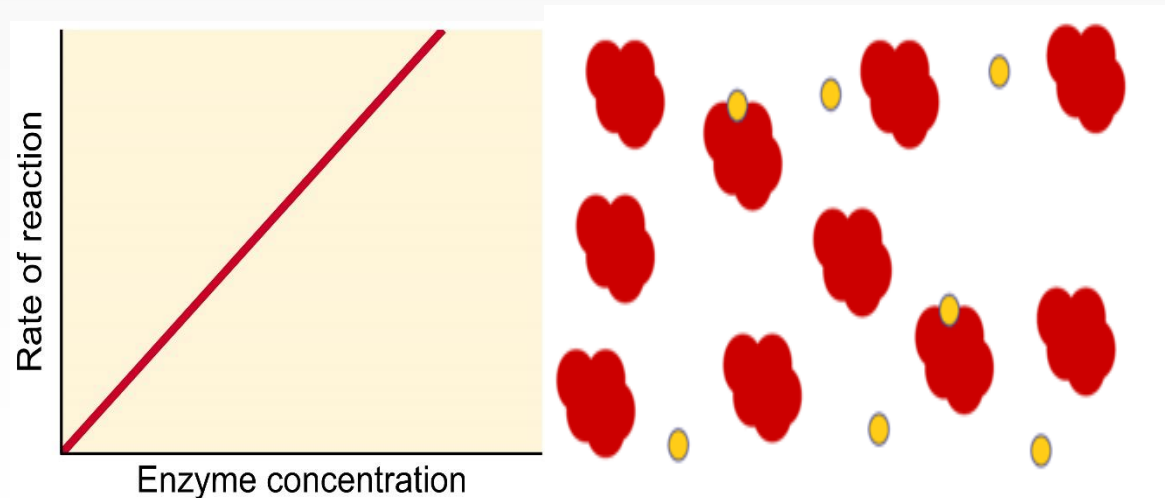
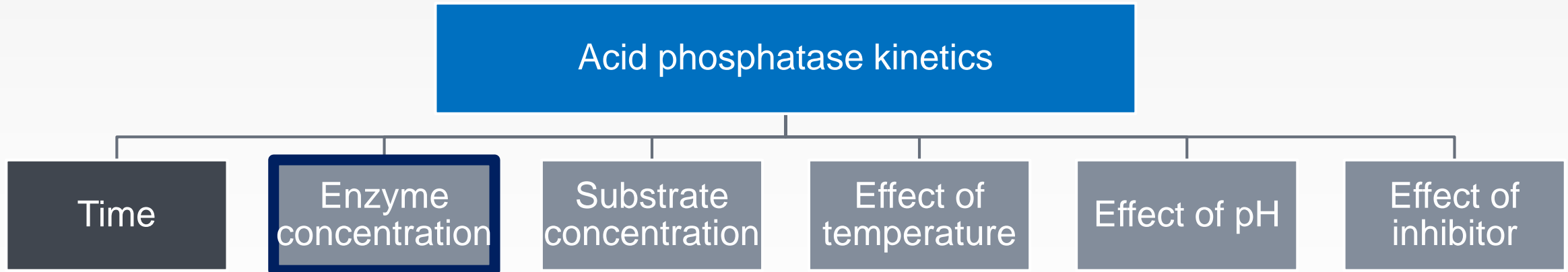


The Effect of Enzyme Concentration on the Rate of an Enzyme Catalyzed Reaction



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



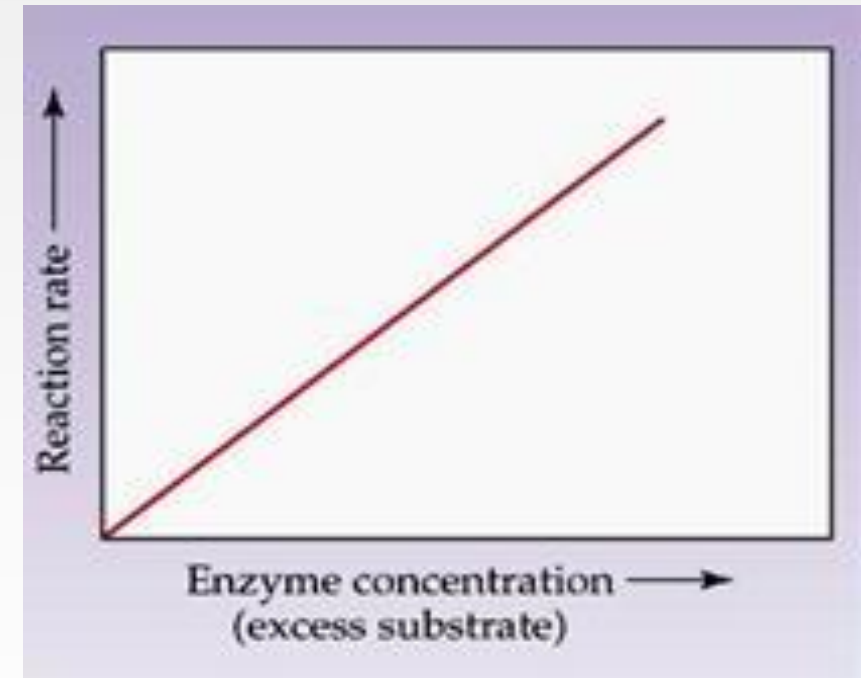


Objective:

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

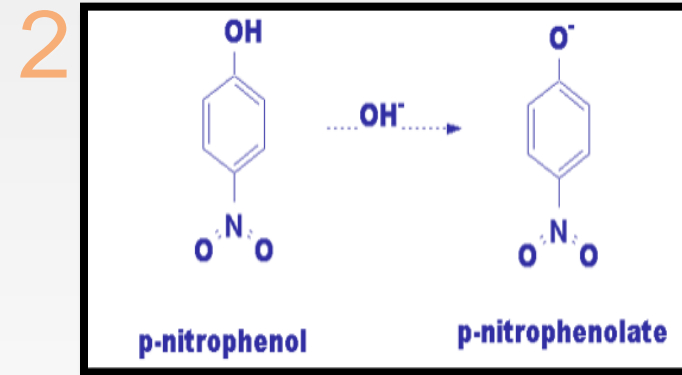
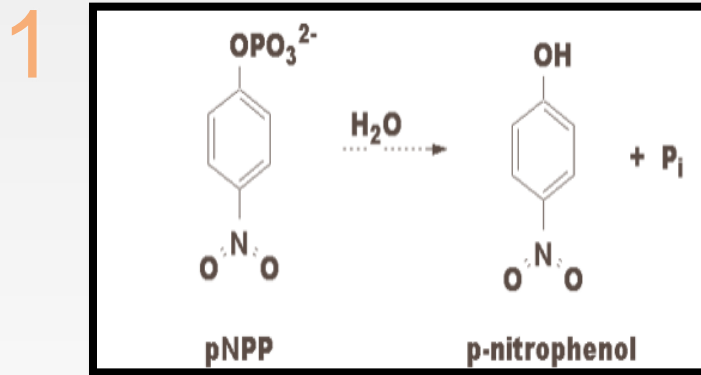
The effect of enzyme concentration on velocity

- The reaction rate will increase as the concentration of enzymes is increased but there must be **a large excess of substrate**
- **This is a linear relationship.**
- The **initial rate of reaction** is directly proportional to the enzyme concentration
- $V \propto [E]$



More Enzyme molecule can react with more substrate molecules, so the **initial rate** will increase.

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.
1. Both p-nitrophenyl phosphate and p-nitrophenol are colorless at **acidic pH** values. Under **alkaline conditions**, p-nitrophenol is converted to a **p-nitrophenolate** (yellow color) and concentration can be measured at 405 nm.

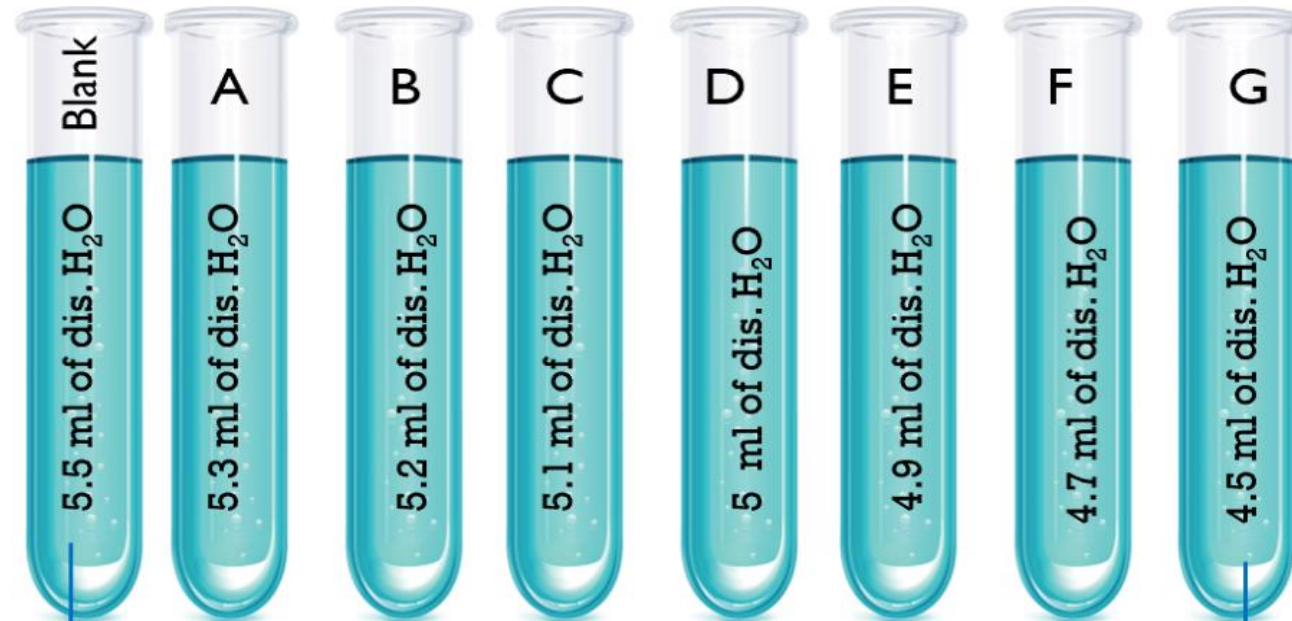
Method:

We want to see the effect of enzyme concentration on the velocity, so every tube will have different enzyme concentration (dilution)
Place in a water bath maintained at 37 °C for 5 minutes.

Add to each tube:

- 0.5 ml of buffer
- 0.5 ml of pNPP
- 0.5 ml MgCl_2
- Water

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



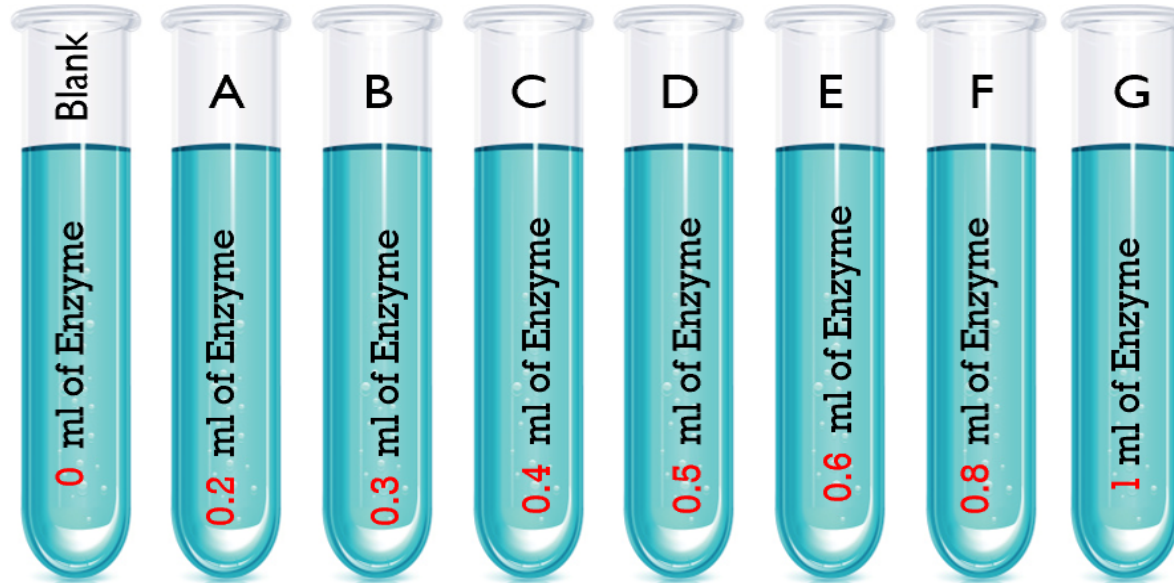
All the factors that affect enzyme kinetics are constant except **enzyme concentration** where it varies in each tube

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 of **E** and **KOH** must be in 37 °C water bath
- Note that the blank do not contain any enzyme



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 200
2	B	Enzyme 300
4	C	Enzyme 400
5	A	KOH
6	D	Enzyme 500
7	B	KOH
8	E	Enzyme 600
9	C	KOH
10	F	Enzyme 800
11	D	KOH
12	G	Enzyme 1000
13	E	KOH
15	F	KOH
17	G	KOH



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



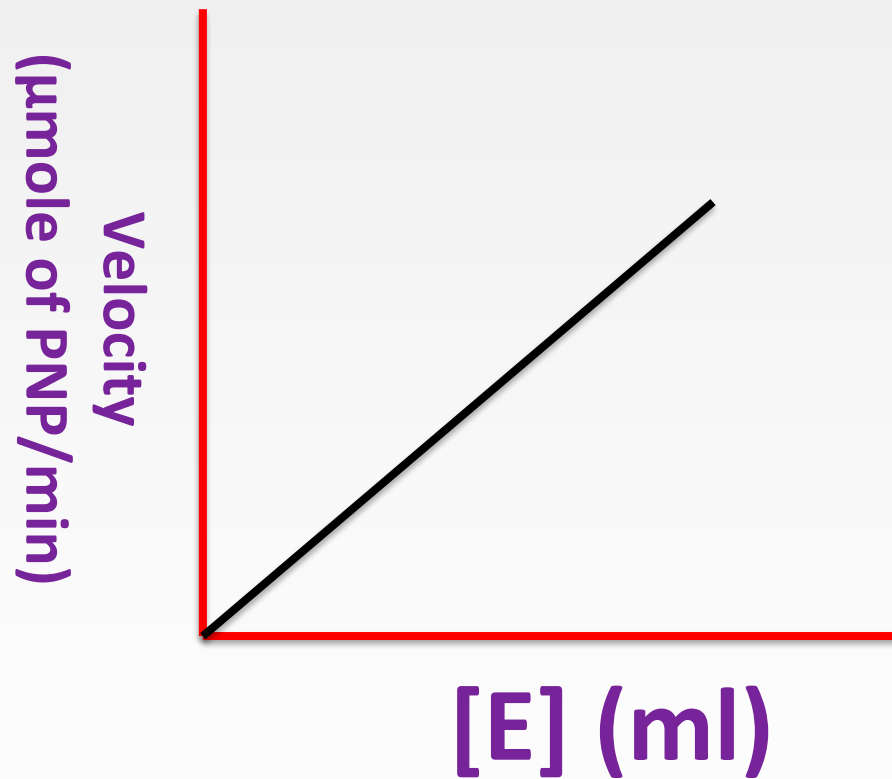
Results :

Tube	Enzyme (ml)	Absorbance 405 nm	Velocity (μ mole 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		

Calculations

- **Velocity (V) = $(A \times 10^6) / (E \times \text{time}) =$ $\mu\text{mole of PNP/min}$**
- A= absorbance
- E= extension coefficient= 18.8×10^3
- Time = 5 min

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



“Linear curve”

Discussion:

- An introductory statement
- Describe the shape of curve you get. WHY?
- Comment on the relationship between $[E]$ and the rate of the reaction.

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

