College of pharmacy

Biochemistry I

Third stage

Dr. Maytham Ahmed

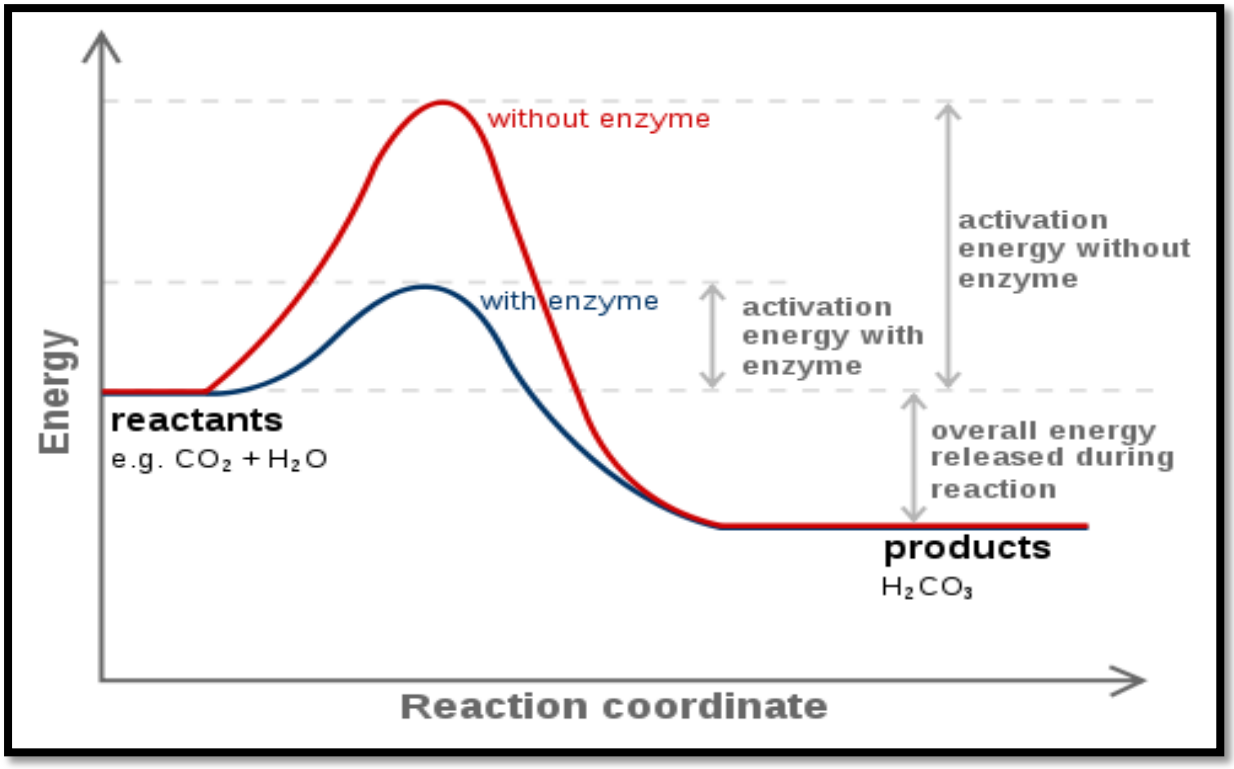
Lecture 6

Mechanism of enzyme action, Factors affecting on reaction velocity and Enzyme kinetics

Mechanism of enzyme action

Virtually all chemical reactions have an energy barrier separating the reactants and the products. This barrier, called the free energy of activation, is the energy difference between that of the reactants and a high energy intermediate that occurs during the formation of product.

For molecules to react, they must contain sufficient energy to overcome the energy barrier of the transition state. In the absence of an enzyme, only a small proportion of a population of molecules may possess enough energy to achieve the transition state between reactant and product (the rates reactions are often slow). An enzyme allows a reaction to proceed rapidly under conditions by providing an alternate reaction pathway with a lower free energy of activation. In general, the lower the free energy of activation, the more molecules have sufficient energy to pass through the transition state, and, thus, the faster the rate of the reaction.



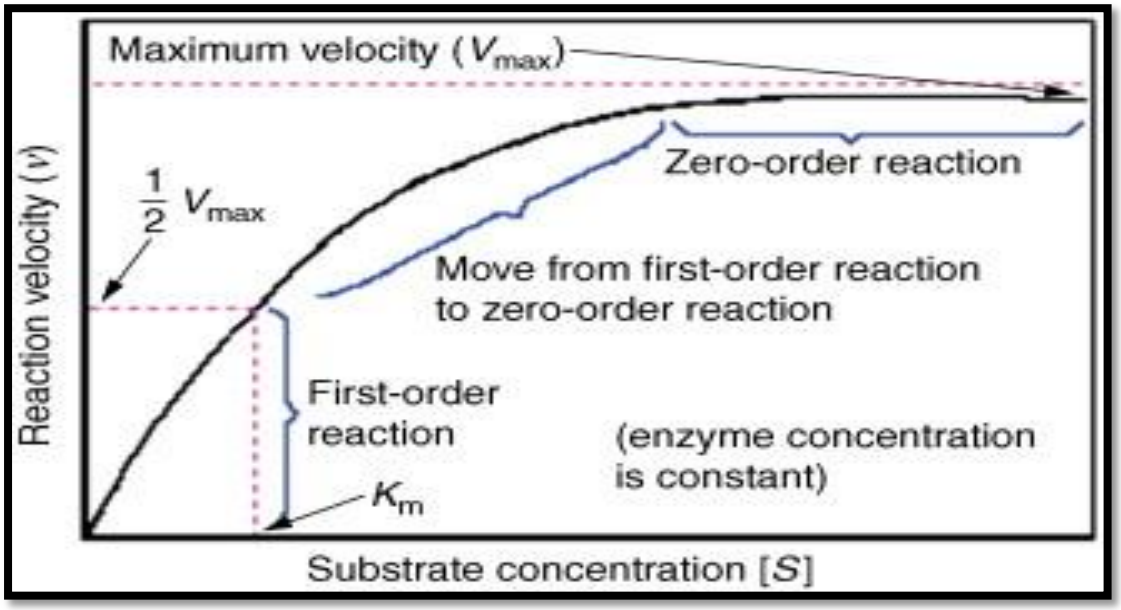
Mechanism of enzyme action

Factors affecting on reaction velocity

The factors affecting on reaction velocity are:

1. Substrate concentration

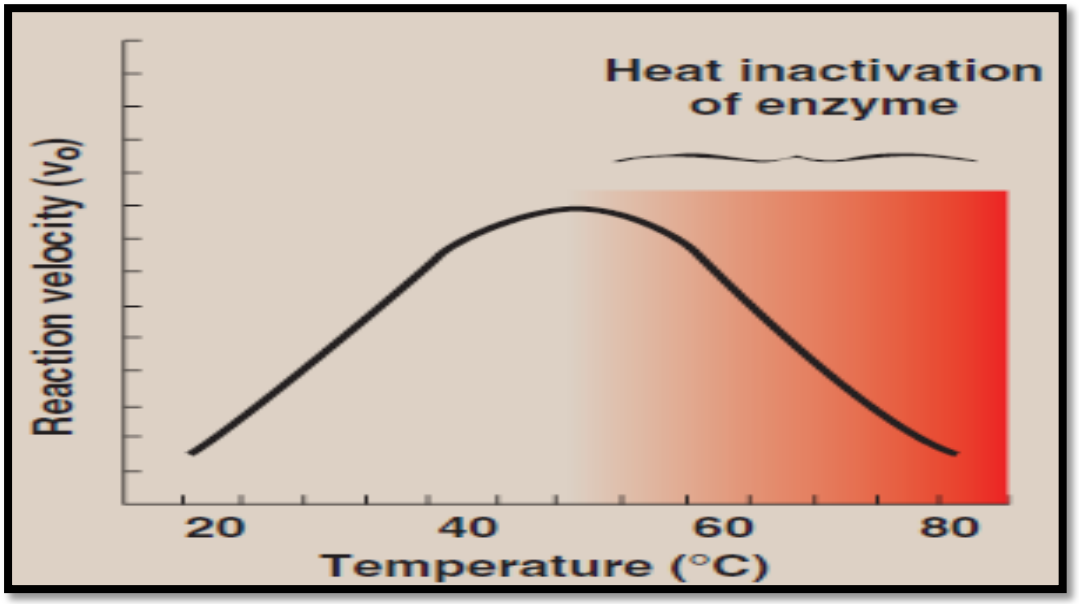
The rate or velocity of a reaction (v) is the number of substrate molecules converted to product per unit time; velocity is usually expressed as μmol of product formed per minute. The rate of an enzyme catalyzed reaction increases with substrate concentration until a maximal velocity (Vmax) is reached. The leveling off of the reaction rate at high substrate concentrations reflects the saturation with substrate of all available binding sites on the enzyme molecules.



Effect of substrate concentration on reaction velocity

2. Temperature

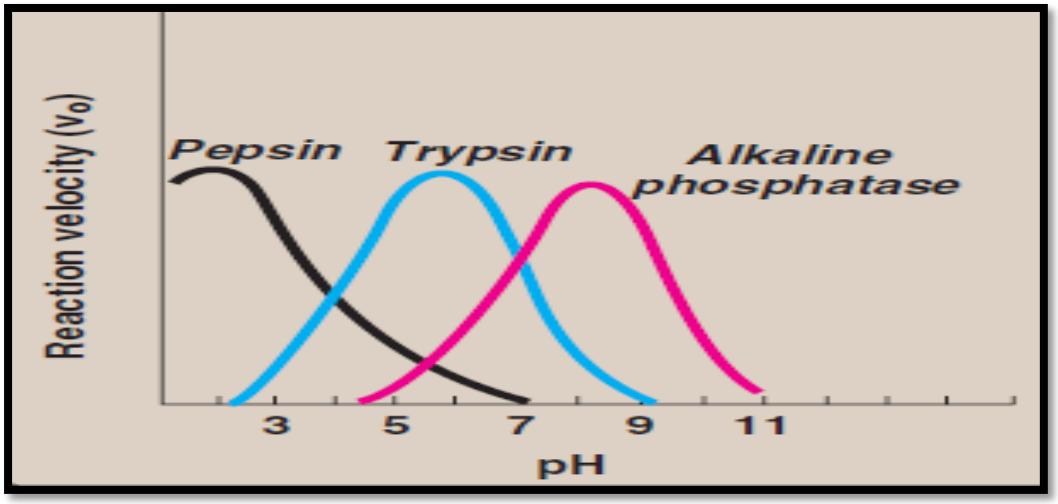
The reaction velocity increases with temperature until a peak velocity is reached. This increase is the result of the increased number of molecules having sufficient energy to pass over the energy barrier and form the products of the reaction. Further elevation of the temperature results in a decrease in reaction velocity as a result of temperature induced denaturation of the enzyme. The optimum temperature for most human enzymes is between 35 and 40°C. Human enzymes start to denature at temperatures above 40°C.



Effect of temperature on an enzyme catalyzed reaction

3. pH

The concentration of H^+ affects reaction velocity in several ways. First, the catalytic process usually requires that the enzyme and substrate have specific chemical groups in either an ionized or un-ionized state in order to interact. The pH at which maximal enzyme activity is achieved is different for different enzymes, and often reflects the $[H^+]$ at which the enzyme functions in the body. For example, pepsin, a digestive enzyme in the stomach, is maximally active at pH 2, whereas other enzymes, designed to work at neutral pH, are denatured by such an acidic environment.



Effect of pH on enzyme-catalyzed reactions

Enzyme kinetics

Michaelis-Menten and Hill equations

1- Michaelis-Menten equation

Most enzymes show Michaelis-Menten kinetics. In this model, the enzyme reversibly combines with its substrate to form an ES complex that subsequently yields product, regenerating the free enzyme:

S is the substrate, **E** is the enzyme, **ES** is the enzyme-substrate complex

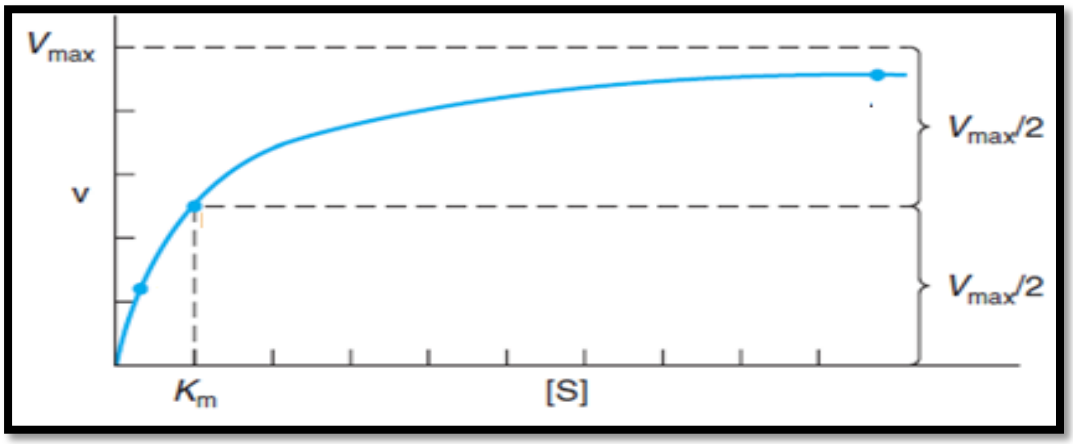
P is the product, $\mathbf{k_1}$, $\mathbf{k_{-1}}$, and $\mathbf{k_2}$ are rate constants

The Michaelis-Menten equation describes how reaction velocity varies with substrate concentration, in which the plot of initial reaction velocity (v) against substrate concentration [S], is **hyperbolic** (similar in shape to that of the **oxygen-dissociation curve of myoglobin**).

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

v = initial reaction velocity $\mathbf{V_{max}}$ = maximal velocity

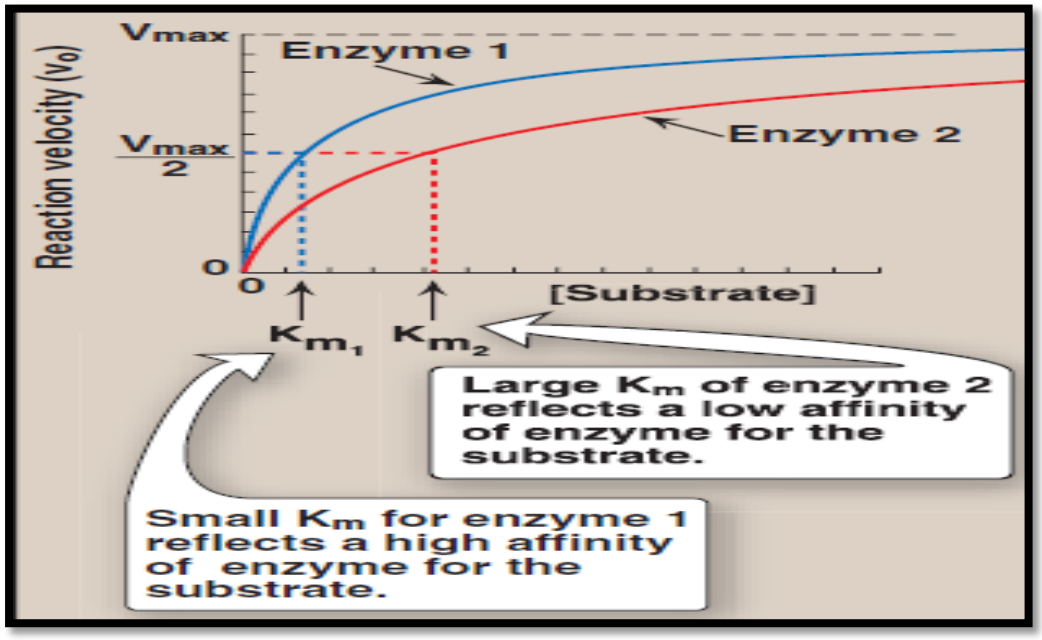
$\mathbf{K_m}$ = Michaelis constant = $(k_{-1} + k_2)/k_1$ **[S]** = substrate concentration



Effect of substrate concentration on the initial velocity

The Michaelis constant (K_m): is the substrate concentration at which initial reaction velocity (v) is half the maximal velocity ($V_{max}/2$) possible at a particular concentration of enzyme. K_m thus has the dimensions of substrate concentration.

- **Small K_m :** A numerically small (low) K_m reflects a **high affinity** of the enzyme for substrate, because a **low concentration** of substrate is needed to half-saturate the enzyme that is, to reach a velocity that is $1/2V_{max}$.
- **Large K_m :** A numerically large (high) K_m reflects a **low affinity** of enzyme for substrate because a **high concentration** of substrate is needed to half-saturate the enzyme.



Enzyme 1 with a small Km, and enzyme 2 with a large Km

Relationship of velocity to enzyme concentration

The rate of the reaction is directly proportional to the enzyme concentration at all substrate concentrations. For example, if the enzyme concentration is halved, the initial rate of the reaction (v), as well as that of V_{max}, are reduced to half that of the original.

Dependence of initial reaction velocity on [S]

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

(1) When [S] is much less than K_m

The term K_m + [S] is essentially equal to K_m. Replacing Km + [S] with K_m reduces equation to:

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

The **initial reaction velocity** therefore is **directly** proportionate to **[S]**. The rate of reaction is then said to be **first order** with respect to substrate.

(2) When [S] is much greater than K_m :

The term K_m + [S] is essentially equal to [S]. Replacing K_m + [S] with [S] reduces equation to:

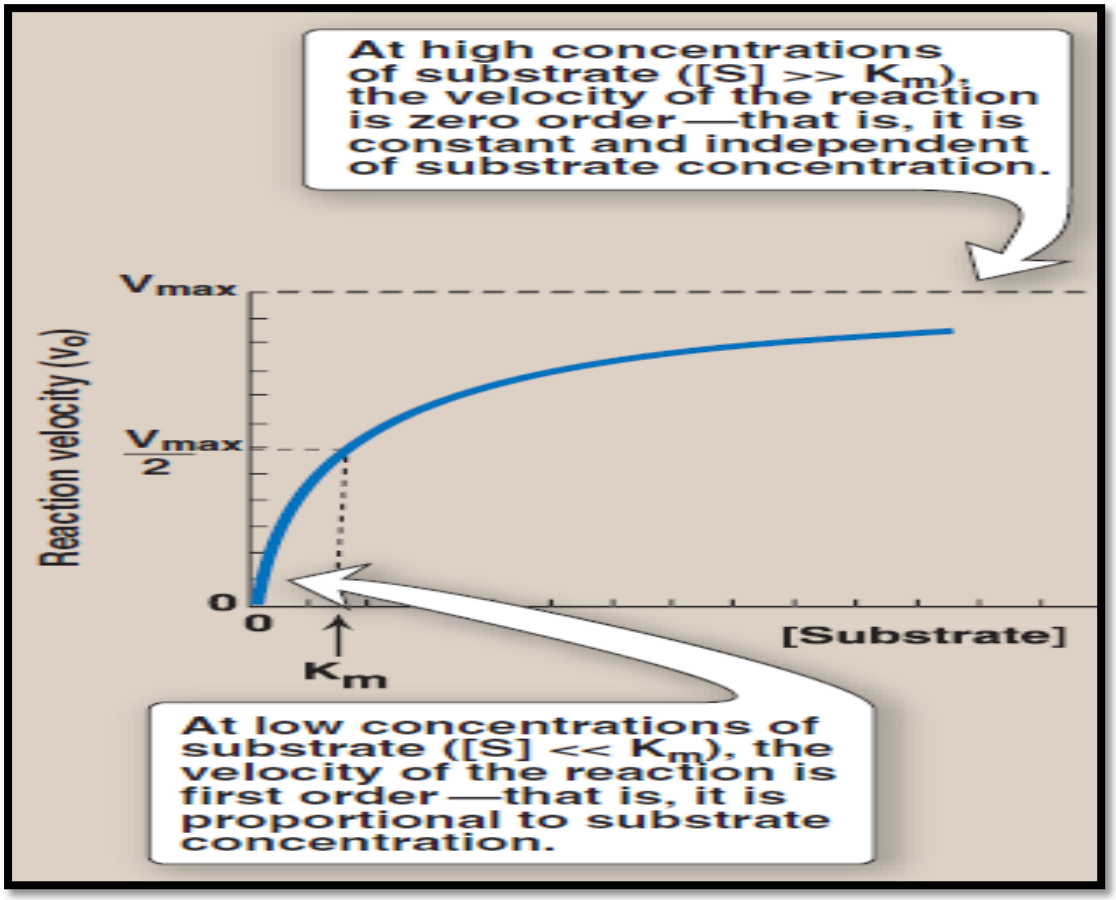
$$v = \frac{V_{max}[S]}{K_m + [S]} \qquad v \approx \frac{V_{max}[S]}{[S]} \approx V_{max}$$

The rate of reaction is then **independent** of **[S]**, and is said to be **zero order** with respect to substrate concentration.

(3) When [S] = K_m

The initial **velocity** is **half maximal**.

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

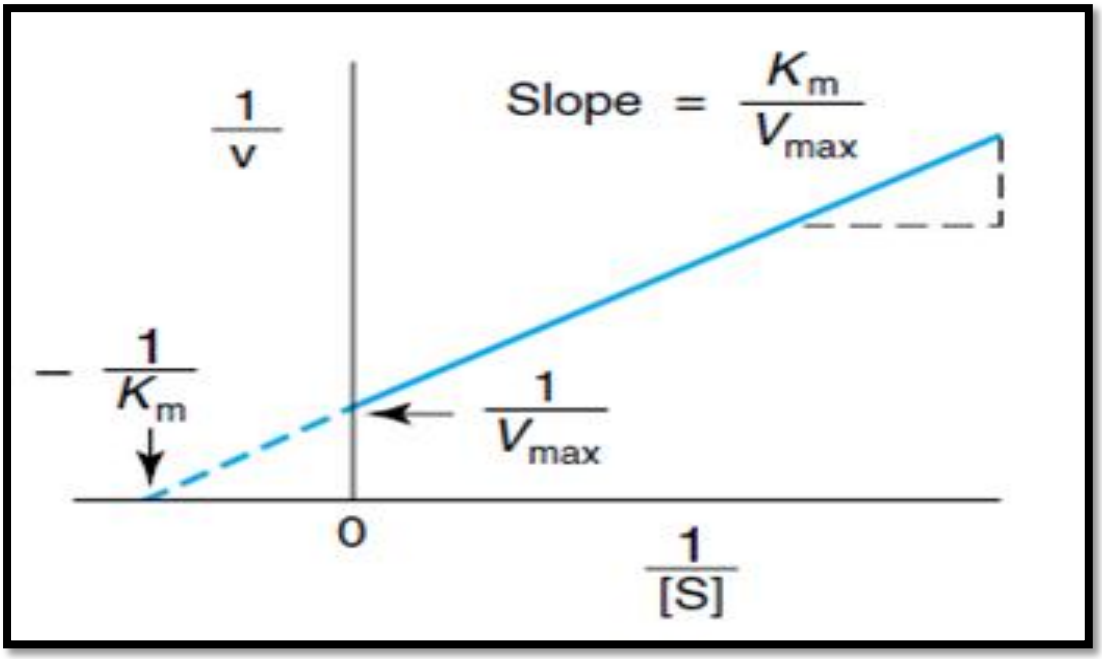


Effect of substrate concentration on reaction velocity

Lineweaver-Burk plot

The direct measurement of the numeric value of Vmax and therefore the calculation of K_m often requires impractically high concentrations of substrate to achieve saturating conditions. However, if 1/v is plotted versus 1/[S], a straight line is obtained. This plot, the **Lineweaver-Burk plot** (also called a **double-reciprocal plot**) can be used to calculate K_m and V_{max}, as well as to determine the mechanism of action of enzyme inhibitors.

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



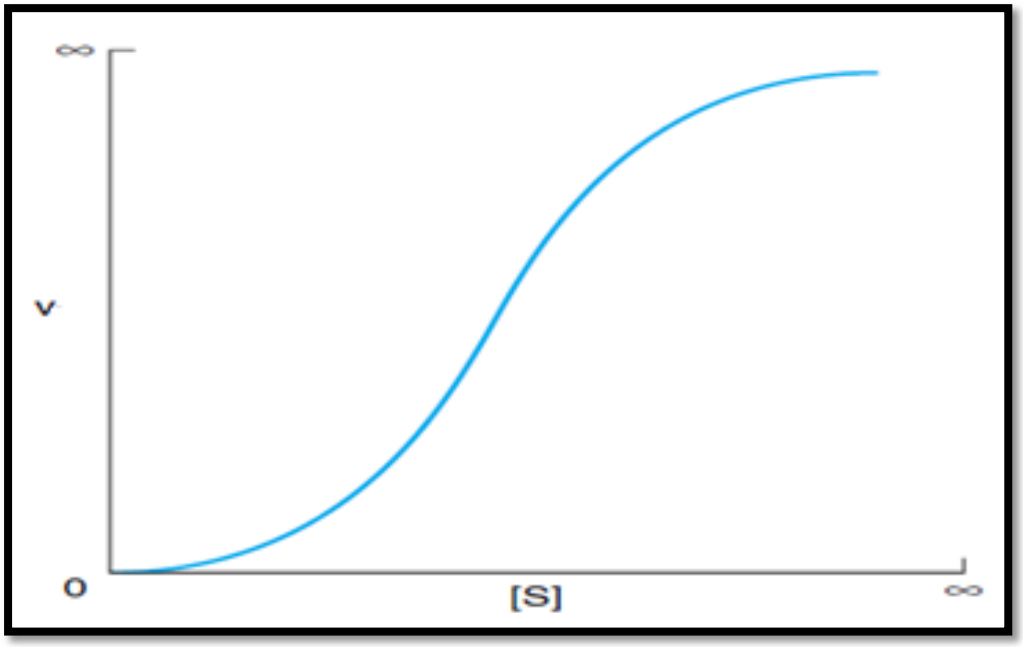
Double reciprocal or Lineweaver-Burk plot of 1/v versus 1/[S] used to evaluate K_m and V_{max}

2- Hill equation

Most enzymes display the simple saturation kinetics and are adequately described by the Michaelis-Menten expression, some enzymes bind their substrates in a **cooperative fashion** analogous to the binding of **oxygen** by **hemoglobin**. Cooperative behavior may be encountered for multimeric enzymes that bind substrate at multiple sites. For enzymes that display positive cooperativity in binding substrate, the shape of the curve that relates changes in v to changes in [S] is **sigmoidal**.

$$\frac{\log v}{V_{max} - v} = n \log[S] - \log k'$$

k′ is a complex constant **n** is the Hill coefficient



Sigmoid substrate saturation kinetics

n (Hill coefficient) is an empirical parameter whose value is a function of the number, kind, and strength of the interactions of the multiple substrate binding sites on the enzyme.

When **n = 1**, all binding sites behave **independently**, and simple **Michaelis-Menten kinetic** behavior is observed. If **n** is **greater** than **1**, the enzyme is said to exhibit **positive cooperativity (Hill kinetic)**. Binding of the first substrate molecule then enhances the affinity of the enzyme for binding additional substrate. **The greater the value for n, the higher the degree of cooperativity**.