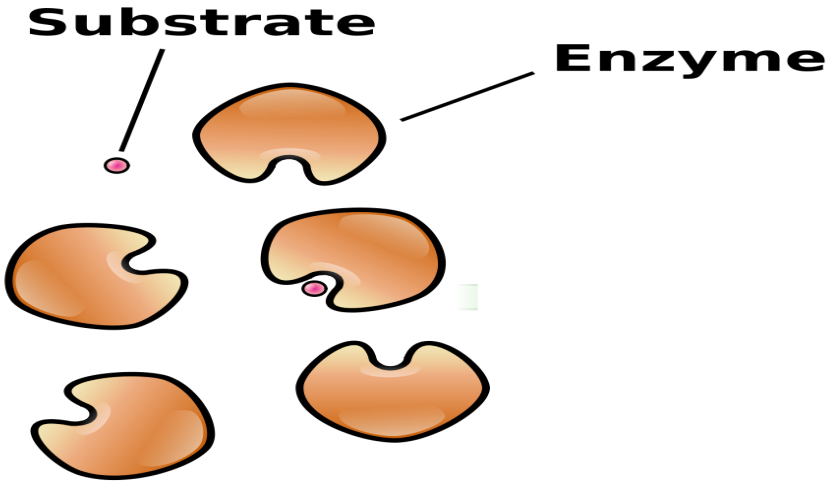
322 BCH

Exp (4)

The Effect of Enzyme Concentration on the Rate of an Enzyme Catalyzed Reaction.

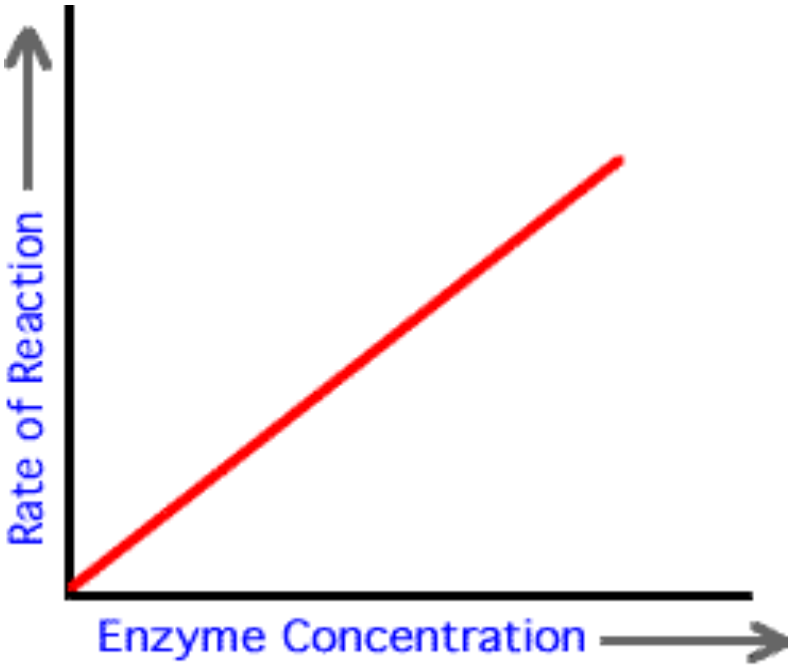
Objectives:

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



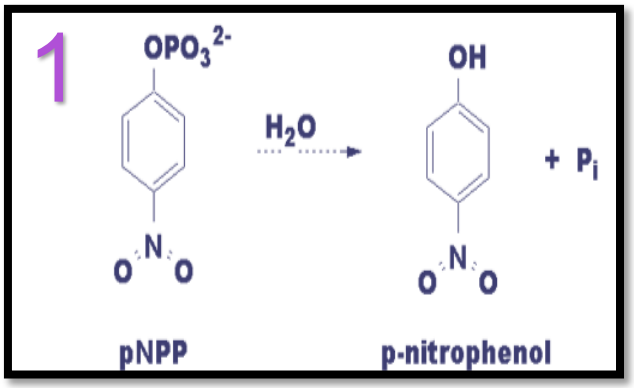
- The rate of enzyme catalyzed reaction is **directly proportional** to enzyme concentration.

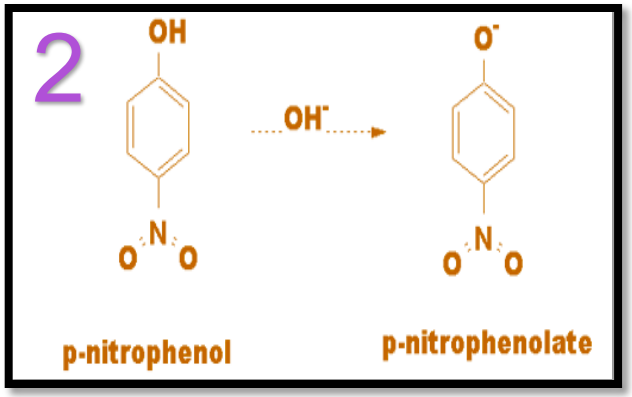
$$\mathbf{V} \propto \mathbf{[E]}$$



As the enzyme concentration increases, the rate of enzymatic reaction increases which gives a **liner curve.**

Principal of the enzyme assay in vitro





1. Under acid conditions, the enzyme catalyzes the hydrolysis of p-nitrophenyl phosphate (pNPP) to inorganic phosphate and p-nitrophenol.

2. Both p-nitrophenyl phosphate and p-nitrophenol are colorless at acidic pH values.

3. Under **alkaline conditions**, p-nitrophenol is converted to a p-nitrophenolate (yellow color) and concentration can be measured at 405 nm.

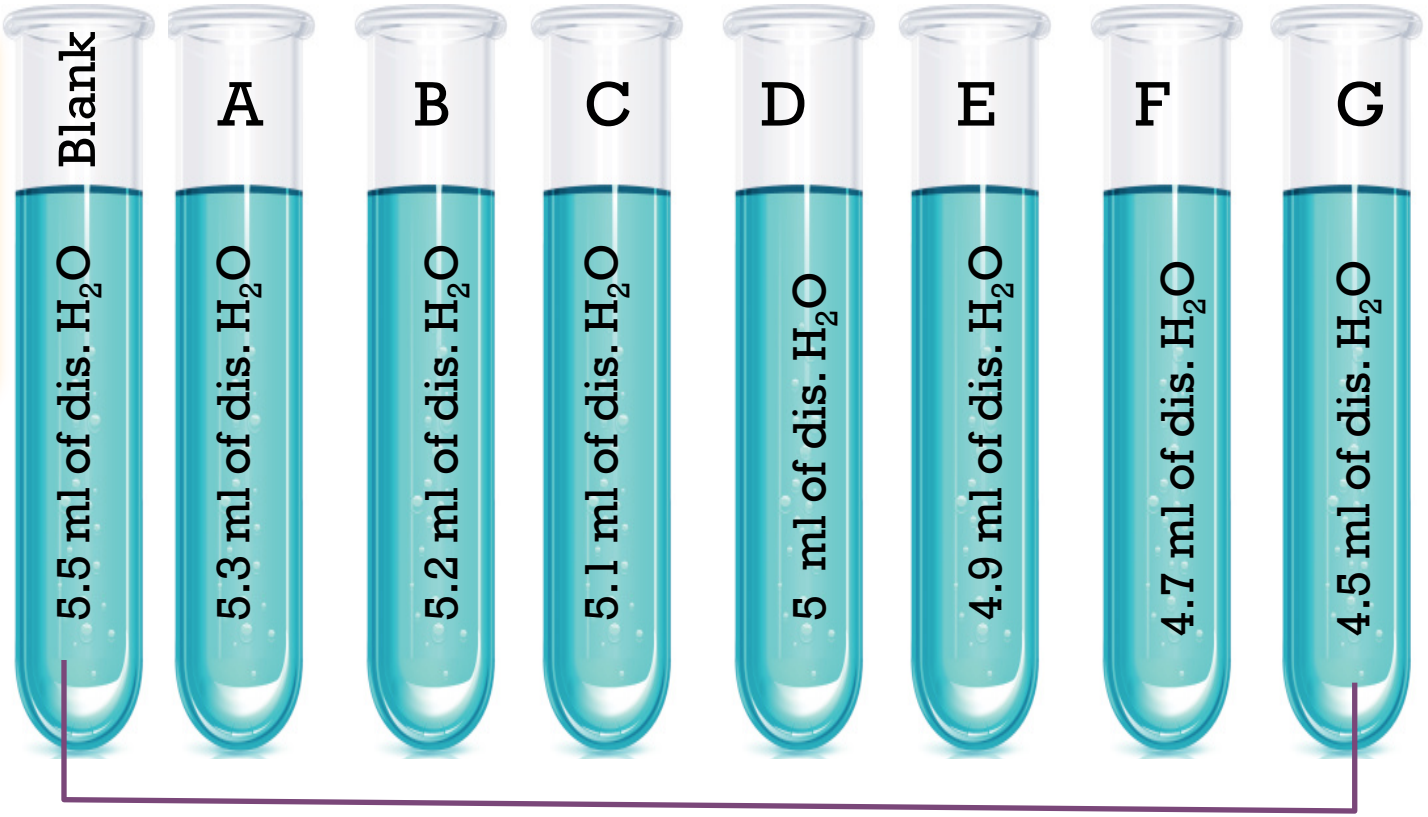
Method:

Add to each tube:

- 0.5 ml of buffer
- 0.5 ml of pNPP
- 0.5 ml $MgCl_2$

PS: Water volume will differ in each tube since each tube have [E].

Place in a water bath maintained at 37 °C for 5 minutes.



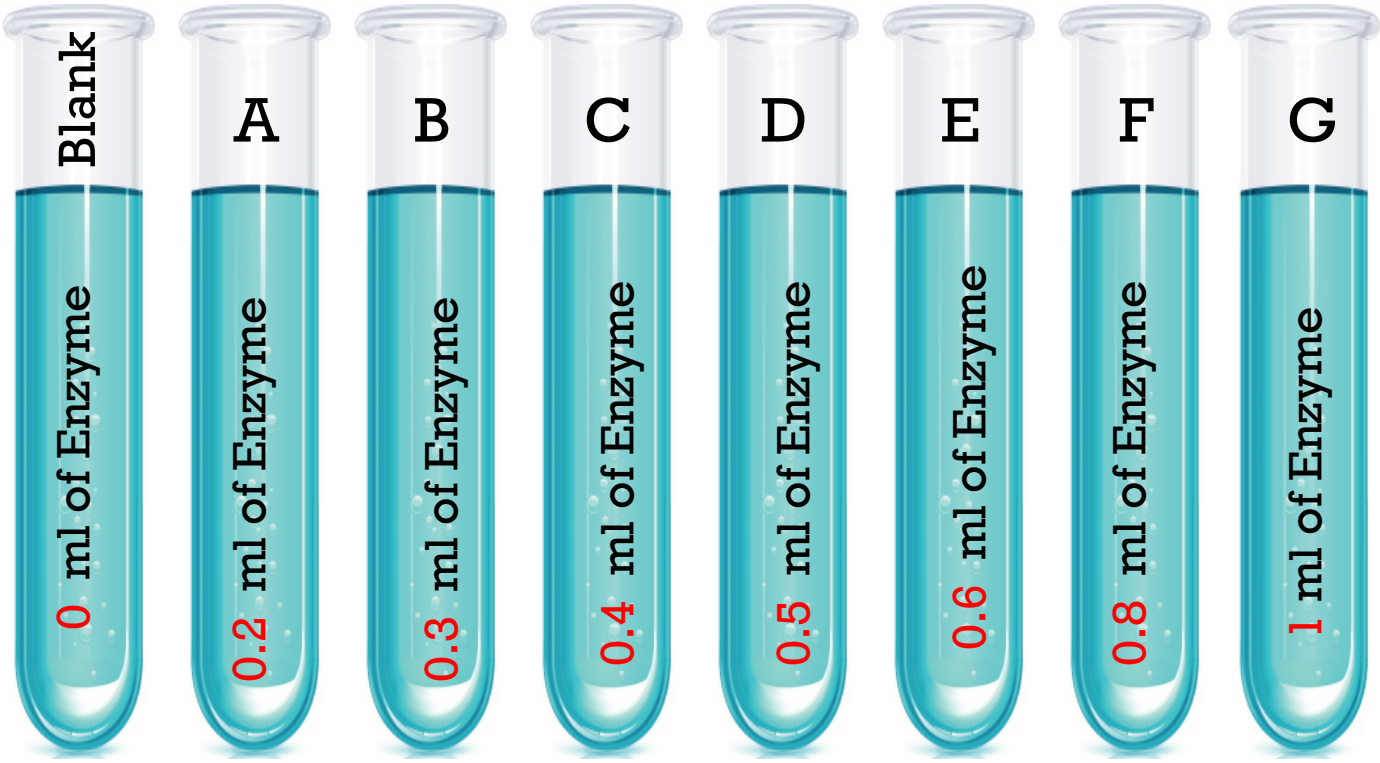
All the factors that affect enzyme kinetics are constant except ***enzyme concentration*** where it varies in each tube

Time = 5 min **pH= 5.7** **Temp= 37 °C** **[S] = 0.05M**

To start the reaction add the corresponding enzyme volume to each tube

To stop the reaction → add **0.5ml of KOH**

All additions f E and KOH must be in 37 ^{0}C water bath



Start at	**0**	**0**	**2**	**4**	**6**	**8**	**10**	**12**
Stop at	**0**	**5**	**7**	**9**	**11**	**13**	**15**	**17**

After all the reactions have been terminated, determine the absorbance at **405 nm** for each sample against blank.

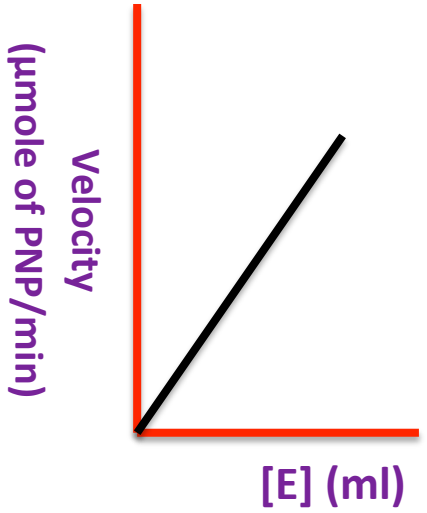
Tube	A	B	C	D	E	F	G
Start at	0	2	4	6	8	10	12
Stop at	5	7	9	11	13	15	17

Time (min)	Tube	Addition
0	A	Enzyme
2	B	Enzyme
4	C	Enzyme
5	A	KOH
6	D	Enzyme
7	B	KOH
8	E	Enzyme
9	C	KOH
10	F	Enzyme
11	D	KOH
12	G	Enzyme
13	E	KOH
15	F	KOH
17	G	KOH

To convert the time table to an easier way try the following

Results :

Tube	[E] (ml)	Absorbance 405 nm	Velocity (µmole of PNP/ min)
Blank	0		
A	0.2		
B	0.3		
C	0.4		
D	0.5		
E	0.6		
F	0.8		
G	1		



Velocity (V) = (A x 10^6) /(E x time)= **µmole of PNP/min**

E= extension coefficient=18.8 x 10^3 Time = 5 min

Discussion:

- An introductory statement (In this experiment, we studied the effect of different enzyme concentrations on the rate of acid phosphatase catalyzed reaction.)
- Principle
- Describe the shape of curve you get.
- Comment on the relationship between [E] and the rate of the reaction.

Question:

Assuming that there is a large excess of substrate. The rate of reaction will increase with increasing enzyme concentration. **WHY?**