Enzyme Chemistry

Enzyme's Kinetics

Introduction

"It is a branch of biochemistry in which we study *the rate of enzyme catalyzed reactions*."

- **Kinetic analysis reveals the number and order of the individual steps by which enzymes transform substrate into products**

- **Why we Study an enzyme's kinetics??**

In this way can reveal the 1. catalytic mechanism of that enzyme, 2. its role in metabolism, 3. how its activity is controlled, and 4. how a drug or an agonist might inhibit the enzyme

Rates of reaction and their dependence on activation energy

- **Activation Energy (Ea):**

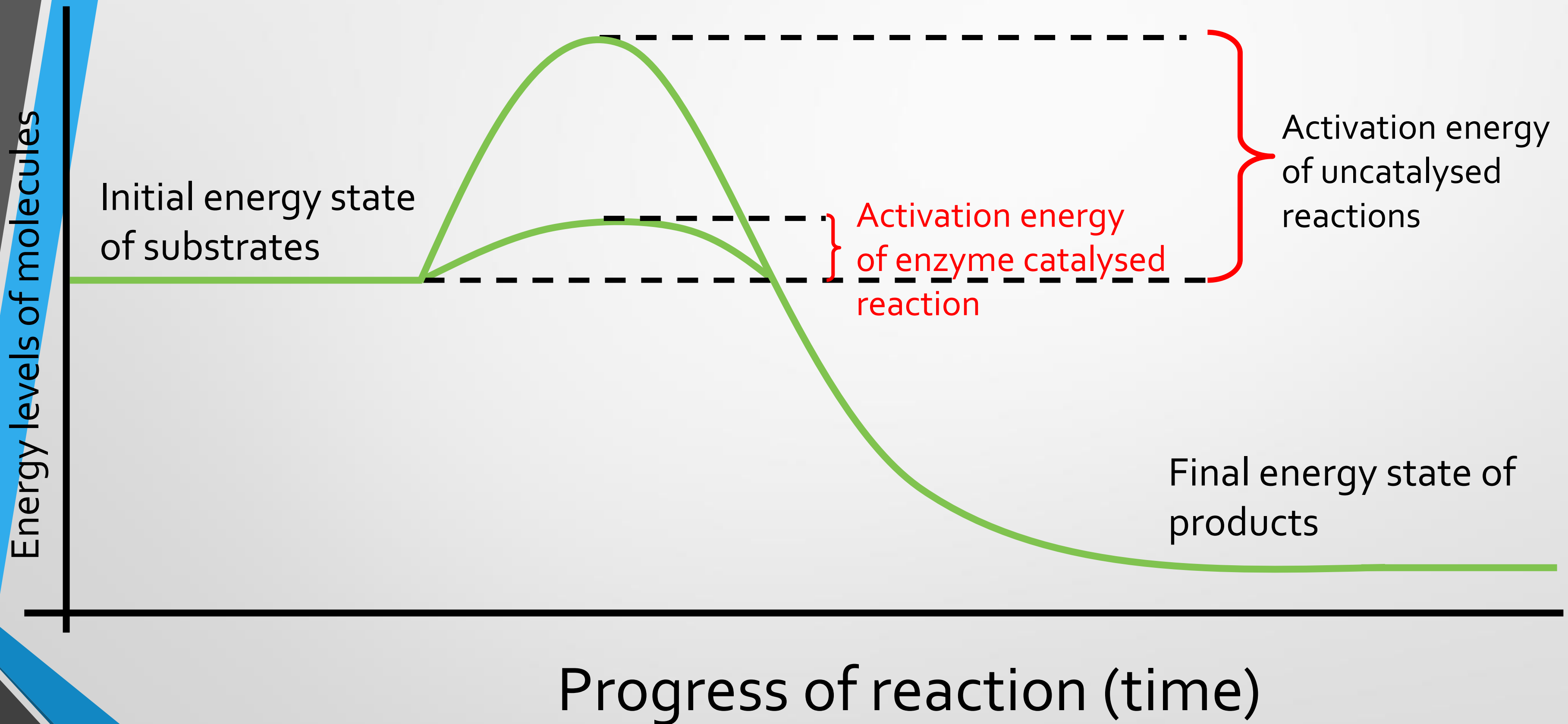
"The least amount of energy needed for a chemical reaction to take place"

- **Enzyme (as a catalyst) acts on substrate in such a way that they lower the activation energy by changing the route of the reaction.**
- **The reduction of activation energy (Ea) increases the amount of reactant molecules that achieve a sufficient level of energy, so that they reach the activation energy and form the product.**

Example:

- ***Carbonic anhydrase*** **catalyses the hydration of 10^6 CO_2 molecules per second which is 10^7x faster than spontaneous hydration.**

Enzymes lower the activation energy of a reaction



Kinetics of enzymes catalysis

- **Enzymes catalysis:**

  “ It is an increase in the rate of reaction with the help of enzyme(as catalyst).”

- **Catalysis by enzymes that proceed via unique reaction mechanism, typically occurs when the transition state intermediate forms a covalent bond with the enzyme(covalent catalysis).**
- **During the process of catalysis enzymes always emerge unchanged at the completion of the reaction.**

Factors affecting rate of enzyme catalyzed reactions

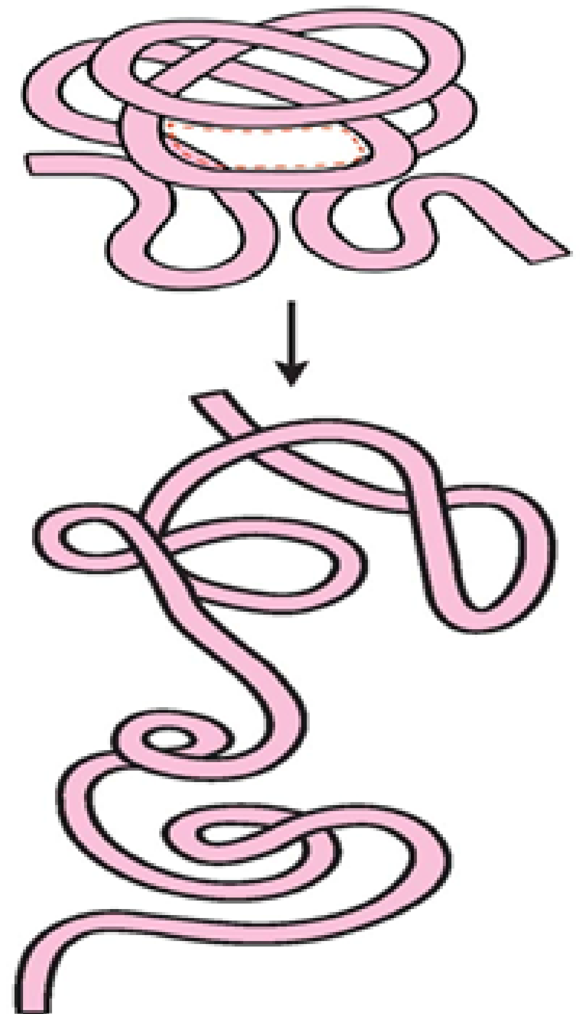
- Temperature
- Hydrogen ion concentration(pH)
- Substrate concentration

Effect of Temperature

- **Raising the temperature increases the rate of enzyme catalyzed reaction by increasing kinetic energy of reacting molecules.**
- **Enzymes work maximum over a particular temperature known as *optimum temperature*. Enzymes for humans generally exhibit stability temperature up to 35-45 °C.**
- **The temperature coefficient is a factor Q_{10} by which the rate of biological processes increases for a 10 °C increase in temperature.**
- **For most biological processes Q_{10} = 2.**
- **However some times heat energy can also increase kinetic energy to a point that exceed the energy barrier which results in denaturing of enzymes.**

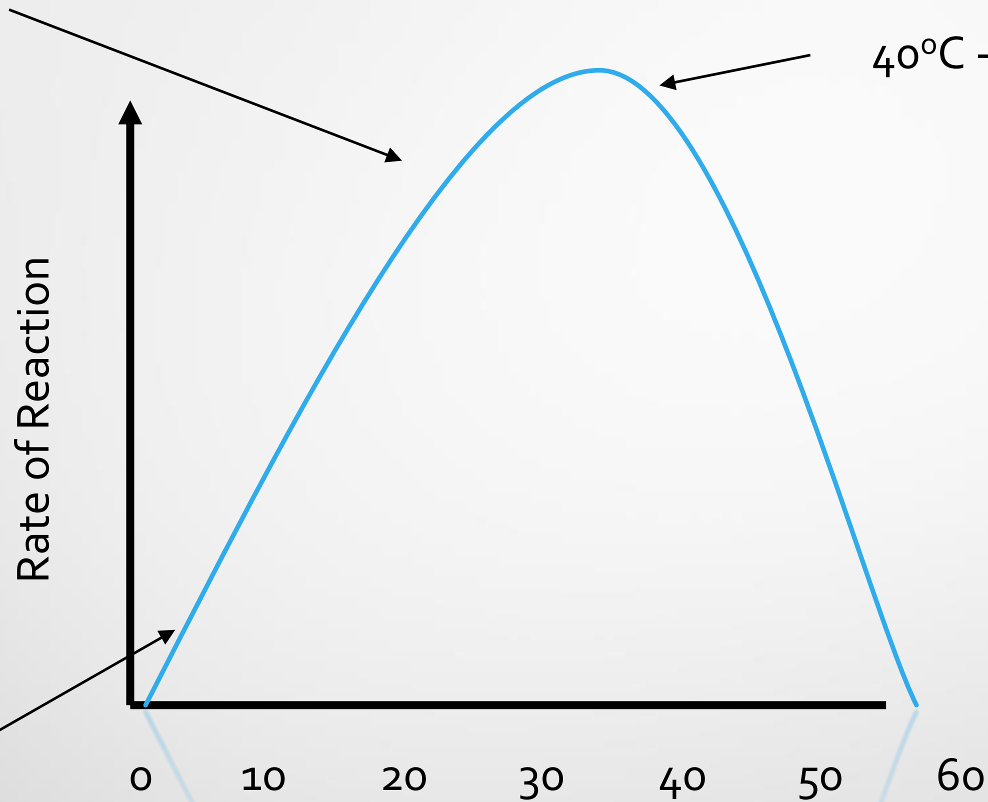
Effect of temperature

- At optimum temp:
  - Rate of reaction is the **highest**.
  - Enzymes are **most active**.
- Beyond optimum temp:
  - Rate of enzyme activity **decreases sharply.**
  - Enzymes are being **denatured.**
  - Hydrogen bonds are easily disrupted by **increasing temperature**.



Temperature

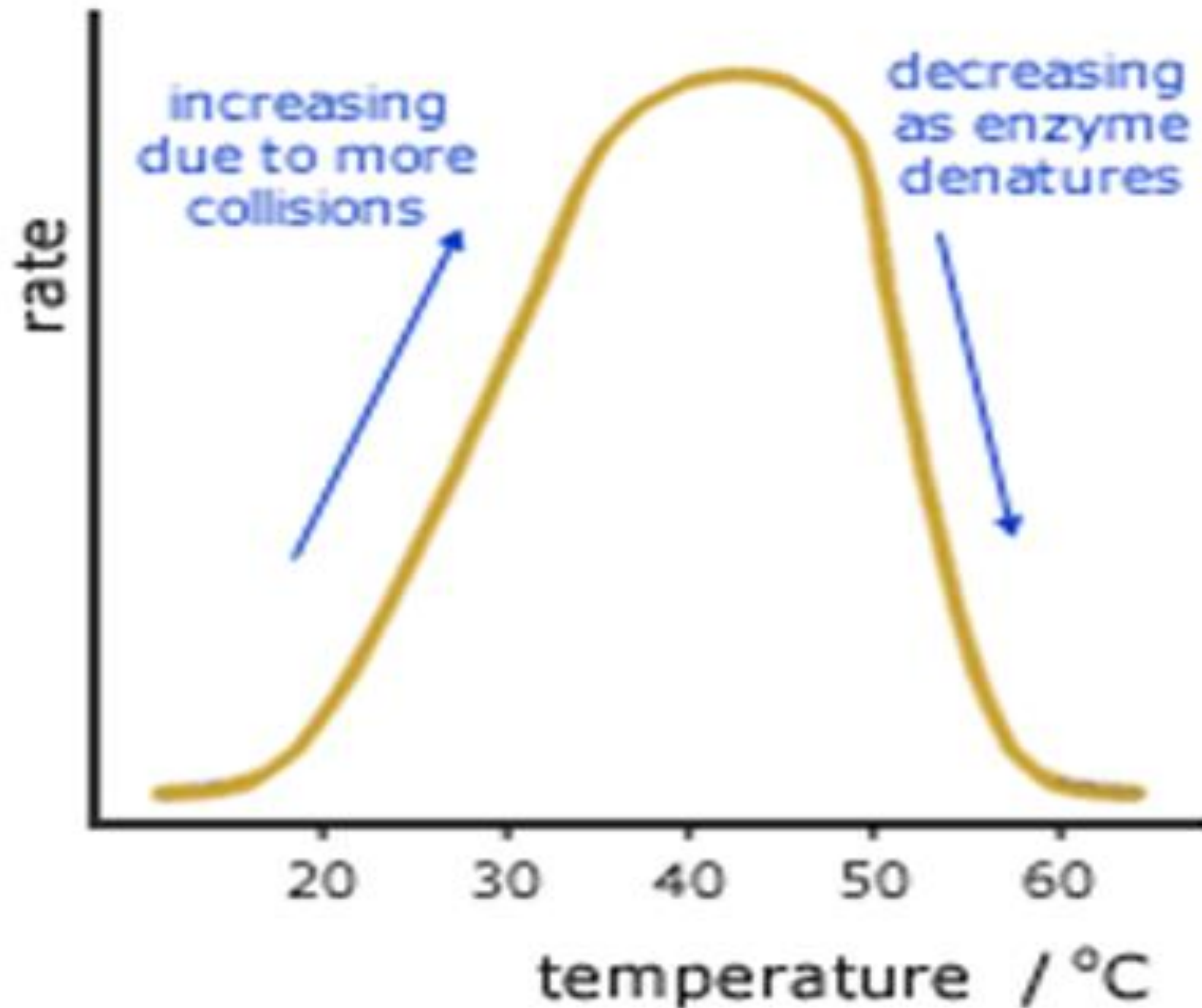
5- 40°C
Increase in Activity

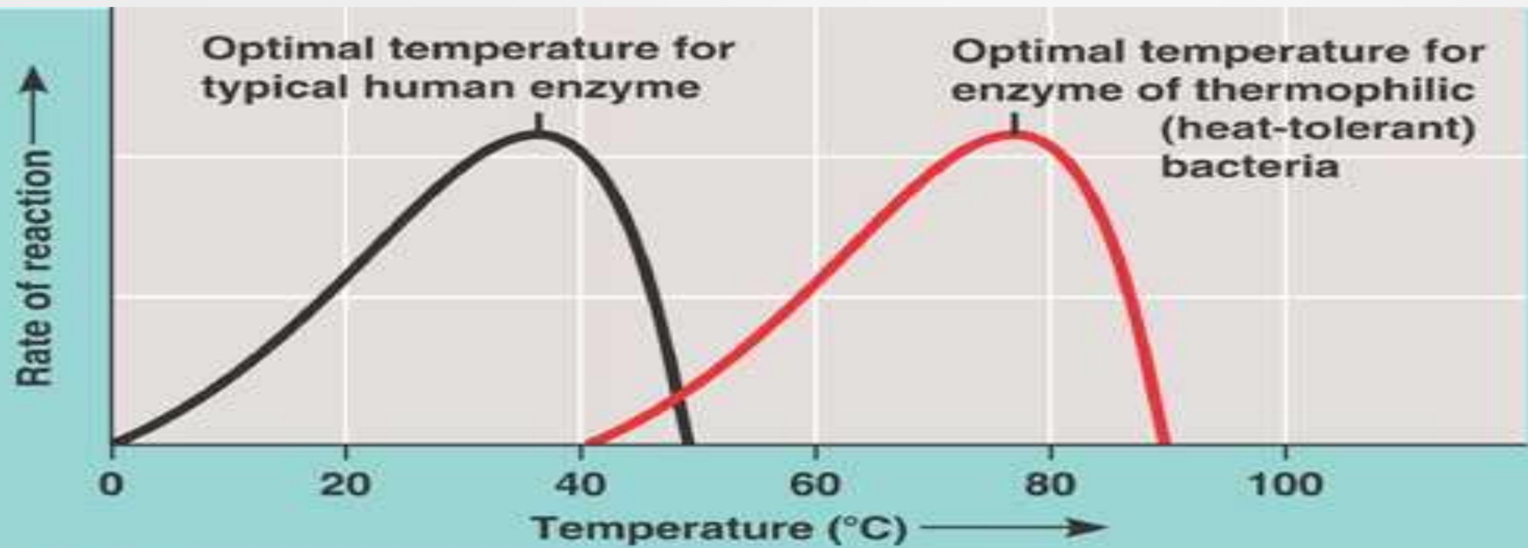


40°C - denatures

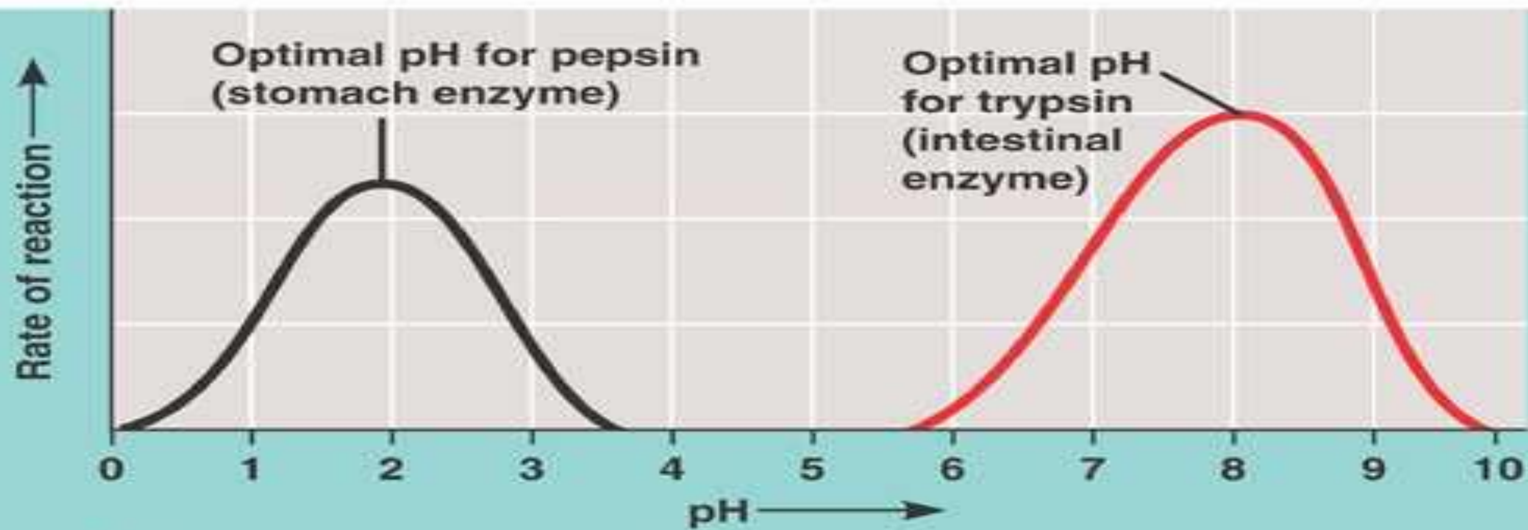
<5°C - inactive

Effect of temperature





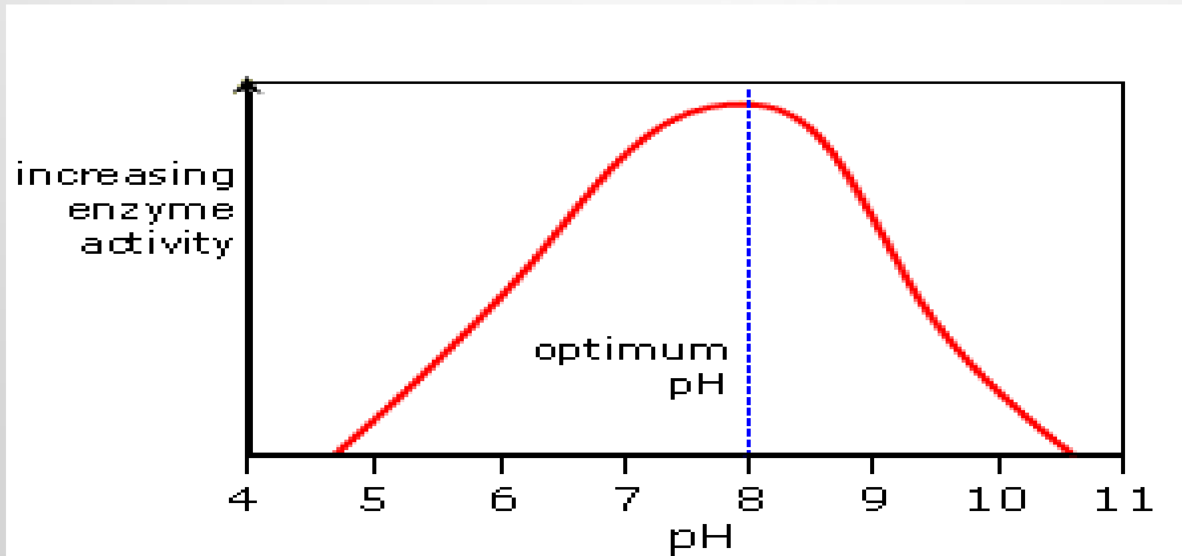
(a) Optimal temperature for two enzymes

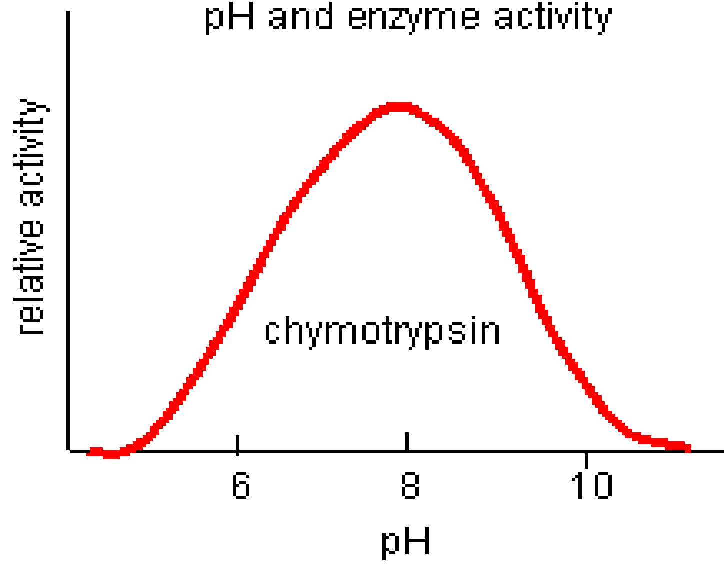


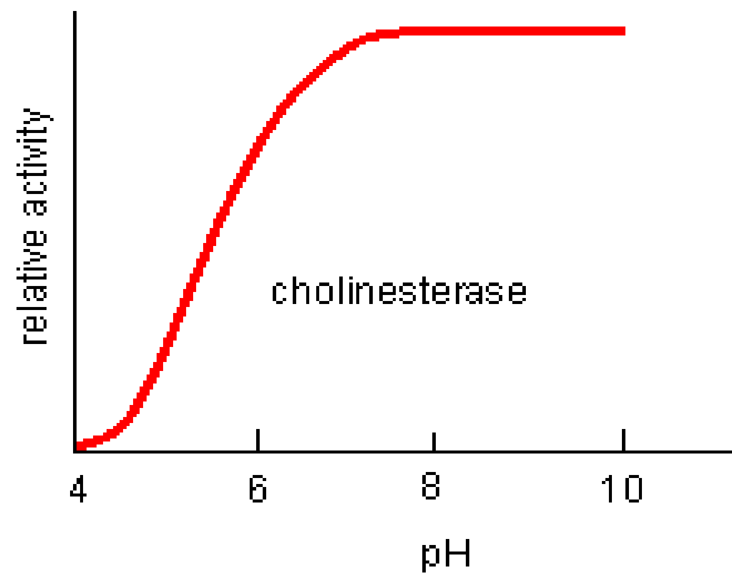
(b) Optimal pH for two enzymes

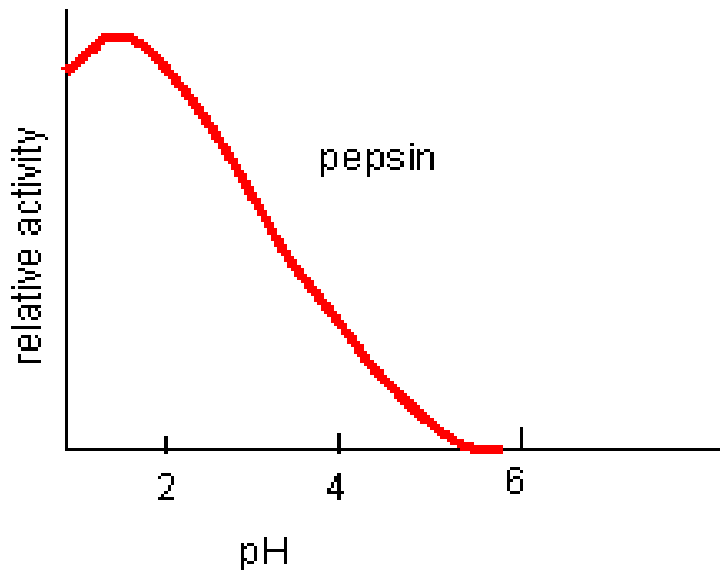
EFFECT OF pH

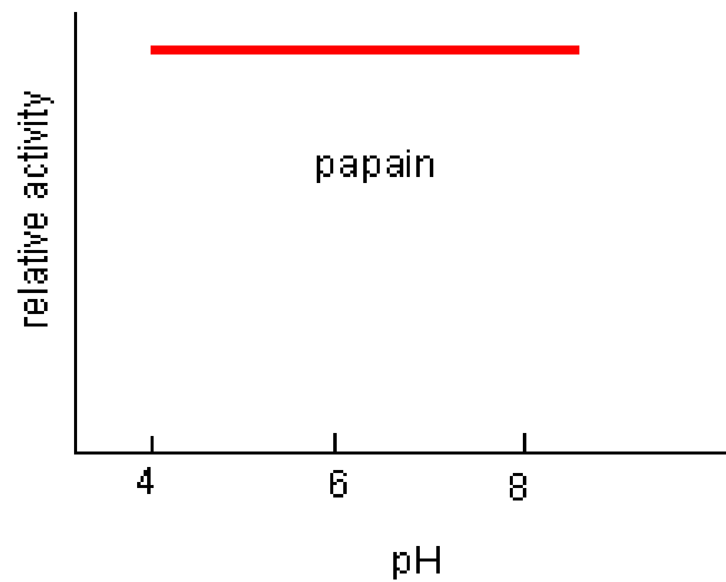
- Rate of almost all enzymes catalyzed reactions depends on pH
- Most enzymes exhibit optimal activity at pH value between *5 and 9*
- High or low pH value than optimum value will cause ionization of enzyme which result in denaturation of enzyme



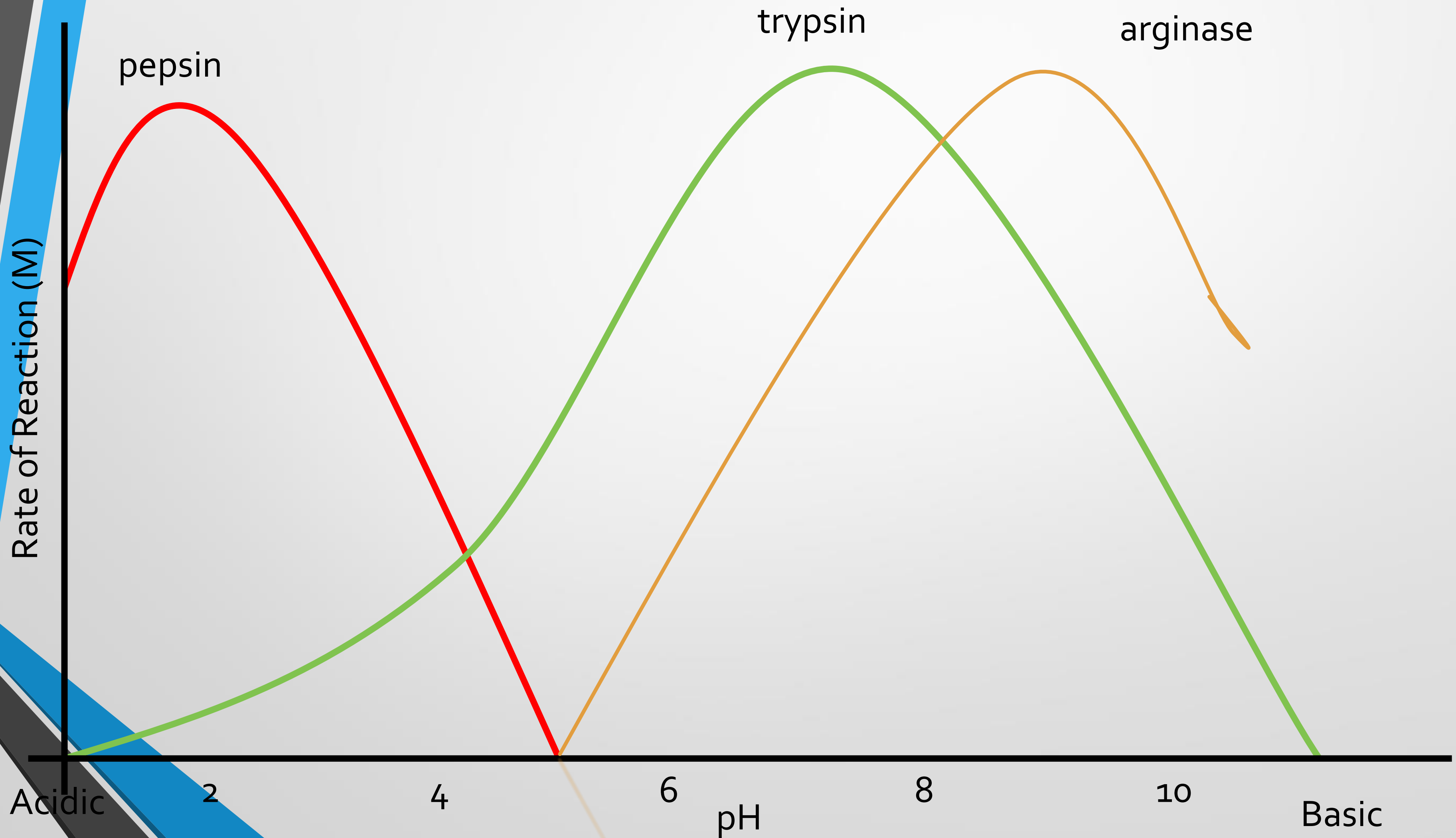

pH and enzyme activity
relative activity
chymotrypsin
6
8
10
pH


relative activity
cholinesterase
4
6
8
10
pH


relative activity
pepsin
2
4
6
pH


relative activity
papain
4
6
8
pH

pH affects the formation of hydrogen bonds and sulphur bridges in proteins and so affects shape.



Michaelis-Menten Model & Effects of Substrate Concentration

- Michaelis-Menten Model:

"According to this model the enzyme reversibly combines with substrate to form an ES complex that subsequently yields product, regenerating the free enzyme."

where:

- S is the substrate
- E is the enzyme
- ES-is the enzyme substrate complex
- P is the product
- K1,K-1 and K2 are rate constants

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3} E + P$$

Two alternative assumptions:

(1) E + S and ES are in equilibrium ($k_2 >> k_3$)

$$k_1[E][S] = k_2[ES] \qquad K_d = \frac{k_2}{k_1} = \frac{[E][S]}{[ES]}$$

(2) Steady-state ($d[ES]/dt = 0$)

$$k_1[E][S] = (k_2 + k_3)[ES]$$

In either case, the reaction velocity $v = k_3[ES]$

Under assumption (1), $v = \frac{k_3}{K_d}[E][S]$

Under assumption (2), $v = \frac{k_3}{K_M}[E][S]$

where K_M is defined as $K_M = \frac{k_2 + k_3}{k_1}$

$$v = k_{CAT}[E \cdot S]$$

$$v_{\substack{\text{formation}\\\text{of } E\cdot S}} = k_1 [E][S]$$

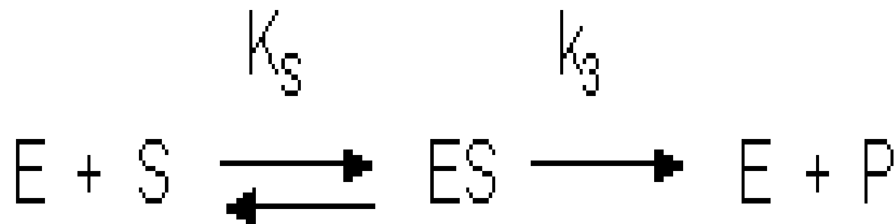
$$v_{\substack{\text{breakdown}\\\text{of } E\cdot S}} = k_2 [E \cdot S] + k_{CAT}[E \cdot S]$$

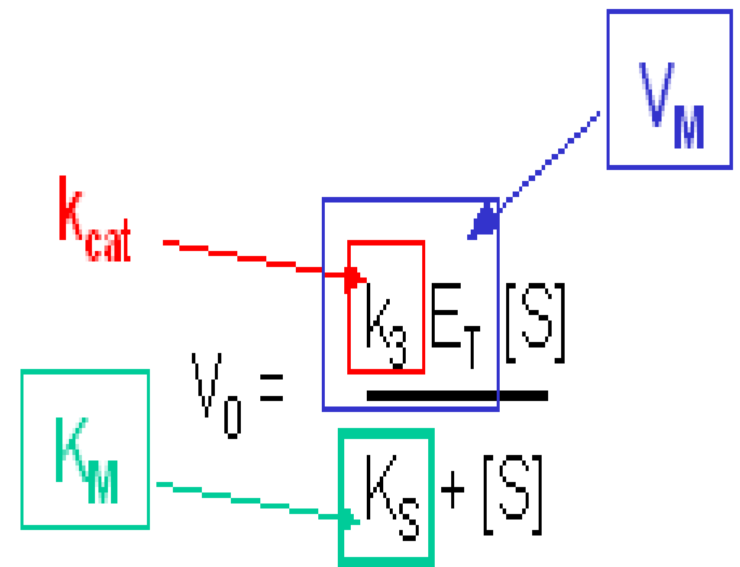
$$K_m = \frac{k_2 + k_{CAT}}{k_1}$$

The Michaelis-Menten equation is

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

1. Rapid Equilibrium Assumption

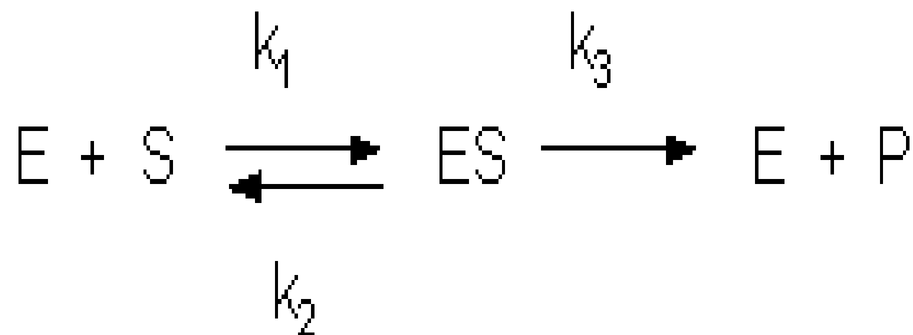




$$V_0 = \frac{k_{cat}\, E_T\, [S]}{K_M + [S]}$$

$$V_0 = \frac{V_M\, [S]}{K_M + [S]}$$

2. Steady-State Assumption



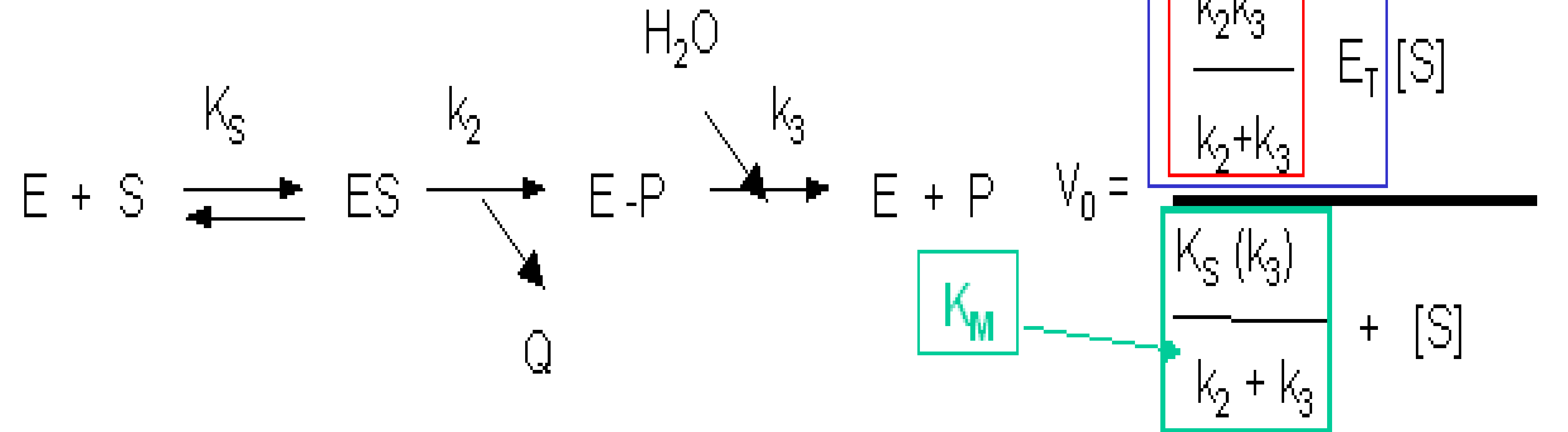
k_{cat} → k_3; V_M → $k_3 E_T$; K_M → $\frac{k_2+k_3}{k_1}$

$$V_0 = \frac{k_3 E_T [S]}{\frac{k_2+k_3}{k_1} + [S]}$$

$$V_0 = \frac{k_{cat} E_T [S]}{K_M + [S]}$$

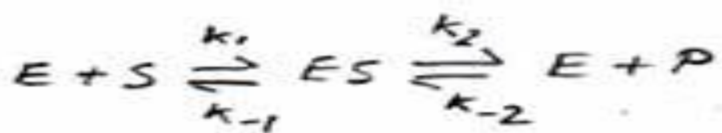
$$V_0 = \frac{V_M [S]}{K_M + [S]}$$

3. Covalent Enzyme-Product



$$V_0 = \frac{k_{cat}\, E_T\, [S]}{K_M + [S]}$$

$$V_0 = \frac{V_M\, [S]}{K_M + [S]}$$



ASSUMPTION I : $k_{-2} = 0$

$$V = \frac{d[P]}{dt} = k_2[ES] - k_{-2}\underbrace{([E]-[ES])}_{\text{FREE }[E]}[P]$$

ASSUMPTION II : $[S] \gg [E]$ $\quad ([S] \gg [ES])$

$$\text{rate of formation of } [ES] = k_1 \underbrace{([E]-[ES])}_{\text{FREE }[E]} \underbrace{([S]-[ES])}_{\text{FREE }[S]}$$

$$\text{rate of breakdown of } [ES] = k_{-1}[ES] + k_2[ES]$$

ASSUMPTION III : STEADY STATE → rate of formation of [ES] = rate of breakdown of [ES]

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

which, when rearranged gives:

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

$$\text{or } V = k_2\frac{[E][S]}{[S]+K_M}$$

maximum V (call this V_{max}) when [S] very high ($[S] \gg K_M$)

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

So,

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

MICHAELIS-MENTEN EQUATION

- Michaelis-Menten Equation:

"It is an equation which describes how reaction velocity varies with substrate concentration."

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

- Where
- V_o is the initial reaction velocity.
- V_{max} is the maximum velocity.
- K_m is the Michaelis constant = $(k_{-1}+k_2)/k_1$.
- [S] is the substrate concentration.

Initial velocity v_o depends on substrate concentration

- Michaelis-Menten equation
- for one-substrate reactions

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

V_{max} = maximal velocity (for the given concentration of enzyme)

K_m = Michaelis constant

Michaelis-Menten kinetics

V_0 varies with [S]

V_{max}

V_{max}

$V_{max}/2$

K_M

Reaction velocity (V_0) ⟶

Substrate concentration [S] ⟶

V_{max} approached asymptotically

V_0 is moles of product formed per sec. when [P] is low (close to zero time)

Michaelis-Menten Model

$V_0 = V_{max}\ x[S]/([S] + K_m)$

Michaelis-Menten Equation

If [S] << K_m

$$v_o = V_{max}\frac{[S]}{\cancel{[S]} + K_m} = \frac{V_{max}}{K_m}[S] = k[S]^1$$

at low substrate concentration the reaction proceeds by the **1st order kinetics**

If [S] >> K_m

$$v_o = V_{max} \frac{[S]}{[S] + K_m} = V_{max} \frac{[S]}{[S]} = V_{max} = k[S]^0$$

at high substrate concentration the reaction proceeds by the **o. order kinetics**

If [S] = K_m

$$v_o = V_{\max}\frac{[S]}{[S]+[S]} = V_{\max}\frac{[S]}{2[S]} = \frac{V_{\max}}{2}$$

Significance of K_m and V_{max}

- the Michaelis constant K_m is the concentration of substrate [S] which gives half the maximal velocity V_{max} (50 % saturation of enzyme)
- the K_m has the dimension of concentration (mol/l)
- K_m is <u>inversely</u> related to the affinity of enzyme for its substrate.

If more substrates with similar structure exist, then the best natural substrate is one with the least value of K_m

- if there is a need to measure the activity of enzyme, the substrate concentration has to be at least several times higher than the K_m value.

V_{max} and K_m describe the kinetic properties of enzyme

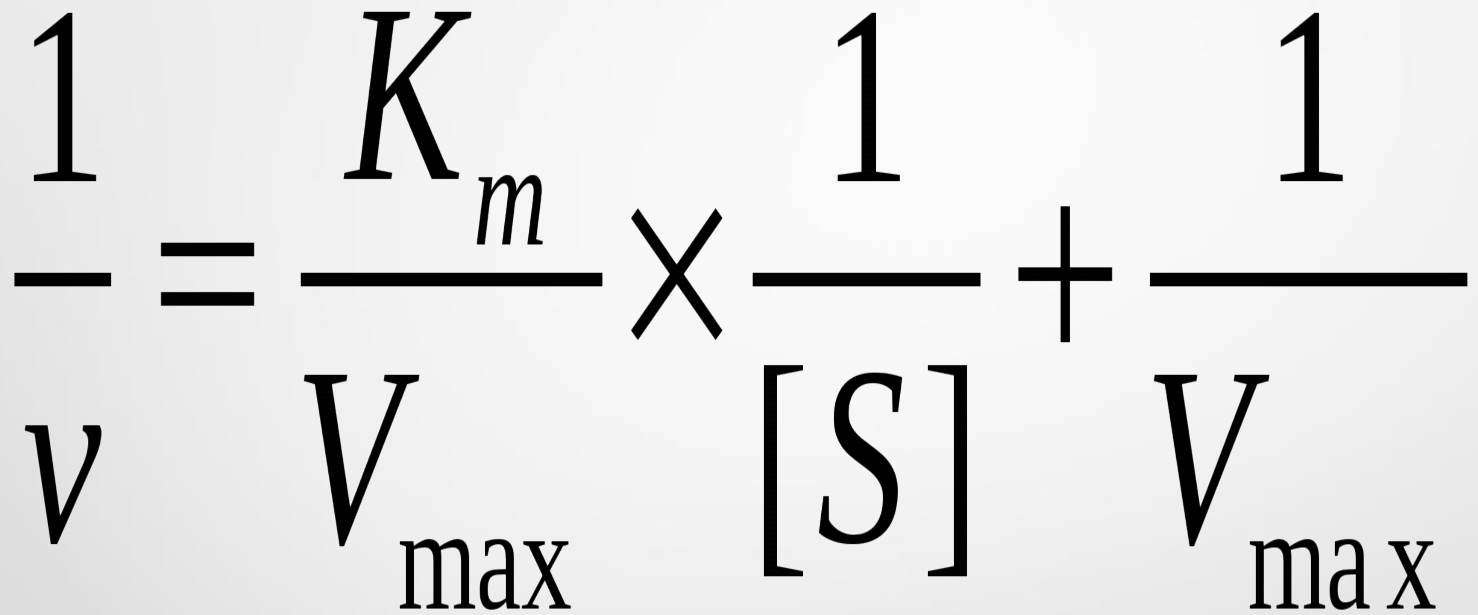
- are hardly obtained from saturation curve
- easily obtained from linear double reciprocal plot
- Lineweaver-Burk: **$1/v_o$** is plotted against **1/[S]**

Reciprocal equation

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

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

$$\frac{1}{v_o} = \frac{1}{V_{max}} + \frac{K_m}{V_{max}} \cdot \frac{1}{[S]}$$


$$\frac{1}{v} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

Reciprocal form is the equation of a line ($y = a x + b$)

$$\frac{1}{v_o} = \frac{1}{V_{\max}} + \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]}$$

$1/v_o$ .... dependent variable (y)

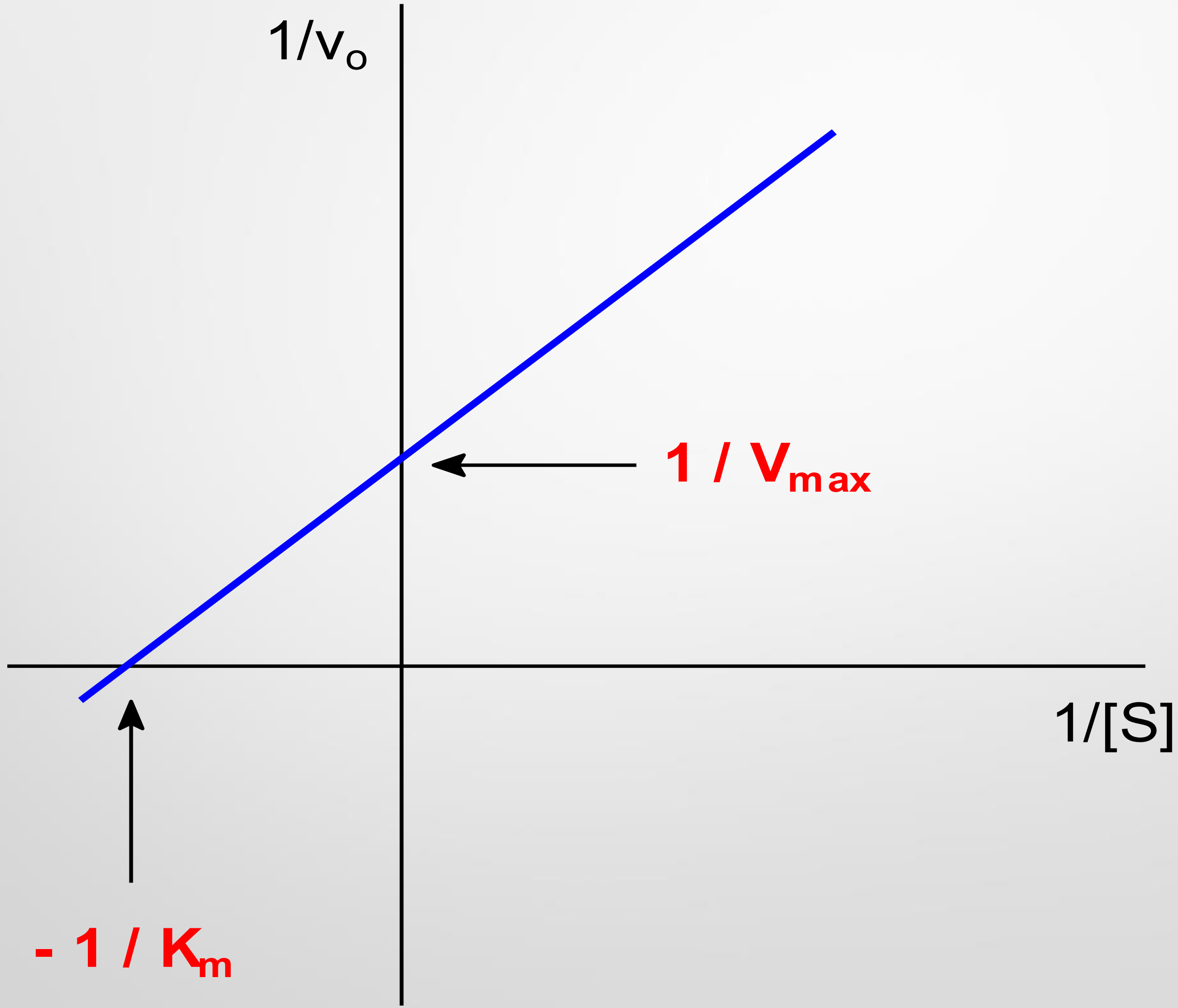
$1/[S]$ .... independent variable (x)

$1/K_m$ ....

$1/V_{max}$ ....

easily determined from the graph

Linear reciprocal plot: 1/v_o is the function of 1/[S]



Eadie–Hofstee diagram

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

V =reaction velocity

K_m = Michaelis–Menten constant

$[S]$ = substrate concentration

V_{max} = maximum reaction velocity.

- **Eadie–Hofstee diagram is a graphical representation of enzyme kinetics in which reaction velocity is plotted as a function of the velocity vs. substrate concentration ratio**:

- It can be derived from the Michaelis–Menten equation as follows:

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

- invert and multiply with :

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

- Rearrange:

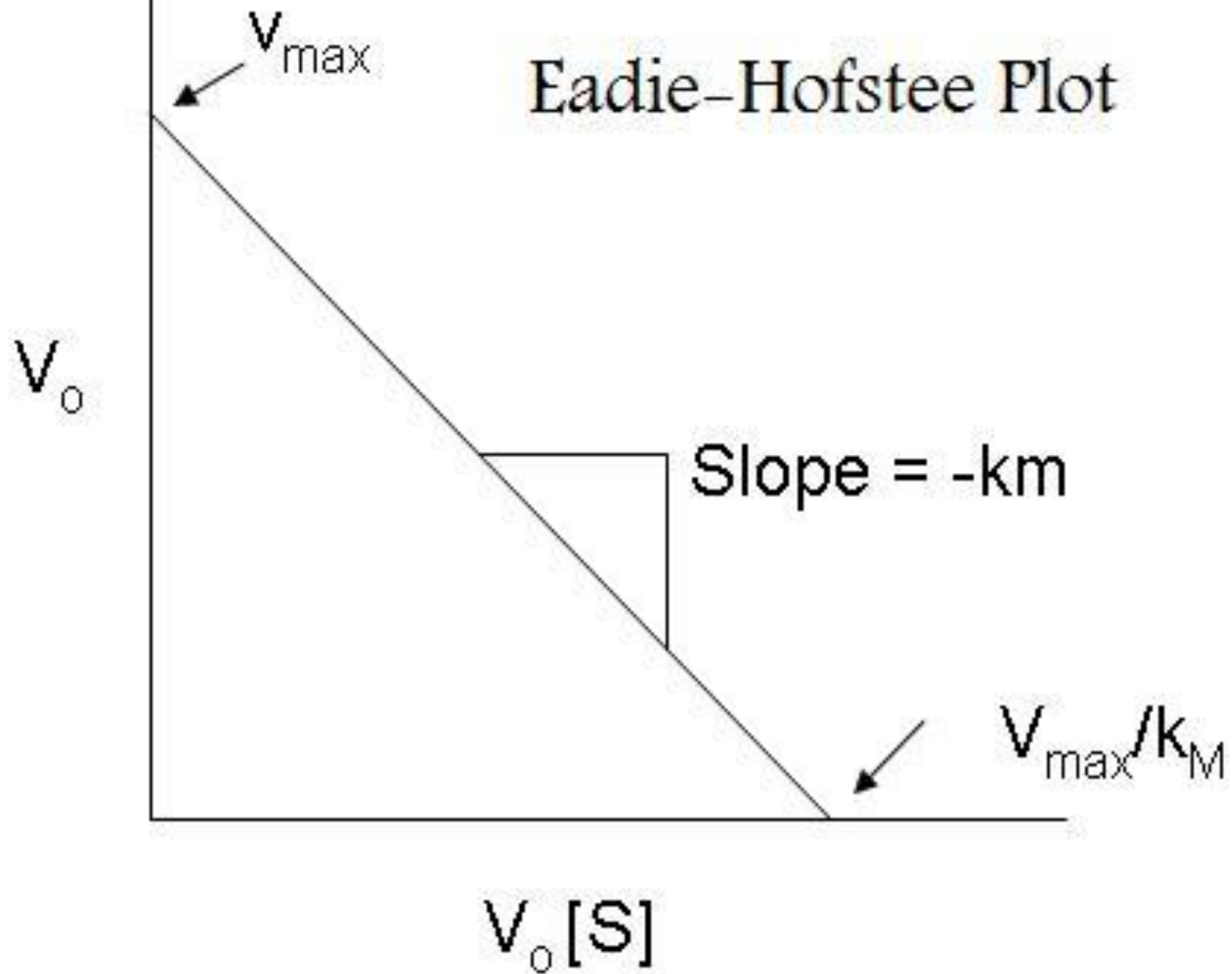
$$V_{\max} = \frac{vK_m}{[S]} + \frac{v[S]}{[S]} = \frac{vK_m}{[S]} + v$$

- Isolate v:

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

By Mohd Anzar Sakharkar

- **A plot of v vs v/[S] will yield V_{max} as the y-intercept, V_{max}/K_m as the x-intercept, and K_m as the negative slope.**
- **Like other techniques that linearize the Michaelis–Menten equation, the Eadie-Hofstee plot was used historically for rapid identification of important kinetic terms like K_m and V_{max}, but has been superseded by nonlinear regression methods that are significantly more accurate and no longer computationally inaccessible.**
- **It is also more robust against error-prone data than the Lineweaver–Burk plot, particularly because it gives equal weight to data points in any range of substrate concentration or reaction velocity.**
- **Both plots remain useful as a means to present data graphically.**



By Mohd Anzar Sakharkar

Problem 1

Enzyme sample (0.1 ml) was added to substrate solution. After 5 min, 0.2 mmol of product was determined.

What is catalytic concentration of enzyme?

$$\frac{\text{amount of product (mmol)}}{\text{time (s)} \times \text{volume (l)}}$$

Solution

t = 5 min = 5 × 60 s = 300 s

in 300 s … 0.2 mmol of product

in 1 s … x = 0.2/300 = 6.7×10^{-4} mmol / 0.1 ml of sample

for 1 litre of sample = $6.7 \times 10^{-4} \times 10^{4}$ = 6.7 mmol/l.s = **6.7 mkat/l**

Problem 2

Reaction mixture contains:

2.5 ml buffer

0.2 ml solution of NADH (optical UV test)

0.1 ml blood serum

0.2 ml substrate solution

After 60 s, the decrease of NADH absorbance is ΔA = 0.03

ε_{NADH} = 6220 l/mol.cm, cuvette width l = 1 cm.

What is catalytic concentration of enzyme?

Solution

Serum sample was diluted: $V_{final}/V_{initial}$ = 3,0 / 0,1 = 30

Lambert-Beer law: $\Delta A = \varepsilon \, \Delta c \, l$

changes of absorbance and concentration expressed per time $\Delta t \Rightarrow$

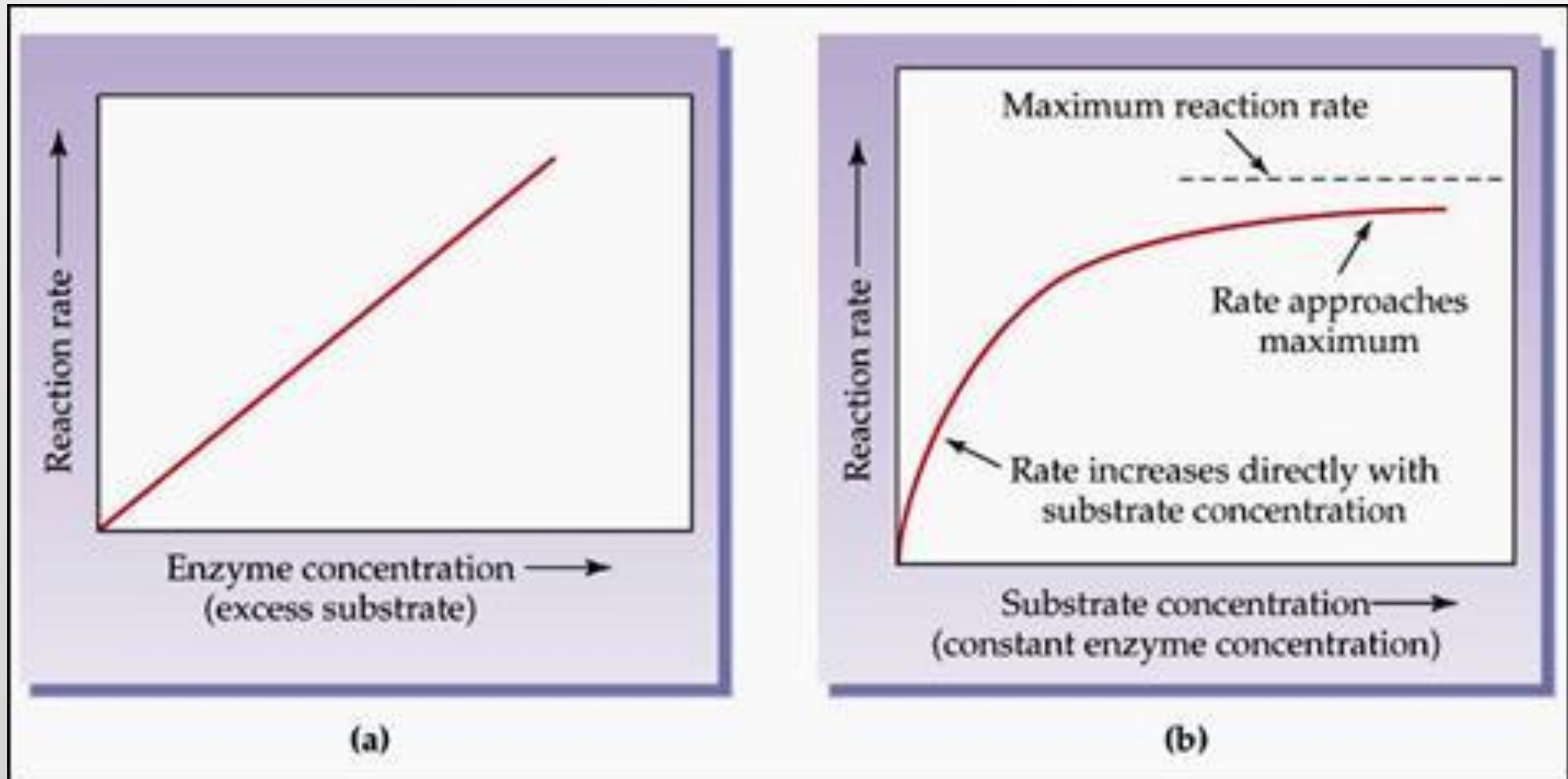
$\Delta A/\Delta t = \varepsilon \, \Delta c \, l/\Delta t$ $\quad\quad$ Δt = 60 s

$$\frac{\Delta c}{\Delta t} = \frac{\Delta A}{\varepsilon \times l \times \Delta t} = \frac{0{,}03}{6220 \times 1 \times 60} = 8.10^{-8} \text{ mol/l.s}$$

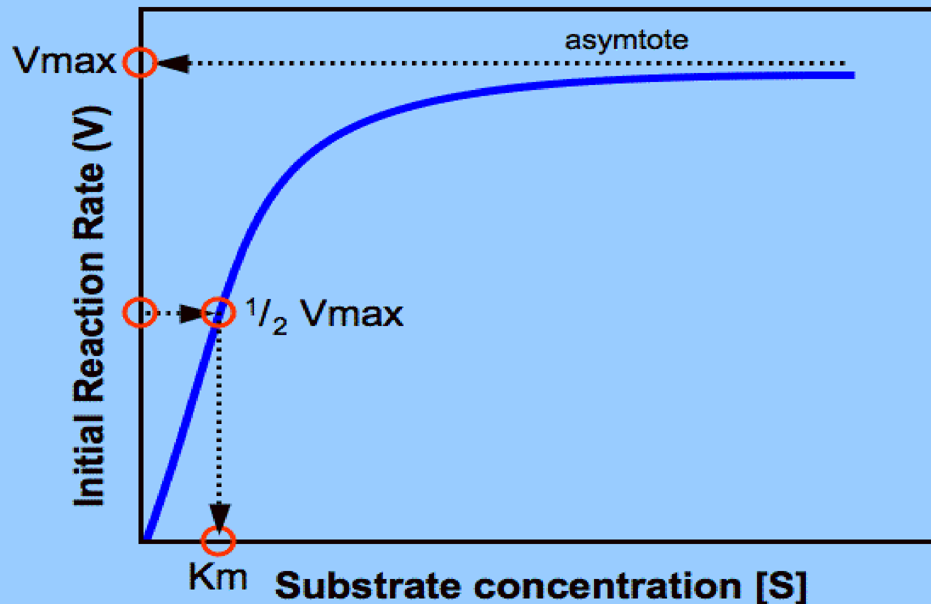
Multiplied by dilution: $30 \times 8 \times 10^{-8} = 2{,}4 \times 10^{-6}$ mol/l.s =

$2{,}4 \times 10^{-6}$ kat/l = **2,4 µkat/l**

SUBSTRATE CONCENTRATION



Substrate Concentration



PHARMACEUTICAL IMPORTANCE

- Enzymes are virtually involved in all physiological processes which makes them the *targets of choice for drugs* that cure or ameliorate human disease.
- Applied enzyme kinetics represents the *principal tool* by which scientist identify and characterize therapeutic agents that selectively inhibit the rates of specific enzymes catalyzed processes.
- Enzymes kinetics thus play a critical role in drug discovery as well as elaborating the mode of action of drugs.