ENZYMOLOGY – III

Original slides: Dr. Samir Mahjoob.
Edited by: Mohannad Al-ja'Awin

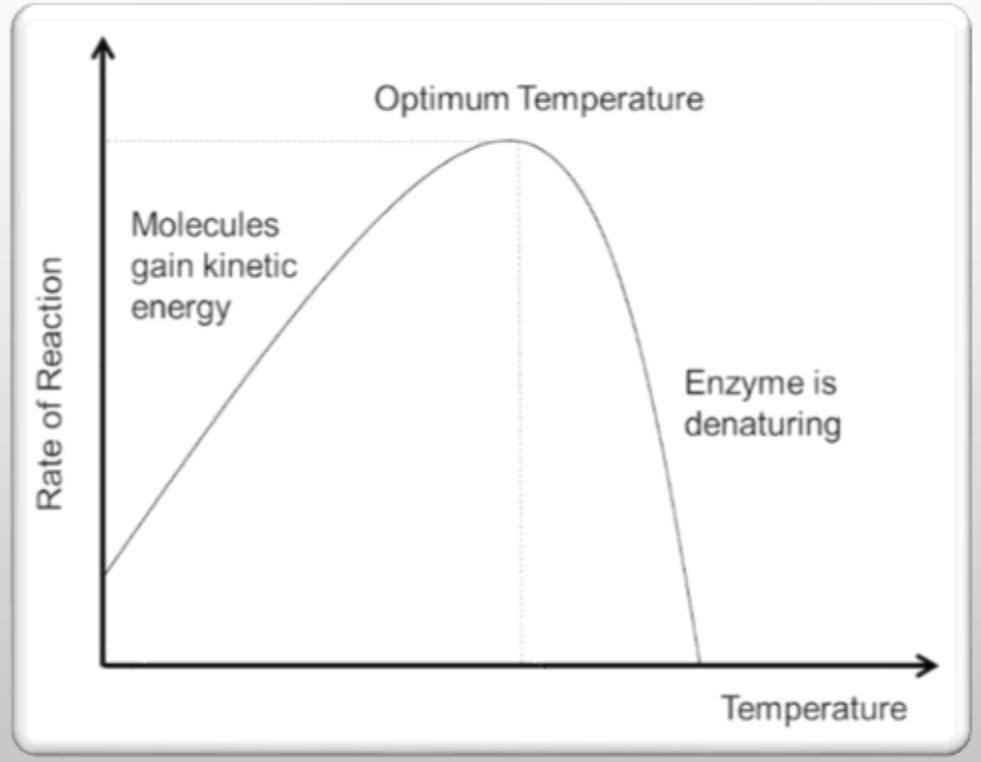
- **FACTORS AFFECTING ENZYME ACTIVITY**
- NUMEROUS FACTORS AFFECT THE REACTION RATE:
- **TEMPERATURE:**

•- THE 1ST FACTOR THAT AFFECT THE ENZYMATIC ACTIVITY.

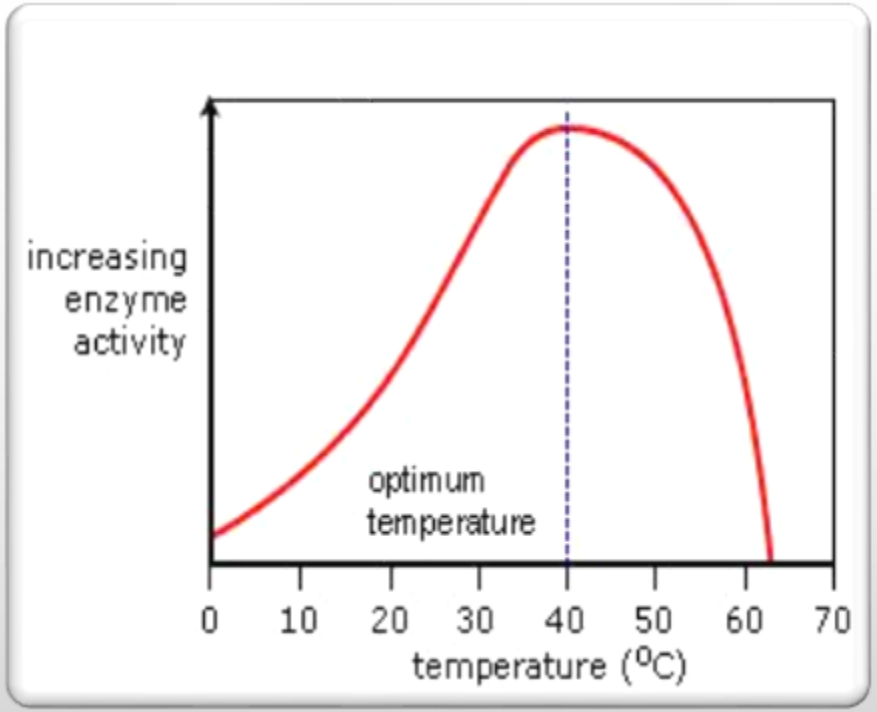
•THE REACTION RATE INCREASES WITH TEMPERATURE TO A MAXIMUM LEVEL, THEN ABRUPTLY DECLINES WITH FURTHER INCREASE OF TEMPERATURE

•MOST ANIMAL ENZYMES RAPIDLY BECOME DENATURED AT TEMPERATURES ABOVE 40 C

•THE OPTIMAL TEMPERATURES OF THE ENZYMES IN HIGHER ORGANISMS RARELY EXCEED 50 °C

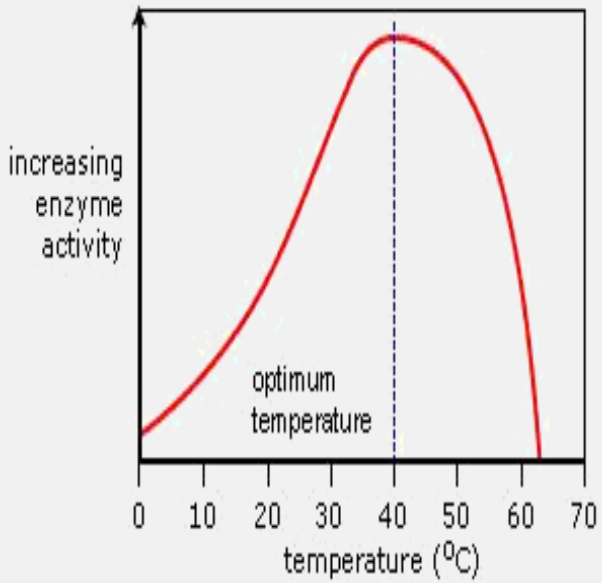


- THE Q10, OR TEMPERATURE COEFFICIENT, IS THE FACTOR BY WHICH THE RATE OF A BIOLOGIC PROCESS INCREASES FOR A 10 °C INCREASE IN TEMPERATURE.
- WE STARTED WITH A ZERO TEMPRETURE, THE TEMPTRETURE IS GRADUALLY INCREASES.
- WHEN THE ENZYMATIC ACTIVITY REACHES TO THE MAXIMAL LEVEL OF TEMP, THERE WOULD BE NO FURTHER INCREASE IN THE ACTIVITY.
- OPTIMUM TEMP: TEPMRETURE AT WHICH THE ENZYME IS ACTING AT MAXIMUM, AT 37C.



Effect of Temperature

- For mammals and other homoeothermic organisms, changes in enzyme reaction rates with temperature assume physiologic importance only in circumstances such as fever or hypothermia.



- - When the enzyme bypass the maximum level, the enzymatic activity decreases.

- Fever and hypothermia may lead to decrease in the enzymatic activity

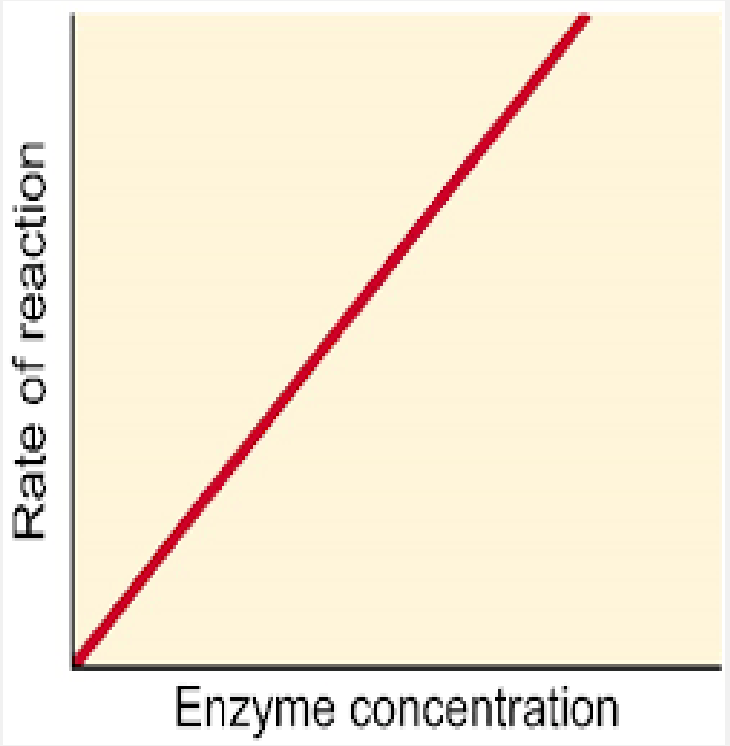
Effect of enzyme concentration

- In this example we stabilize all the factors except the concetration of the enzymes

-As the amount of enzyme is increased, the rate of reaction increases.

-If there are more enzyme molecules than are needed, adding additional enzyme will not increase the rate.

- Reaction rate therefore increases then it levels off.



-The more the enzyme concentration increases, the activaty of the enzyme inrecases until it reaches a platue, where the turn over number decreases, which means the substrate concetration is less than the enzymatic concetration

Effect of pH on enzyme activity

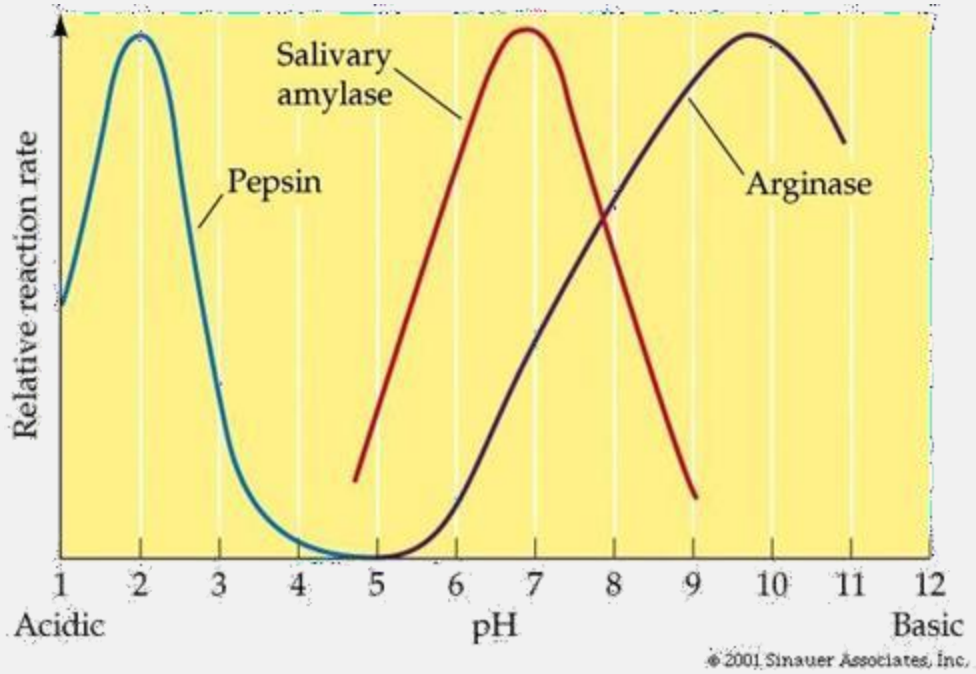
-The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration.

- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.

- The maximum enzymatic activity in the blood is at PH 7.35

-The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation at high or low pH and effects on the charged state of the enzyme, the substrates, or both.

-Except for Pepsin, acid phosphatase and alkaline phosphatase, most enzyme have optimum pH between 5 to 9.



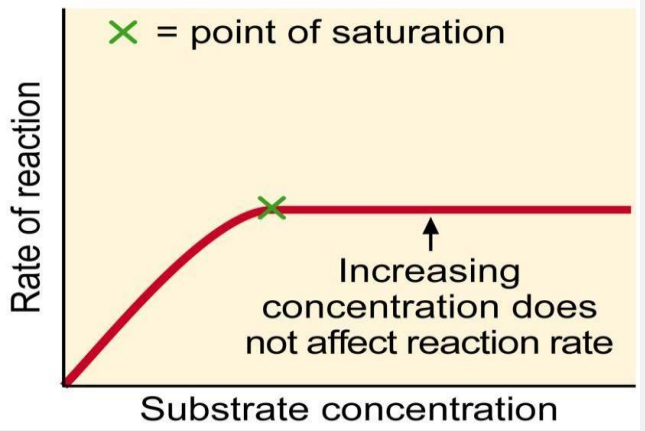
Effect of substrate concentration

- At lower concentrations, the active sites on most of the enzyme molecules are not filled because there is not much substrate.

- Higher concentrations cause more collisions between the molecules.

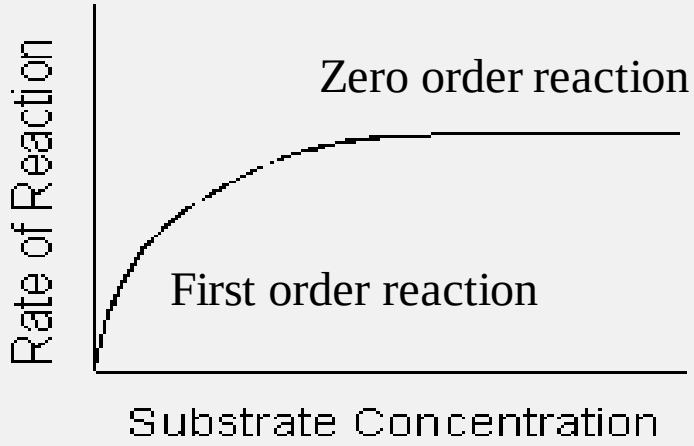
-The rate of reaction increases (First order reaction).

-The maximum velocity of a reaction is reached when the active sites are almost continuously filled.



Effect of substrate concentration Cont:

- Reaction rate therefore increases as substrate concentration is increased but it levels off (Zero order reaction).



- Increased substrate concentration after this point will not increase the rate. All the molecules are occupied.

- The shape of the curve that relates activity to substrate concentration is hyperbolic.

- **Zero Order Recation:** **No EFFECT** regarding the enzymatic activity (Completely saturated).
- **First Order Reaction:** **Direct** relationship between the substrate and the enzyme concentration.

Enzyme kinetics

- It is the study of the chemical reactions that are catalyzed by enzymes.

- In enzyme kinetics, the reaction rate is measured and how get changes in response to changes in experimental parameters such as substrate concentration, enzyme concentration etc.

- This is the oldest approach to understanding enzyme mechanisms and remains the most important.
- The initial rate (or initial velocity), designated V0, when [S] is much greater than the concentration of enzyme [E] can be measured by Michaelis–Menten kinetics. It is one of the simplest and best-known models of enzyme kinetics.

- **Note**# Michaelis-Menten equation, the rate equation for a one-substrate enzyme-catalyzed reaction.

هالسلايد بتفهموه بآخر المحاضرة، و فاضي ما فيو شي)بس للإحتياط(

Michaelis-Menten Kinetics

-The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme-catalyzed reaction [V_i], the concentration of substrate [S] and two constants, V max and km (which are set by the particular equation).

- Each enzyme molecule is going to catalyze one reaction.

- Each enzyme molecule is going to convert one substrate molecule into one product.

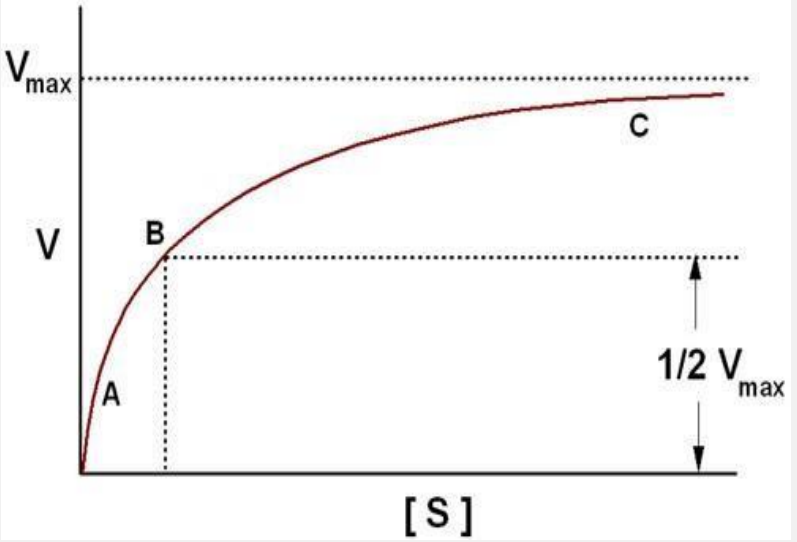
-Michaelis-Menten equation

-The symbols used in the Michaelis-Menten equation refer to:

- the reaction rate [V_i]
-maximum reaction rate (V max)
-substrate concentration [S]
-Michaelis-Menten constant (km).

$$V_1 = \frac{V_{max}[S]}{\{K_m + [S]\}}$$

KM: Value of substrate concentration when the activity of the enzyme is half the max



Michaelis-Menten equation

-The dependence of initial reaction velocity on [S] and Km may be illustrated by evaluating the Michaelis-Menten equation under three conditions:

$$v_1 = \frac{V_{max}[S]}{\{K_m + [S]\}}$$

1- When [S] is much less than km (Km>S) , the term km + [S] is essentially equal to km.

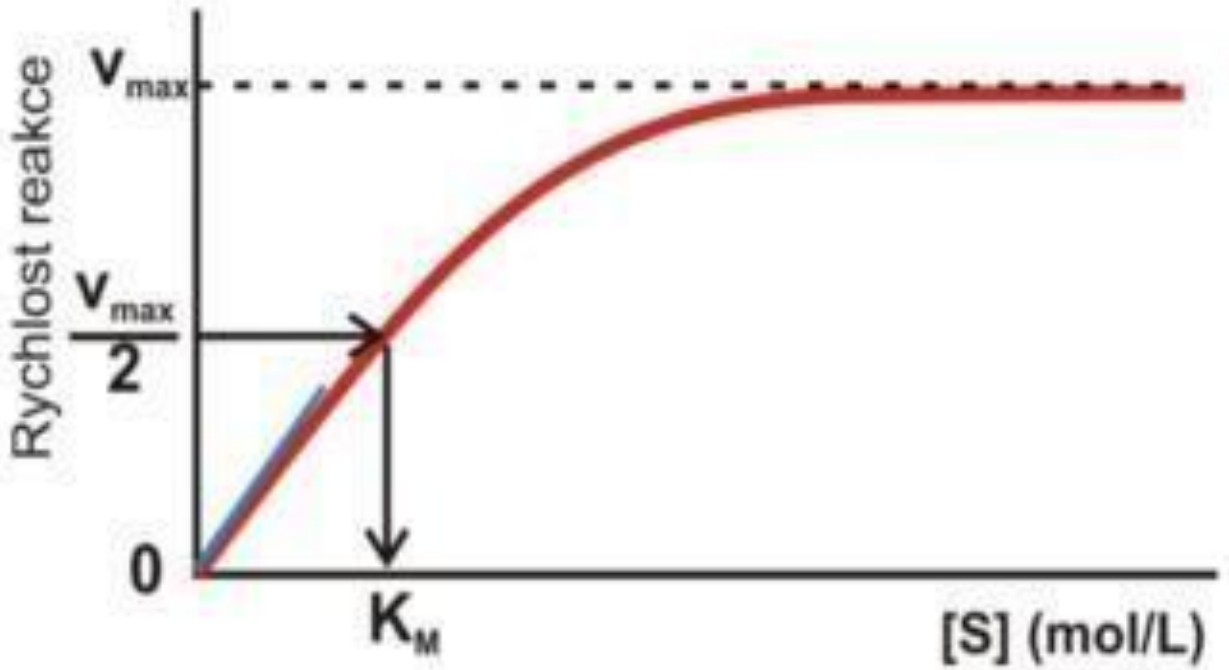
- Since V max and km are both constants, their ratio is constant (k).
- In other words, when [S] is considerably below km, V max is proportionate to k[S].
- The initial reaction velocity therefore is directly proportionate to [S].

Illustration regarding the 1st point:

- We ignored [S] because it is very little compared to the Km.

- So the formula is converted into:
  V1 = K * [S]

Michaelis-Menten Equation



[S] low
$v = (v_{max}/K_M).[S]$

$[S] = K_M$
$v = v_{max}/2$

[S] high
$v = v_{max}$

2- When [S] is much greater than km (Km<[S])
the term km + [S] is essentially equal to [S].

- [S] is very big compared to the Km, so we ignore the Km.

- Replacing km + [S] with [S] reduces equation to:
  Vi = Vmax

And here we are passed the point of saturation

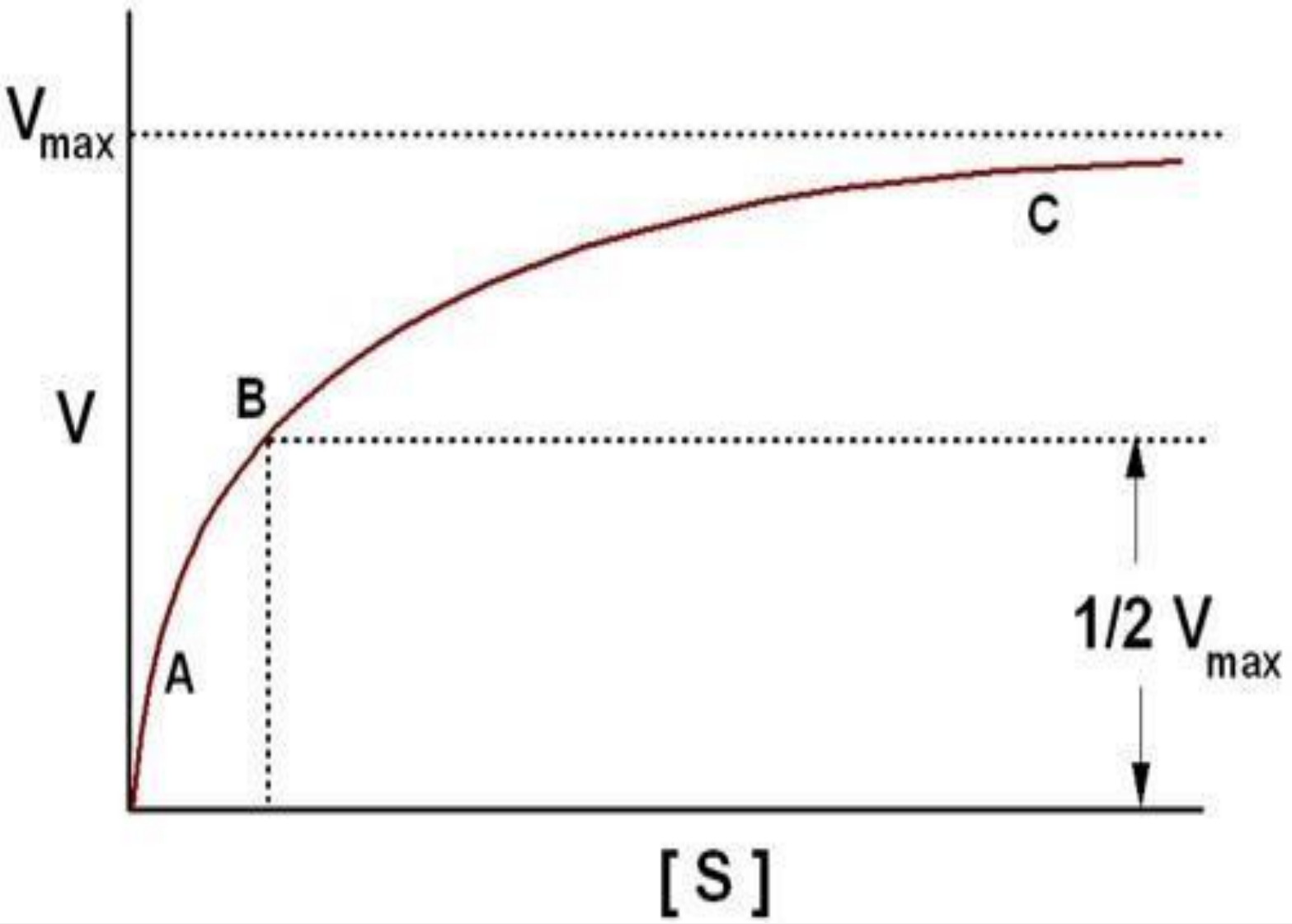
- Thus, when [S] greatly exceeds km, the reaction velocity is maximal (V max) and unaffected by further increases in substrate concentration (Saturation).

3- When [S] = km

Equation states that when [S] equals km, the initial velocity is half-maximal.

•Equation also reveals that km is a constant and may be determined experimentally from—the substrate concentration at which the initial velocity is half-maximal.

Plot of substrate concentration versus reaction velocity



Lineweaver-Burk Plot:

- **It's the inversion of the Michaelis-Menten equation**

- There is 3 types of curves:

1. **Linear:** Every variable is proportional to the other.

2. **Hyperbolic:** 1st and zero order reactions. Where at point of saturation we can't take any value.

3. **S-shaped curve (Sigmoidal Curve):** looks like the letter S curve.

- **At the Lineweaver-Burk plot, we DON'T IGNORE any value whatever it's small.**

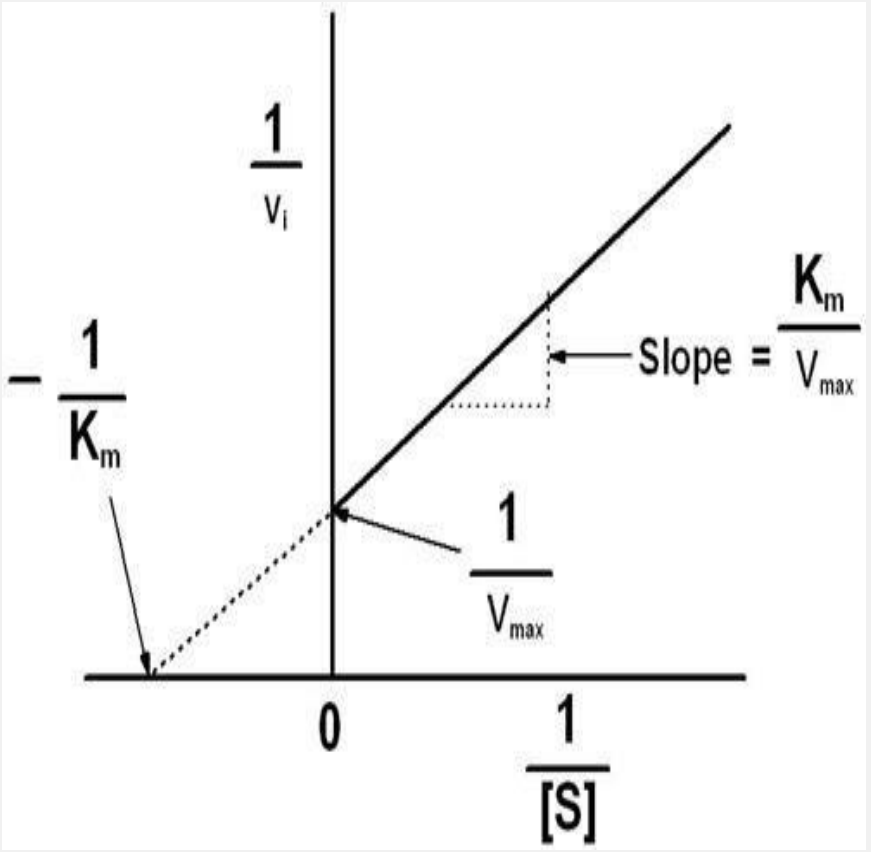
Lineweaver-Burk Plot

- A Linear Form of the Michaelis-Menten Equation is used to determine km & V max.

$$v_i = \frac{V_{max}[S]}{K_m + [S]}$$ Invert $$\frac{1}{v_i} = \frac{K_m + [S]}{V_{max}[S]}$$ factor $$\frac{1}{v_i} = \frac{K_m}{V_{max}[S]} + \frac{[S]}{V_{max}[S]}$$ and simplify $$\frac{1}{v_i} = \left(\frac{K_m}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Lineweaver-Burk Plot

- A plot of $1/V_i$ as *y* as a function of $1/[S]$ as *x* therefore gives a straight line whose *y* intercept is $1/V$ max and whose slope is km / V max.
- Such a plot is called a double reciprocal or Lineweaver-Burk plot.



Importance of Km:

1. Specific and constant for a particular enzyme.

- If we estimated a Km value for every enzyme, we will find that each enzyme has it's own Km value, even if there is 2 enzymes are acting on the same substrate!
- But under one condition: both temp and PH are constant.

2. Determines the affinity of an enzyme for it's substrate.

- Affinity: The amount of substrate needed to give the enzyme the maximum activity.

- There is inverse relationship between the Affinity and the Substrate.
- The less we needed the substrate to reach the maximum activity of the substrate, the more the affinity the [ES] has.

Km and its significance

-The Michaelis constant K_m is the substrate concentration at which V_i is half the maximal velocity (Vmax/2) attainable at a particular concentration of enzyme

- It is specific and constant for a given enzyme under defined conditions of time , temperature and pH

- K_m determines the affinity of an enzyme for its substrate, lesser the Km for is the affinity and vice versa, it is inversely proportionate to the affinity

- K_m value helps in determining the true substrate for the enzyme.

THANK YOU