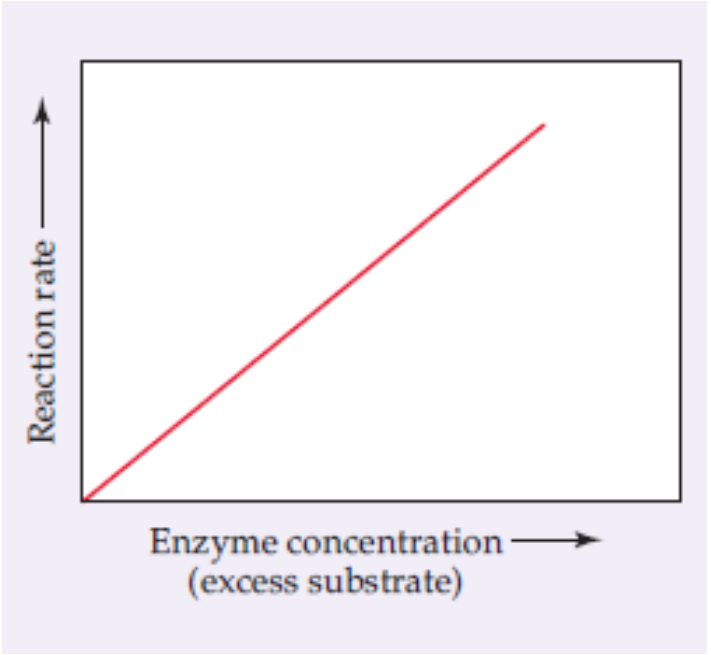
K_M & K_D
[E], K_M & V_{max}

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

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

- **When you increase the enzyme concentration, what will happen to V_{max} & K_m?**





V_{max} & k_{cat}

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

- **For the enzymatic reaction**

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

- **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**

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

- **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**

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 6×10^5 s^{-1}**
  - ✓ **10^4 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

$$V = \frac{V_{max}[S]}{K_M + [S]} = \frac{k_{cat}[E_T][S]}{K_M + [S]} \qquad V = (k_{cat}/K_M)[E][S]$$

- **Specificity constant (k_{cat}/K_M): determines the relative rate of the reaction at low [S]**
- **k_{cat}/K_M (M^{-1} 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 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 wide range

K_M values also vary over a wide range

K_{cat}/K_M, the range is narrower

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**

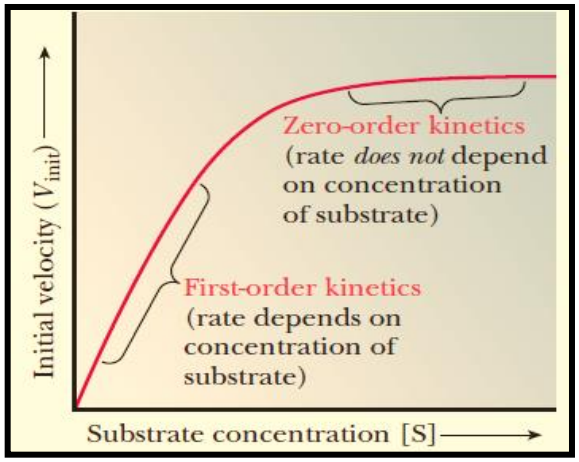
Sample calculations:

A solution contains initially **25.0×10^{-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	= 6.4×10^{-4} mol L^{-1} in 10 minutes
Rate of reaction	= **6.4×10^{-5} mol L^{-1} min^{-1}**
Enzyme activity	= 6.4×10^{-5} mol L^{-1} min^{-1} x 2.5×10^{-3} L
(rate × volume)	= **1.6×10^{-7} mol min^{-1}**
Specific activity	= 1.6×10^{-7} mol min^{-1} / 1.50 µg
(activity / mass)	= **1.1×10^{-7} mol $µg^{-1}$ min^{-1}**
Turnover number	= 1.1×10^{-7} mol $µg^{-1}$ min^{-1} x 25,000 g mol^{-1} x 10^{6} µg g^{-1}
(sp. act. × molar mass)	= 2.7×10^{3} min^{-1} = **45** 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 $1/v_0$ 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 $-1/K_M$**



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

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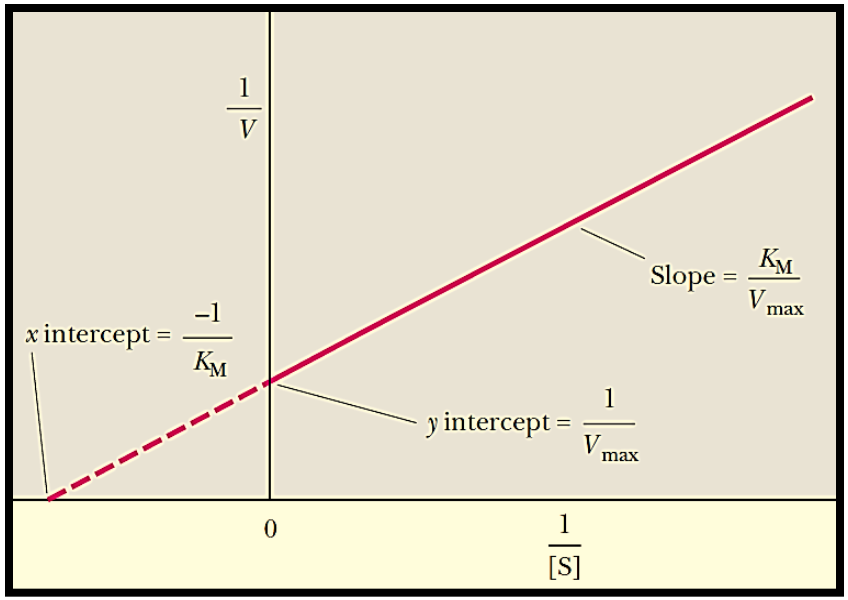
$$\frac{1}{v} = \left[\frac{K_m(1)}{V_{max}[S]} + \frac{1}{V_{max}}\right]$$



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 (µmol/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}**