Enzymology- An overview-2

Factors affecting Enzyme activity

-Numerous factors affect the reaction rate:

factors affecting enzymes:
- temperature, pH, enzyme concentration, substrate concentration, inhibitors, activators, cofactors

Temperature

-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

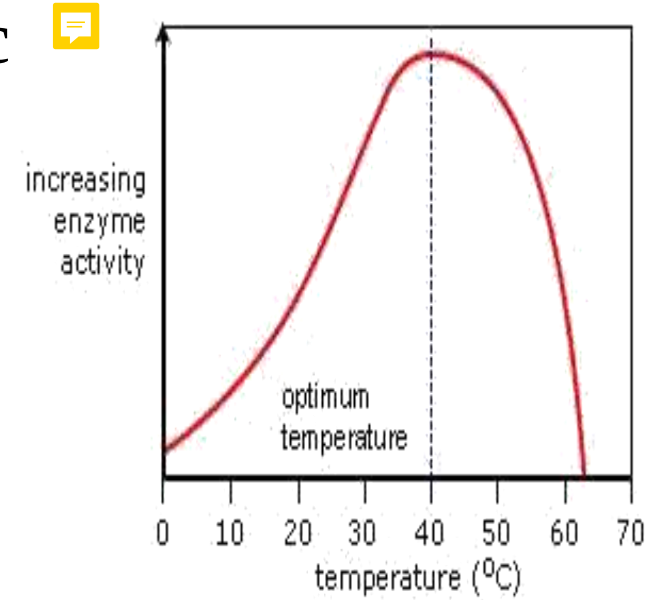
-The optimal temperatures of the enzymes in higher organisms rarely exceed 50 °C

some plants have the optimal temp of 50C

-The Q_{10}, or temperature coefficient, is the factor by which the rate of a biologic process increases for a 10 °C increase in temperature.

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.



Effect of enzyme concentration

- 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.

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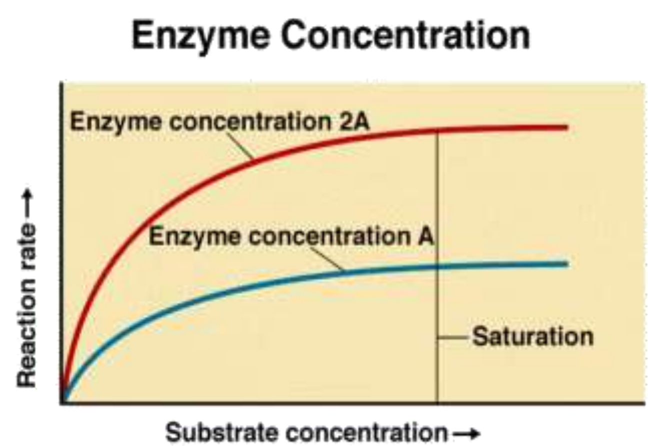
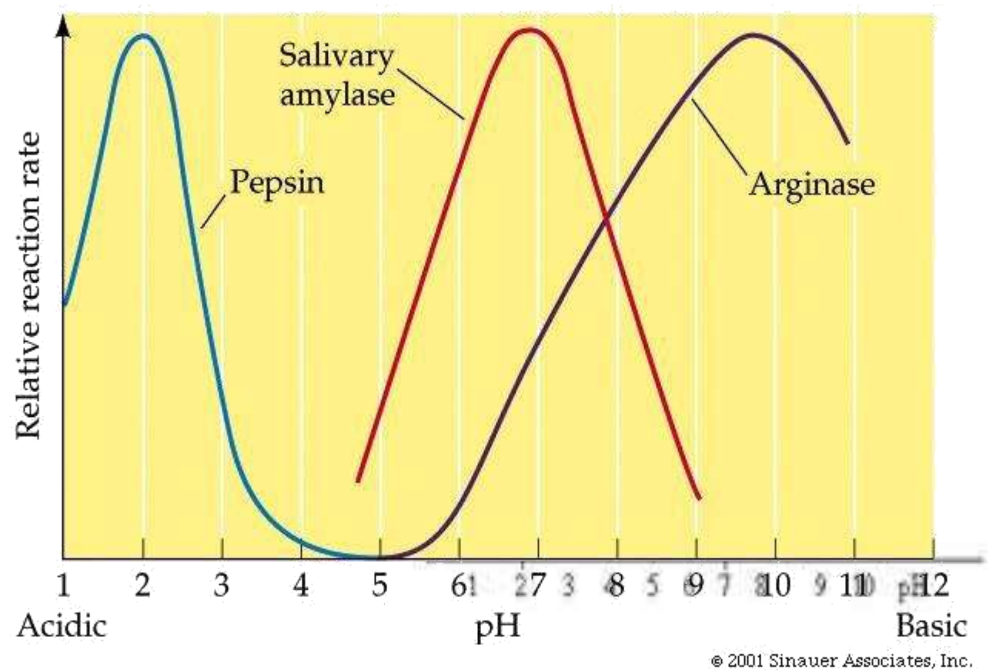


<u>Effect of pH on enzyme activity</u>

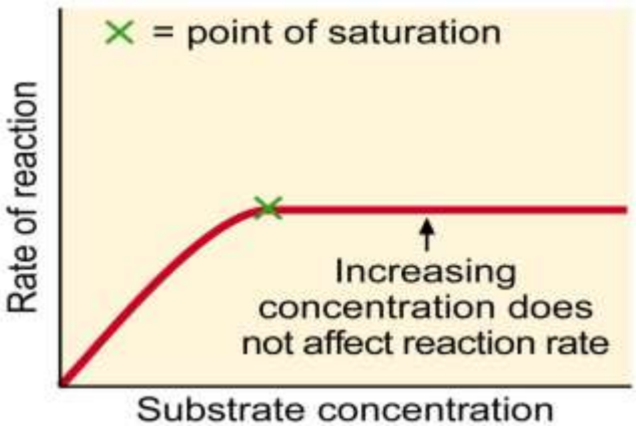
- The rate of almost all enzyme-catalyzed reactions exhibits a significant dependence on hydrogen ion concentration
- Most intracellular enzymes exhibit optimal activity at pH values between 5 and 9.
- The relationship of activity to hydrogen ion concentration reflects the balance between enzyme denaturation 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.

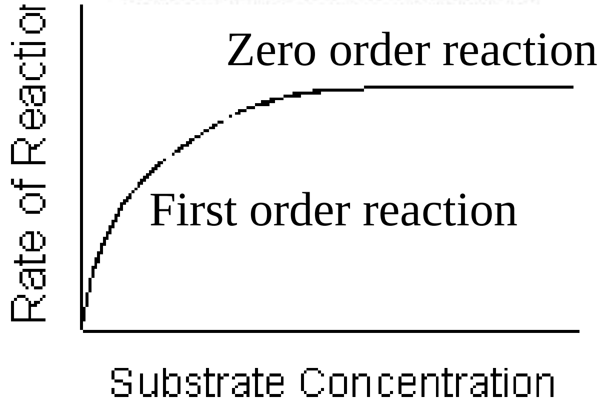


Effect of substrate concentration

most important factor

- 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.
- Increased substrate concentration after this point will not increase the rate.
- Reaction rate therefore increases as substrate concentration is increased but it levels off (Zero order reaction).





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

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.

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).

-The symbols used in the Michaelis-Menten equation refer to the reaction rate [V_i], maximum reaction rate (V max), substrate concentration [S] and the Michaelis-Menten constant (km).

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 initial

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

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

Since V max and km are both constants, their ratio is a 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].

2- When [S] is much greater than km, the term km + [S] is essentially equal to [S].

Replacing km + [S] with [S] reduces equation to

$$Vi = Vmax$$

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

3- When [S] = km

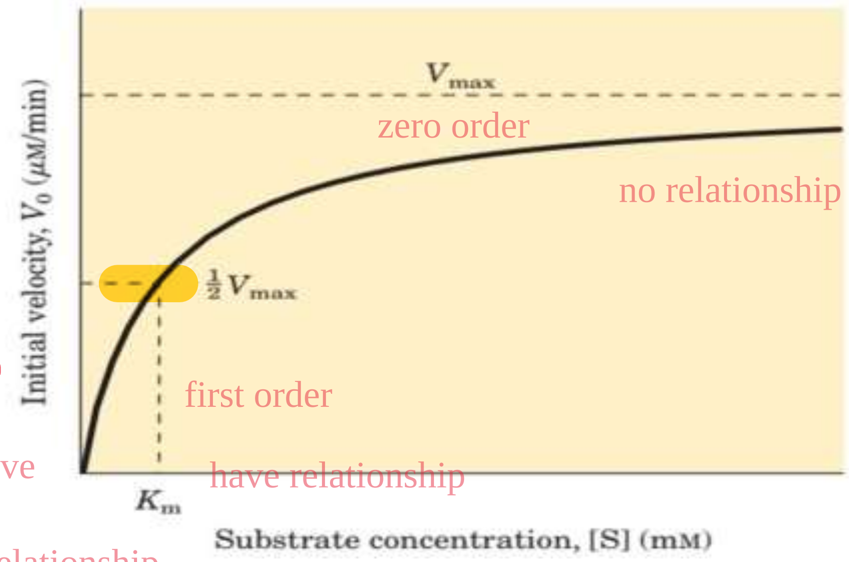
Equation states that when [S] equals km, the initial velocity is half-maximal.

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

Types of curves:
-Linear
-S shape
-hyperbolic

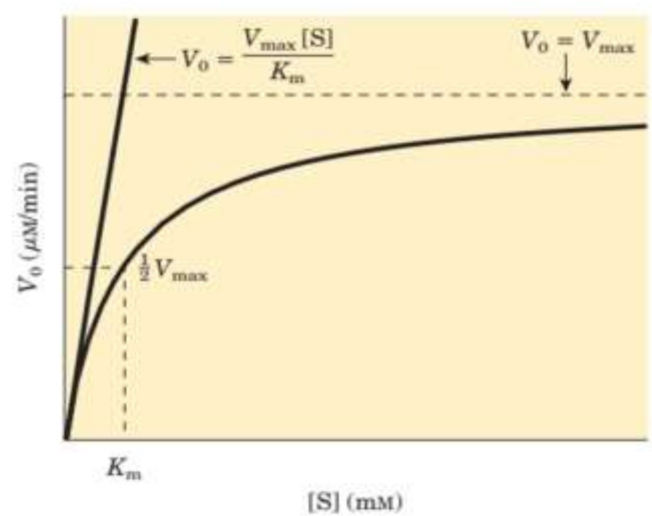
this curve is a hyperbolic curve



hyperbolic have 1 part with direct relationship and 1 part with no relationship

Graphical Representation of Michaelis-Menten equation

- The equation describes the kinetic behavior of all enzymes that Michaelis Menten kinetics.
- This equation practically determine the value of Km and Vmax and, also describe the analysis of inhibitor action.
- But double-reciprocal plot is more convenient procedure, to determine an approximate value of Km.



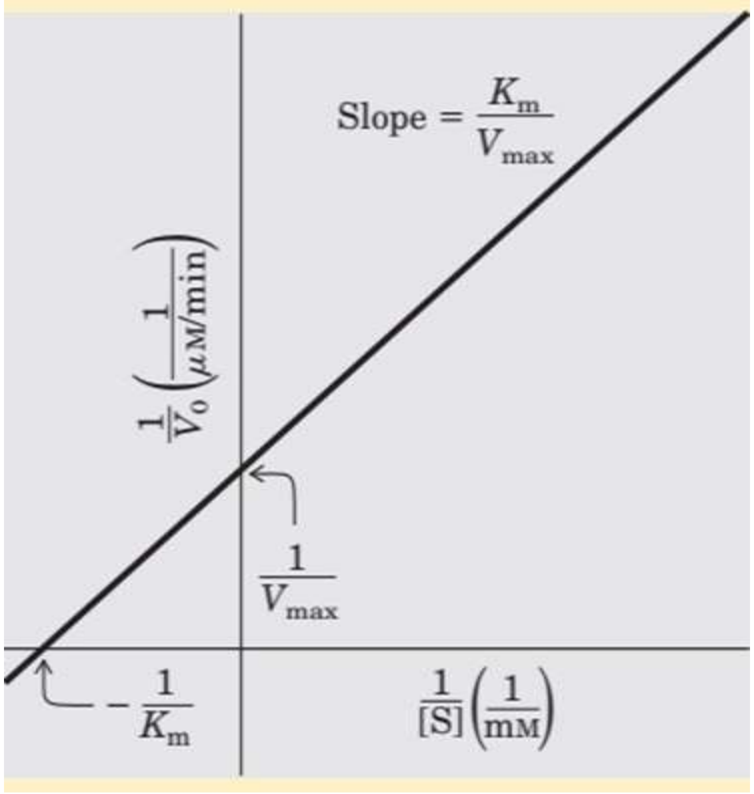
Lineweaver-Burk Plot

- 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, has the great advantage of allowing a more accurate determination of Vmax, and Km.
- The double-reciprocal plot is very useful to determine the mechanism of enzymatic reaction
- This line has a slope of Km/Vmax, an intercept of 1/Vmax on the 1/V0 y -axis, and an intercept of -1/Km on the 1/[S] x-axis .



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

relationship

- 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.