LAB 6

The Effect of Substrate Concentration on the Rate of Enzyme Catalyzed Reaction

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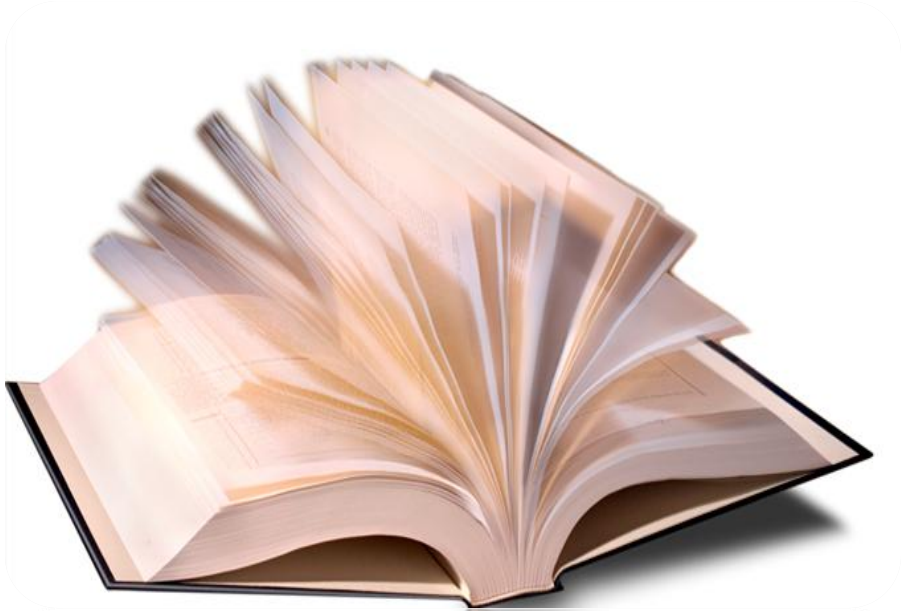


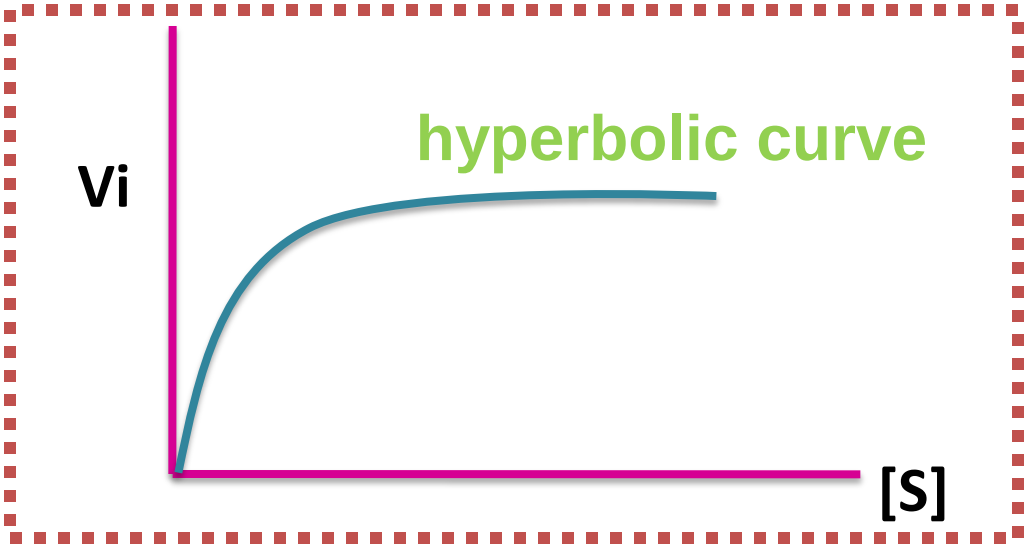
Aims :

1- To establish the relationship between substrate concentration and the rate of an enzyme catalyzed reaction.

2- To determine the K_M and V_{MAX} of the enzyme for a particular substrate.

•The rate of an enzyme catalyzed reaction depends **directly** on the concentration of the enzyme.

But with fixed concentration of enzyme and other factors effecting in the enzymatic reaction and increasing substrate concentration, a rapid increase in the rate of the reaction is observed which give **hyperbolic curve.**



Relationship between the rate of reaction and substrate concentration.

A: At low concentration of [s] the rate of the reaction is proportional to [s] which give straight line , that called **first order.**

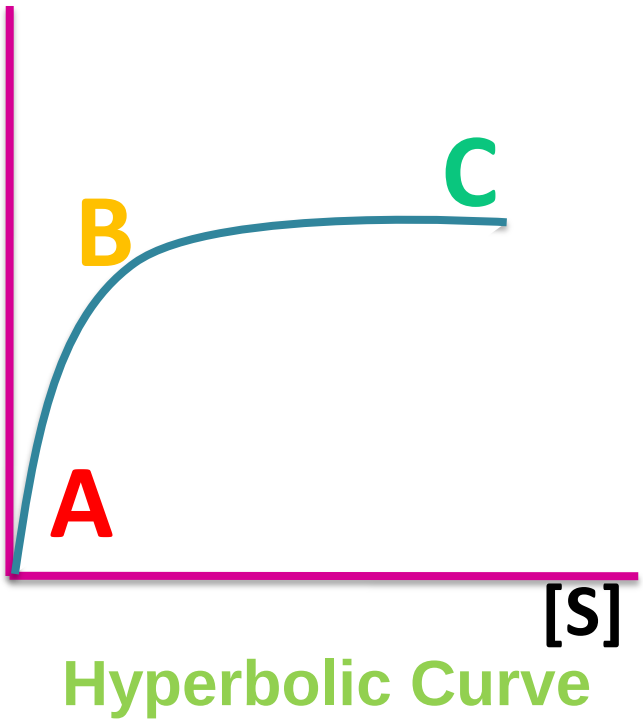
B: As the substrate concentration continues to increase, the increase in the rate of reaction begins to slow down, which give the curve called **mixed order.**

C: With a high substrate concentration, no further increase in the rate of the reaction is obtained.

- Rate of the reaction is independent to [S] and constant .

Because :

1. The substrate consumed (i. e all the active site of the enzyme saturated)
- Called **zero order**



Hyperbolic Curve

• At the zero order the rate of the reaction equal to maximum velocity which called **V max.**

•**V max:** maximum velocity of the enzymatic reaction.

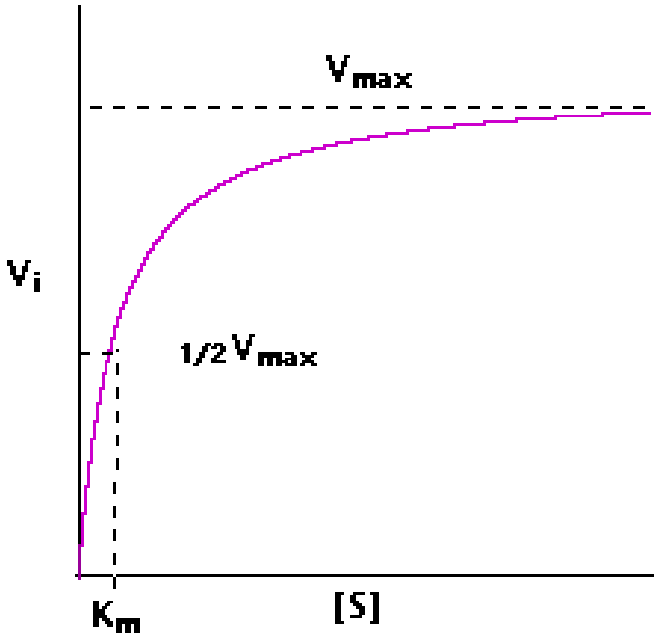
•**1/2Vmax:** half maximum velocity of the enzymatic reaction.

•**Km:** the substrate concentration at half V max.

• Km a illustrated the relationship between S and E(i. e affinity of binding S with E)

•Km ↑ = ↓ affinity

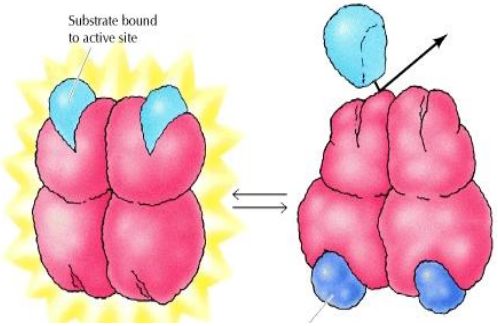
•Km ↓ = ↑ affinity

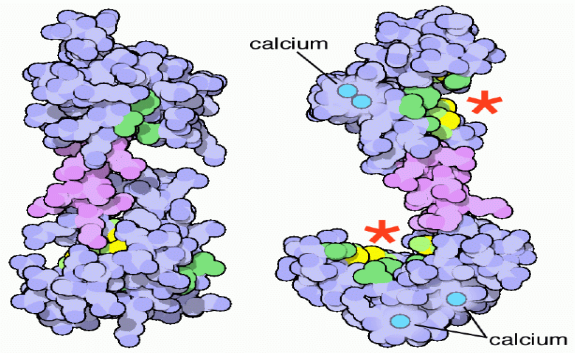


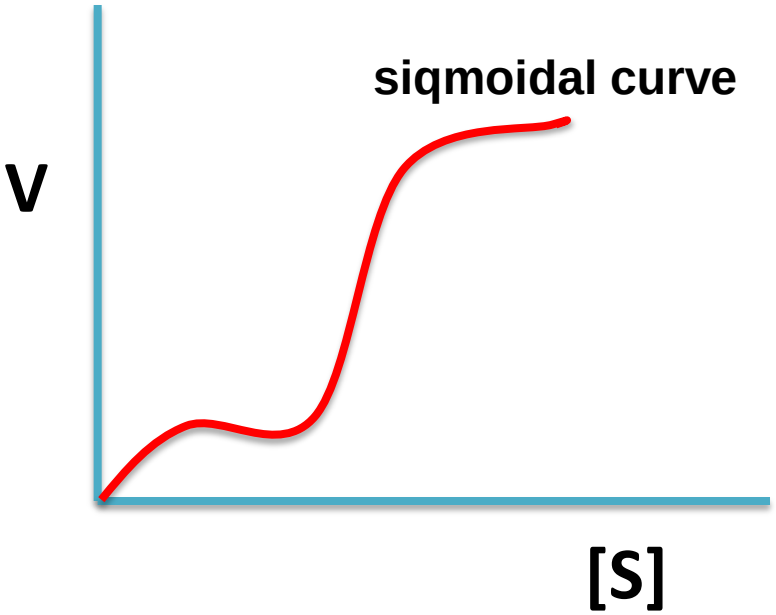
Michaelis–Menten equation

•The enzymatic reaction give hyperbolic curve with substrate are follow Michaelis–Menten kinetics.

Some enzyme give siqmoidal curve
Because have more than one site (active site and modulator site)
This enzyme called allosteric enzyme(i.e. it is regulatory enzyme)
This enzyme can not follow the Michaelis–Menten equation.

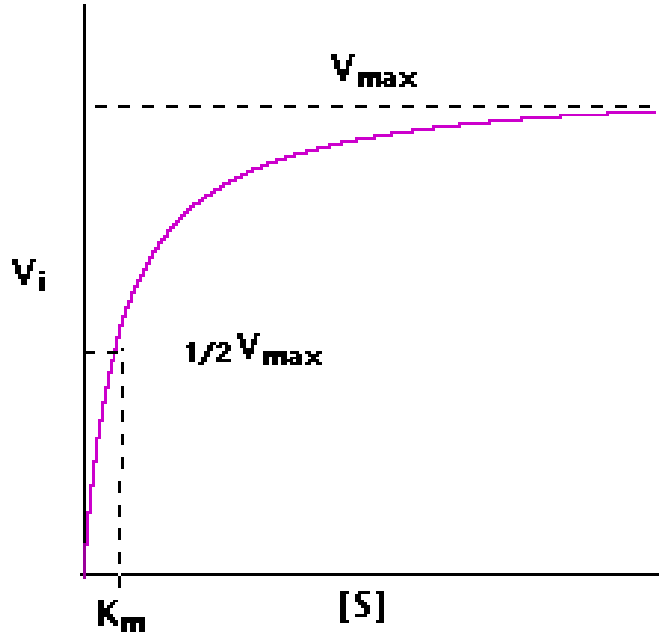






•Michaelis-Menten equation give the relationship between [S] and velocity of enzymatic reaction.

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



Where

Vi = initial velocity

$V_{MAX=}$ is the maximum velocity

Km= Michaelis constant

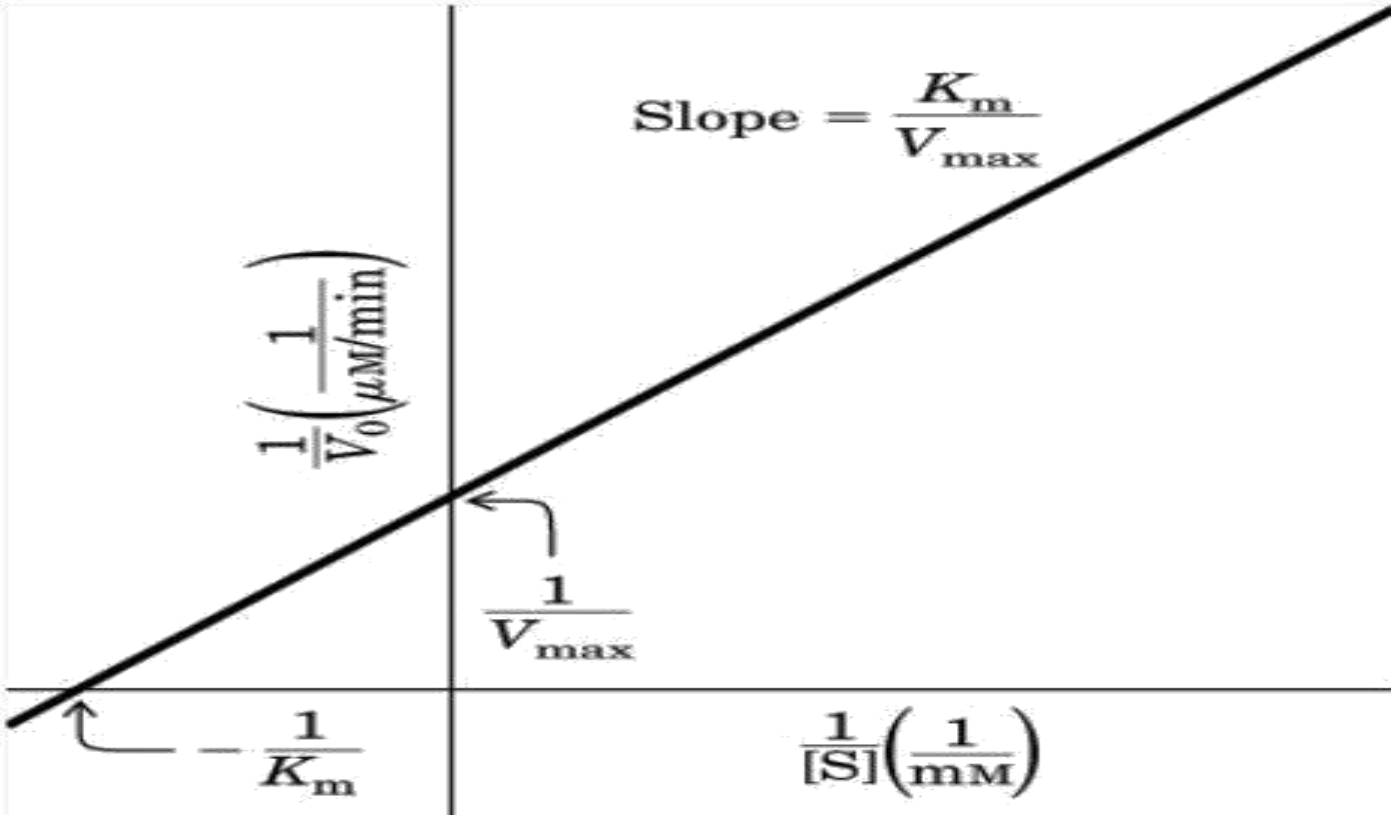
[S]= substrate concentration

Both K_M and V_{MAX} are constants for any particular combination of enzyme and substrate. These constants may be obtained by preparing a graph of the type shown above.

•Abetter method of measuring V_{MAX} and K_M is obtained by Lineweaver-Burk equation.
•Can be obtained by taking the reciprocal of both sides of Michaelis Menten equation
•Rearranging the Michaelis Menten equation in the following form

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

By plotting $1/v_i$ against 1/[S], a straight-line plot, the Lineweaver-Burk plot, is obtained,



Both Vmax and Km may be obtained accurately from the intercepts of the straight line with the axes of the above plot.

The object of this exercise is :

To demonstrate the effect of performing the standard 5-minute assay in the presence of substrate concentrations ranging from 0 to 5.0 mM p-nitrophenyl phosphate.

The results should provide classic Michaelis-Menten date from which approximations of V_{MAX} and K_M can be made.

Double-reciprocal plots of the same data should be done to arrive at even more exact values for V_{MAX} and K_M.

calculations

velocity (v) =($(A*10^{6})$ /(E* 5)

E= extension coefficient=$18.8*10^{3}$

A= absorbance

Results:

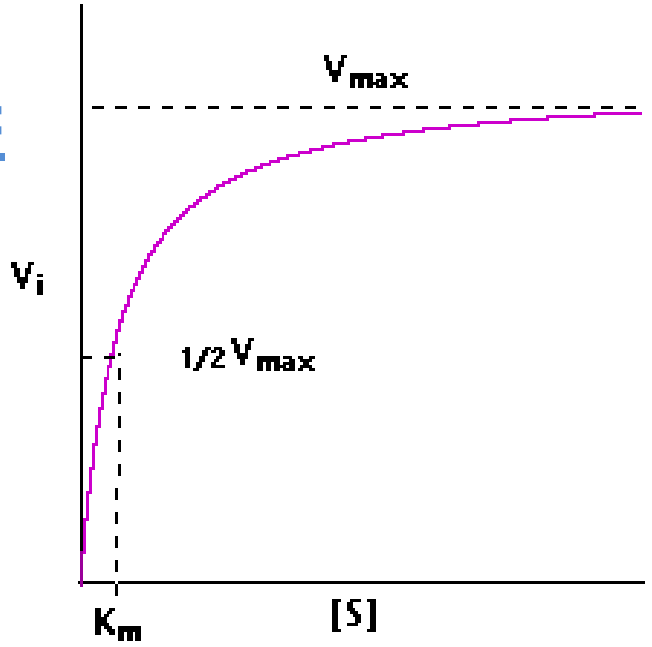
Tube	Absorbance at 405nm	Velocity µm/min	1/V	[S] M	[S] mM	1/[S] mM
A						
B						
C						
D						
E						
F						
G						
H						

Michaelis–Menten equation=

Vmax=

Vmax/2=

Km=



Lineweaver-Burk equation =

1/Vmax=

Vmax=

- 1/Km=

Km=

Slope= Km/Vmax