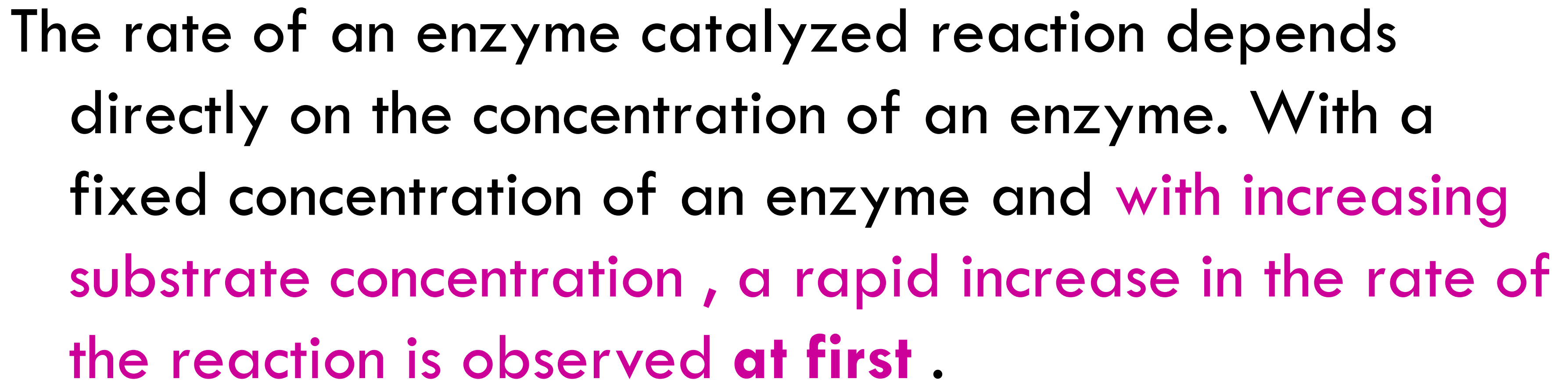
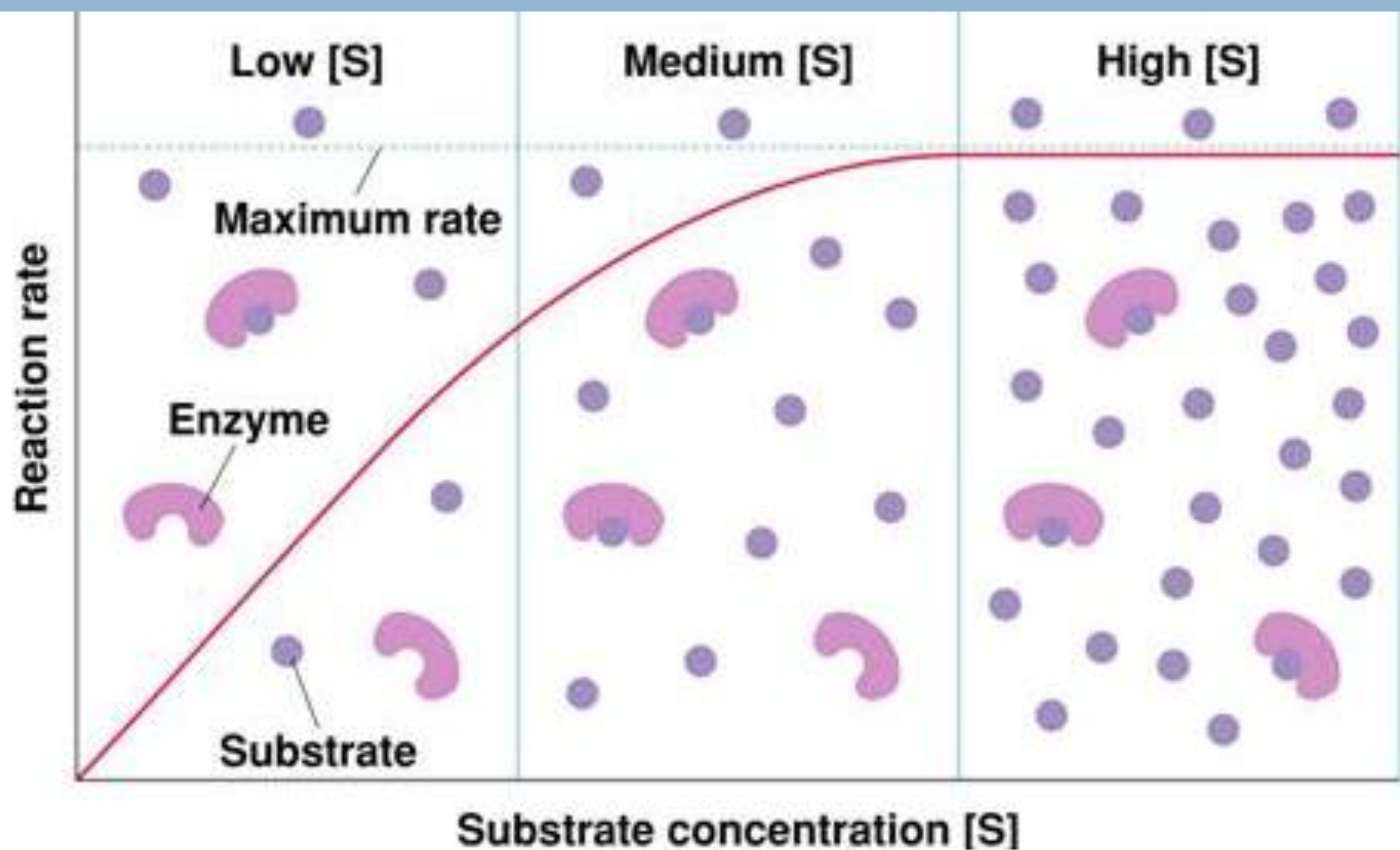
The effect of substrate concentration on the rate of an enzyme catalyzed reaction



The rate of an enzyme catalyzed reaction depends directly on the concentration of an enzyme. With a fixed concentration of an enzyme and with increasing substrate concentration , a rapid increase in the rate of the reaction is observed **at first** .

But as substrate concentration continues to increase , the increase in rate of reaction *being slow down until* , *with a large substrate concentration, no further increase in the rate of the reaction obtained.*



Low [S]
Medium [S]
High [S]
Maximum rate
Enzyme
Substrate
Reaction rate
Substrate concentration [S]

As the substrate level is increased , the velocity increases in a hyperbolic fashion.

At relatively low concentration of substrate, the rate of reaction increase linearly with an increase in substrate concentration.

At higher substrate concentration the rate of reaction increase *smaller and smaller* amount in response to increase in substrate concentration.

Finally , a point is reached beyond which there is only small increase in the rate of the reaction with increasing substrate concentration. **This plateau is called maximum velocity, V max.**

The hyperbolic shape of this curve can be expressed algebraically by the Michaelis -Menten equation:

Vo= V max [S] / Km+[S]

Vi= intial velocity

V max= maxiumm velocity

[S] = substrate concentration

Km= Michaelis -Menten constant

Km: is a substrate concentration at half V max(Km indicate the affinity of an enzyme for its substrate)

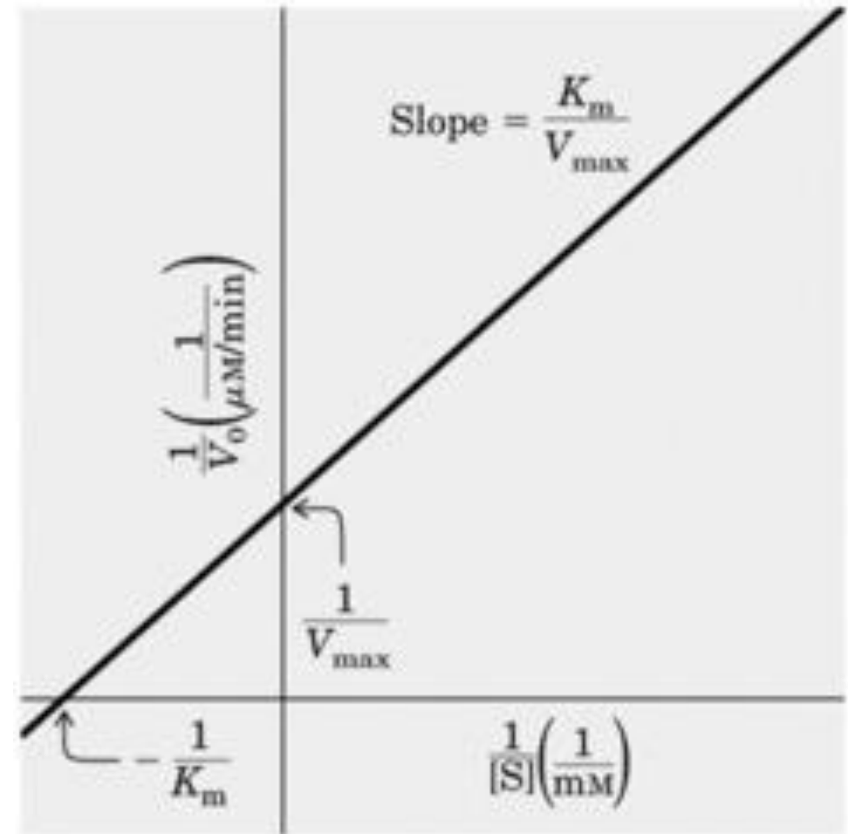
Note that the larger the K_M (the weaker the binding), the larger the [S] needed to reach the half maximum rate.

The Km can vary greatly from enzyme to enzyme , and even for different substrates of the same enzyme. **The Michaelis -Menten equation can be algebraically transformed** into forms that are useful in the practical determination of Km an V max. One common transformation is derived simply **by taking the reciprocal of both sides of the Michaelis -Menten equation to give Lineweaver – Burk equation:**

$$\frac{1}{Vi} = \frac{1}{Vmax} + \frac{Km}{Vmax} \cdot \frac{1}{[S]}$$

By plotting 1/v against 1/ [S] a straight line plot, Lineweaver – Burk plot is obtained.

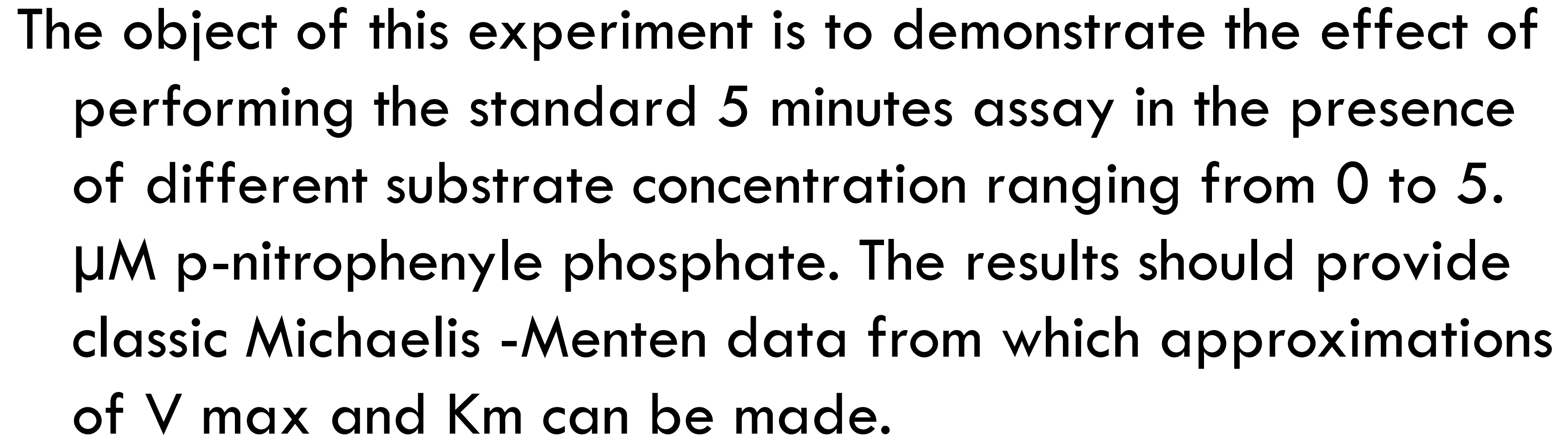
Both V max and Km can be obtained accurately from intercepts of the straight line with the y – axis and x-axis



Objectives:

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

2) To determine the Km and V max of the enzyme for a particular substrate.



Double-reciprocal plots of the same data should be done to arrive at even more exact values for Km and V max.

Test tubes

Pipettes

Cuvettes

Water bath

Stopwatch

Spectrophotometer

Chemicals:

Acid phosphatase enzyme

0.05 M PNPP Different substrate concentration

1 M sodium acetate buffer

0.1 M $MgCl_2$

0.5 M KOH

Method:

1. Prepare a series of substrate dilutions according to the protocol outlined in Tables I .

Reagent	A	B	C	D	E	F	G	H
0.05MPNPP ml	0	0.05	0.1	0.25	0.5	1	1.5	2
H2O(ml)	5	4.95	4.9	4.75	4.5	4	3.5	3
[S] M	......	......	......	......	....	....	....	....

Concentration of stock Substrate 0.05M

1. Set up eight assay tubes. To each of these tubes **add 0.5 ml of 1.0 M sodium acetate buffer (pH 5.7),**

0.5 ml of 0.1 M MgCl2, and

3. **add 0.5ml of the enzyme, run each reaction for 5 minutes**
4. Place the tubes in a test tube rack situated in a **37oC water bath and let stand for 5 minutes. stop it by adding 0.5ml of 0.5M KOH.**
5. **Let the tube stand for 20 min. before determine the absorbance at 405** nm for each reaction mixture against the blank. (The tube containing no substrate should be used as the blank).

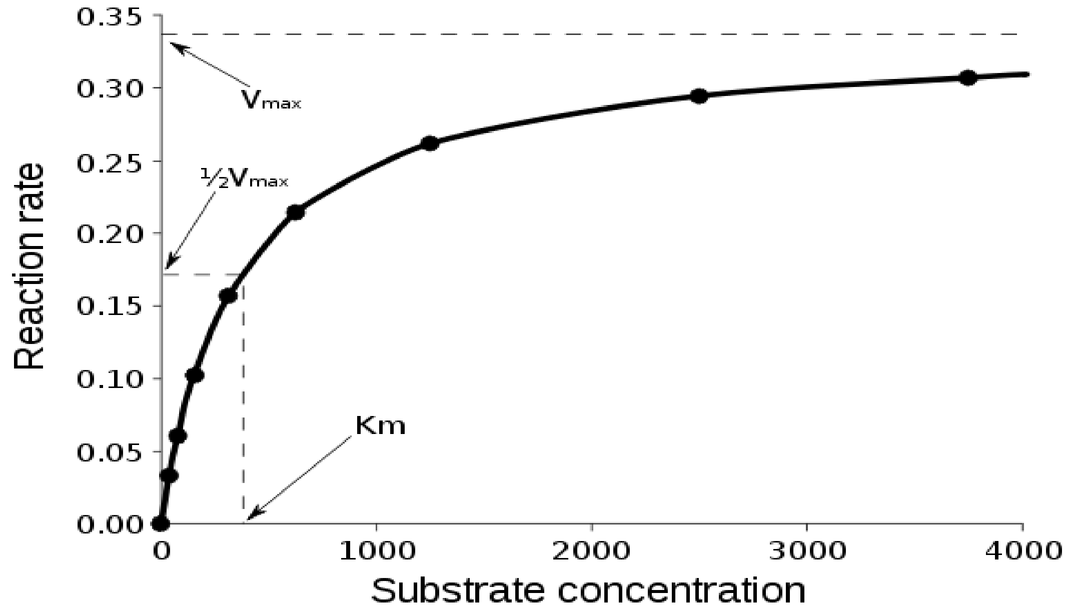
Result:

Tube	Absorbance at 405 nm	Substrate Conc.(x-values)	Velocity of reaction* (y- values)
B		………………………	………………………
C		………………………	………………………
D		………………………	………………………
E		………………………	………………………
F		………………………	………………………
G		………………………	………………………
H		………………………	………………………

1- Calculate the velocity (*M* of p-nitrophenol/minute)

2- Plot velocity against substrate concentration in the standard manner of *Michaelis* and *Menten*.

I) Michaelis –Menten Curve



3-Determine Vmax and Km for acid phosphatase.

***Velocity/Rate of reaction= Ax10^3/(εxT)**

Where:

A= Absorbance

ε= extinction coefficient for ACP enzyme = 18.8 x 10^3

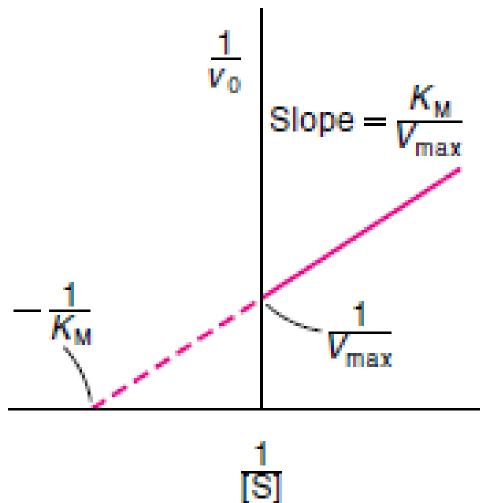
T=Time = 5 min.

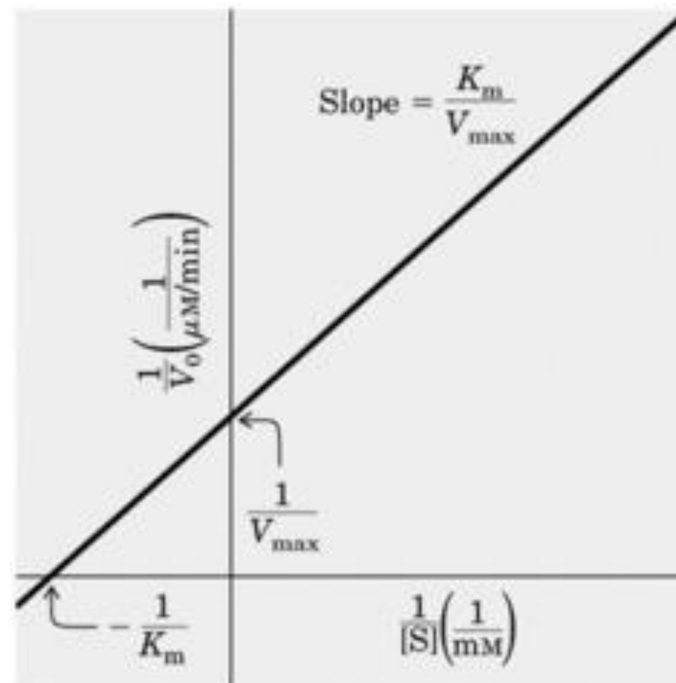
4-**Calculate the reciprocals of velocity (1/v) and substrate concentration (1/[S]) and present these data as a table.**

Tube	Substrate Conc.	1/(Substrate Conc.) (x-values)	Velocity of reaction	1/(Velocity of reaction) (y- values)
B		……………………..		……………………..
C		……………………..		……………………..
D		……………………..		……………………..
E		……………………..		……………………..
F		……………………..		……………………..
G		……………………..		……………………..
H		……………………..		……………………..

II)Lineweaver and Burk Curve

5-Prepare the double –reciprocal plot of Lineweaver and Burk and determine the Km and V max from the x and y intercepts.





Thank You