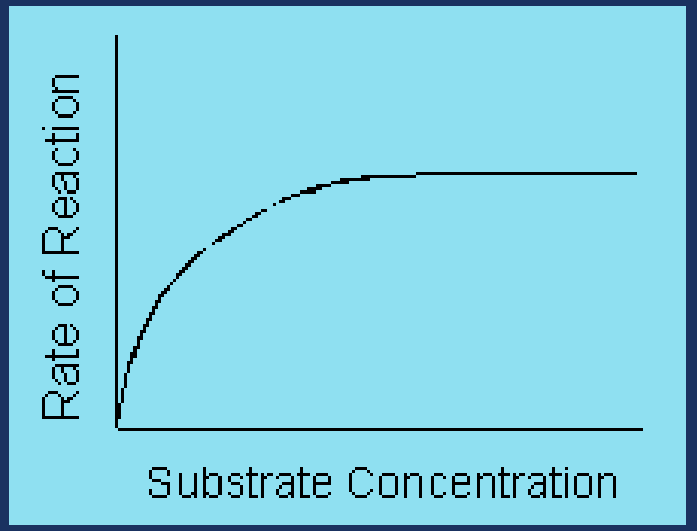
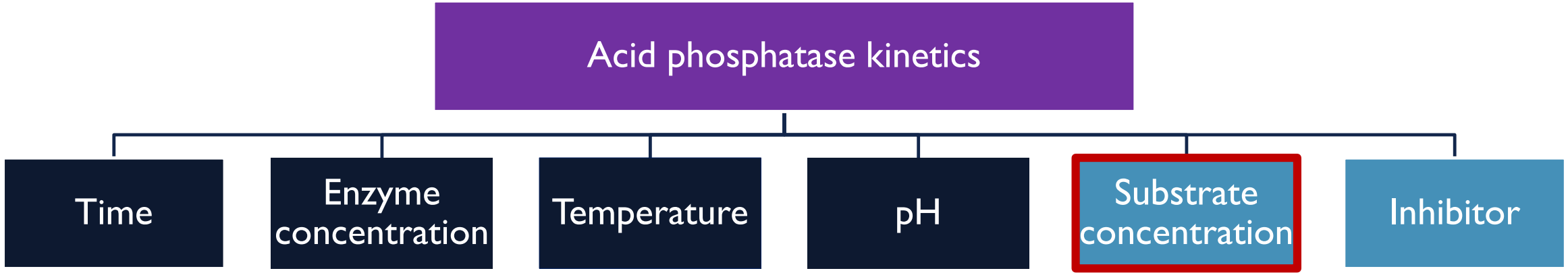
322 BCH

EXP (7)

THE EFFECT OF SUBSTRATE CONCENTRATION ON THE RATE OF AN ENZYME CATALYZED REACTION



In this experiment, we will continue to study acid phosphatase kinetics.



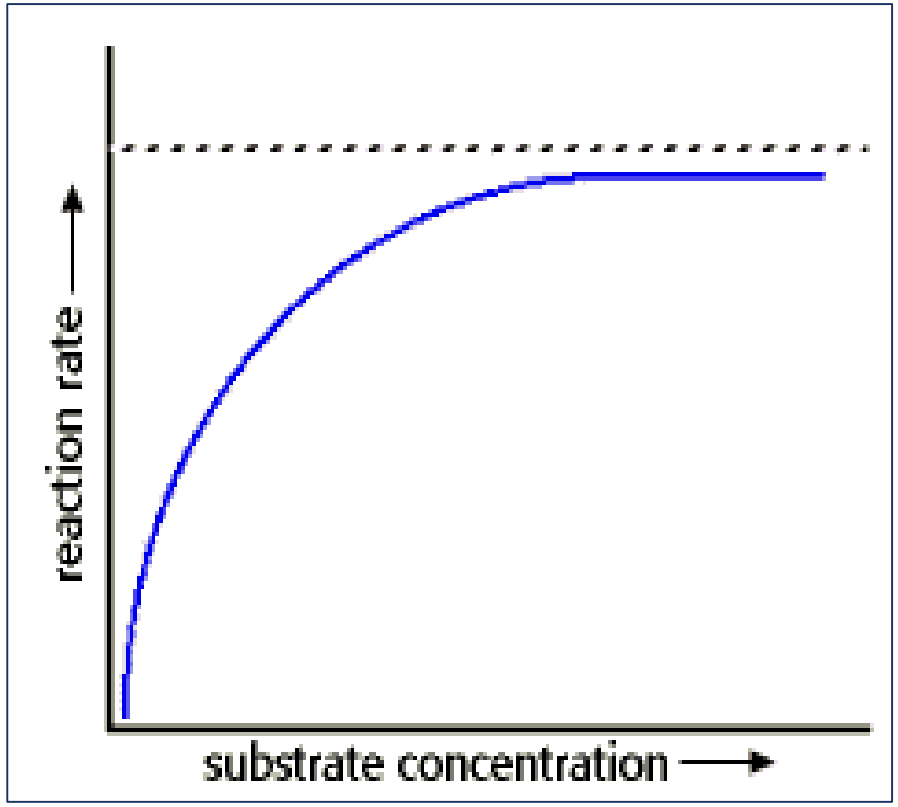
OBJECTIVES

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

- To determine the Km and Vmax of the enzyme for a particular substrate.

THE EFFECT OF SUBSTRATE CONCENTRATION

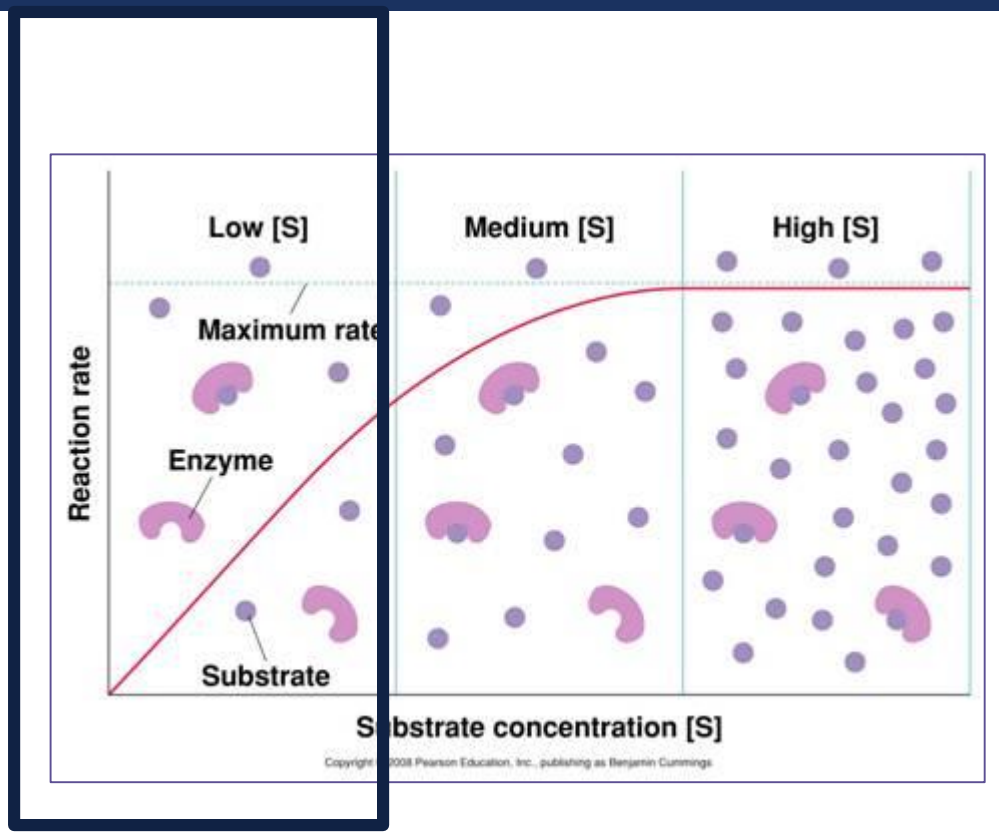
- One of the important parameters affecting the rate of a reaction catalyzed by an enzyme is the substrate concentration, [S].
- During enzyme substrate reaction, the initial velocity V_0 gradually increases with increasing concentration of the substrate. Finally a point is reached, beyond which the increase in V_0 will not depend on the [S].



THE EFFECT OF SUBSTRATE CONCENTRATION

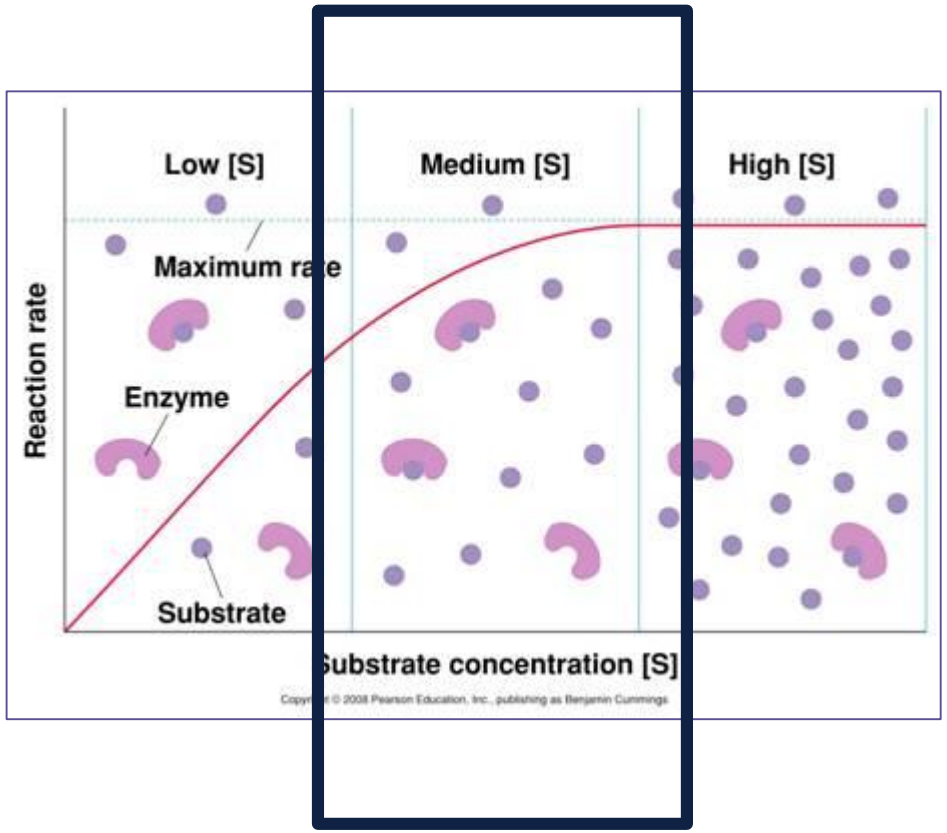
- **At relatively low concentration of substrate, the rate of reaction increase linearly with an increase in substrate concentration.**

→The catalytic site of the enzyme is empty, waiting for substrate to bind, for much of the time, and the rate at which product can be formed is limited by the concentration of substrate which is available.



THE EFFECT OF SUBSTRATE CONCENTRATION

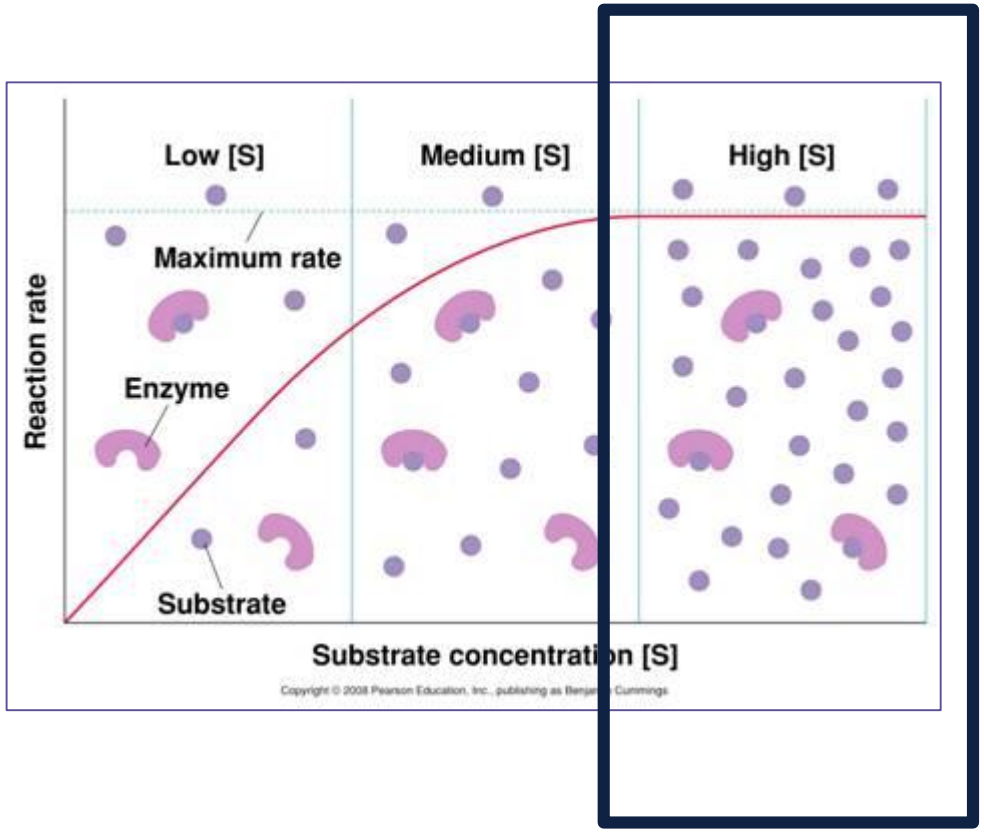
- **At higher substrate concentration, the rate of reaction increases by smaller and smaller amounts in response to increase in substrate concentration.**



THE EFFECT OF SUBSTRATE CONCENTRATION

- ***However*** **beyond a particular substrate concentration, the velocity remains constant without any further increase. This plateau is called the maximum velocity, Vmax**

→ This is because as the concentration of substrate increases, the enzyme becomes saturated with substrate.



So there is usually a hyperbolic relationship between the rate of reaction and the concentration of substrate

MICHAELIS–MENTEN EQUATION

- The rate of reaction when the enzyme is saturated with substrate is the **maximum rate** of reaction,(**maximum velocity**) **Vmax**.
- Michaelis-Menten equation give the relationship between [S] and velocity of enzymatic reaction.
- The hyperbolic shape of this curve can be expressed algebraically by the Michaelis – Menten equation:

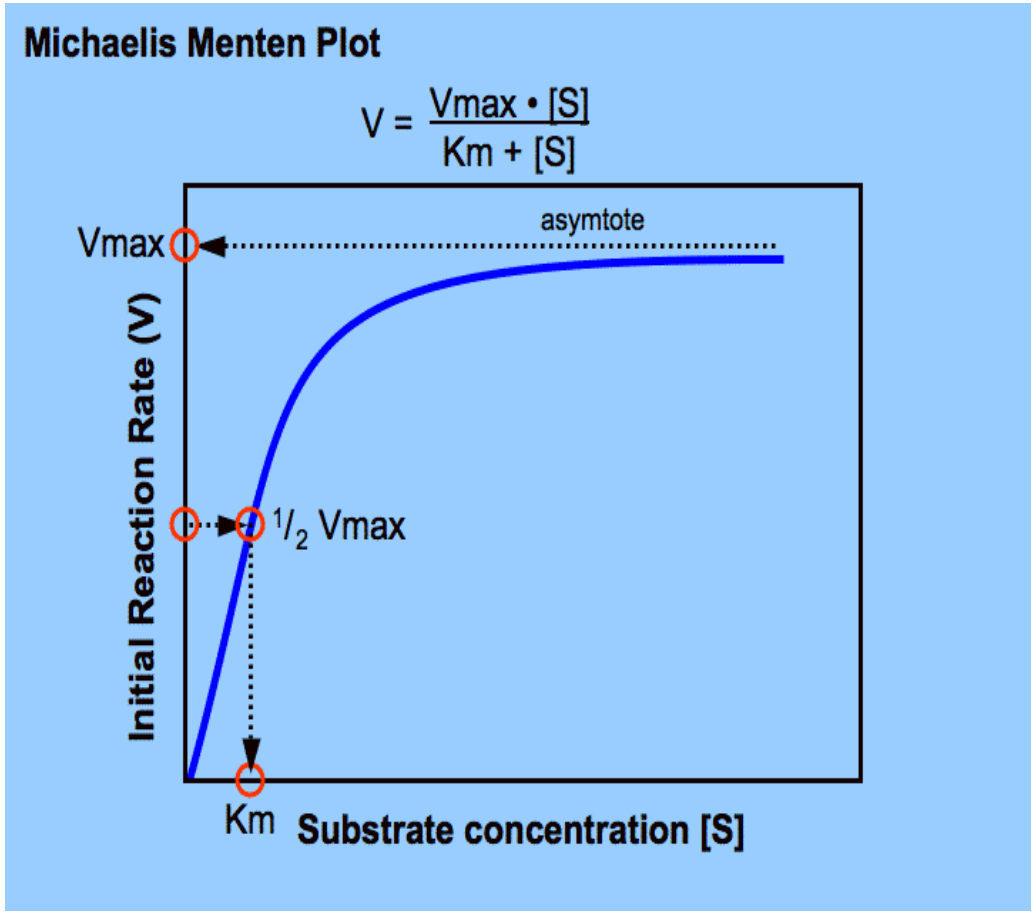
$$V = \frac{V_{max}\,[S]}{K_m + [S]}$$

Vi= intial velocity

V max= maxiumm velocity

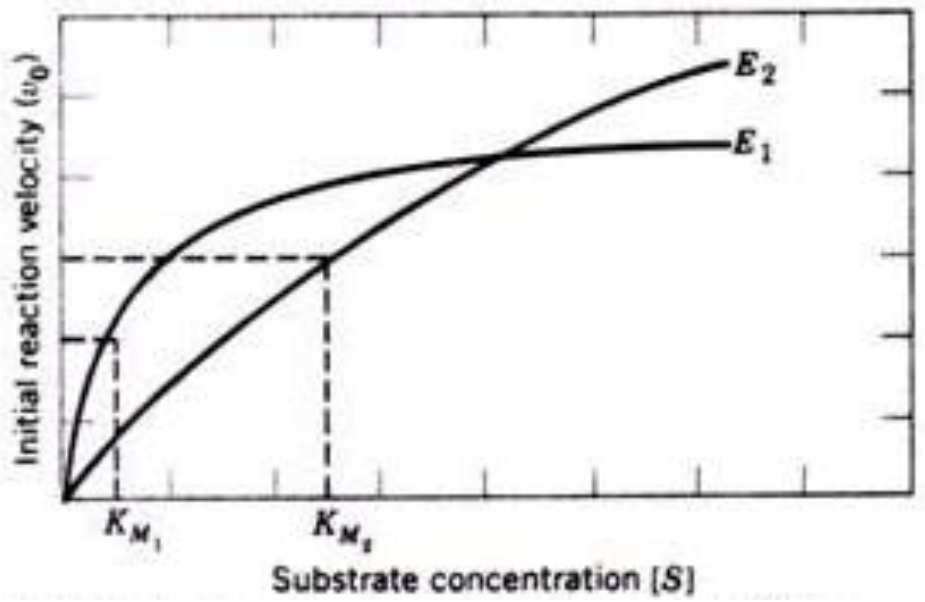
[S] = substrate concentration

Km= Michaelis constant



MICHAELIS CONSTANT (Km)

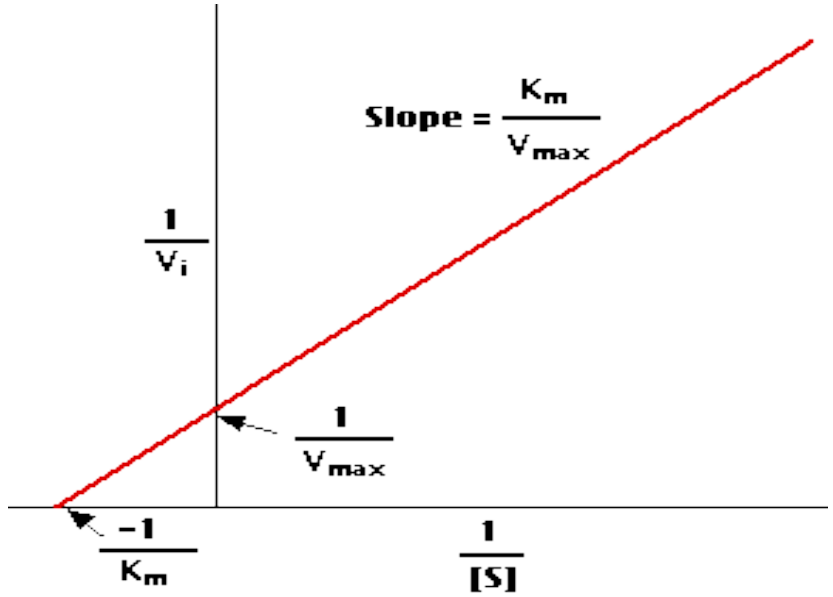
- Km is the substrate concentration at half V max.
- The relationship between rate of reaction and concentration of substrate depends on the affinity of the enzyme for its substrate. This is usually expressed as the **Km** of the enzyme, an inverse measure of affinity
- The larger the km, the weaker the binding and the larger the [S] needed to reach the half the maximum rate
- The Km can vary greatly from enzyme to enzyme, and even for different substrates of the same enzyme



LINEWEAVER – BURK EQUATION

- 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

Method

In order to detect the effect of substrate concentration you must fix all the component except the [S]	
Time (5 minutes)	constant
Enzyme concentration	constant
Substrate concentration	Variable
Temperature (37˚C)	constant
pH 5.5	constant

Method

- Prepare 8 tubes labeled as follows

Tube	A	B	C	D	E	F	G	H
[S] mM	0	0.5	1	2.5	5	10	25	50

- To each of these tubes add

Chemical	**Volume (ml)**
pH sodium acetate buffer	0.5
0.1M $MgCl_2$	0.5
Corresponding p-nitrophenyl phosphate (pNPP)	0.5
Water	5

- Place the tubes in a test tube rack situated in 37°C water bath and let stand for 5 min.

- Start the reaction by adding 0.5 ml enzyme and stop it by adding 0.5 ml KOH as in the following table:

Tube	Start the reaction	Stop the reaction
A	0 min	0 min
B	0 min	5 min
C	2 min	7 min
D	4 min	9min
E	6 min	11 min
F	8 min	13 min
G	10 min	15 min
H	12 min	17 min

Notes:

(The tube containing no substrate should be used as the blank).

RESULTS

Record the absorbance and calculate the velocity:

Tube	Absorbance at 405 nm	Velocity µM/min	[S] mM
A			0
B			0.5
C			1
D			2.5
E			5
F			10
G			25
H			50

In order to draw using Lineweaver – Burk: calculate the following:

1/V	1/[S]

CALCULATIONS

Velocity (V) = $(A \times 10^6) / (E \times \text{time}) =$ **µmole of PNP/min**

A= absorbance

E= extension coefficient= 18.8×10^3

Time = 5 min

RESULTS

- Draw the curve using Michaelis -Menten and determine Vmax and Km for acid phosphatase.
- Prepare the double –reciprocal plot of Lineweaver and Burk and determine the Km and V max from the x and y intercepts.

DISCUSSION

- Describe the curve of the effect of substate concentration on the enzymatic activity
- Comment on the value of Vmax and km and define each of them