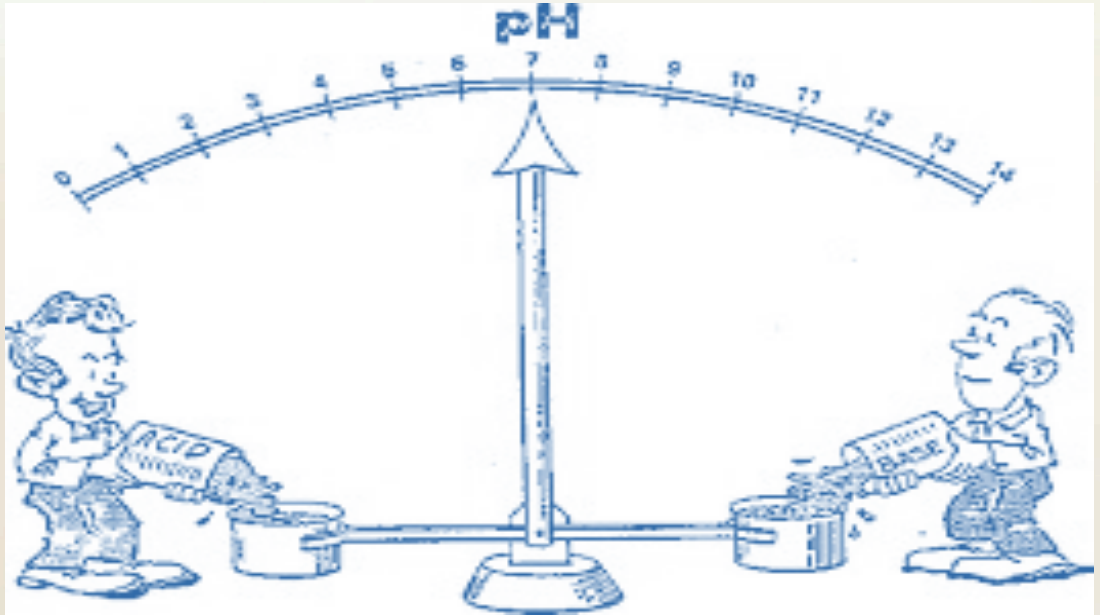
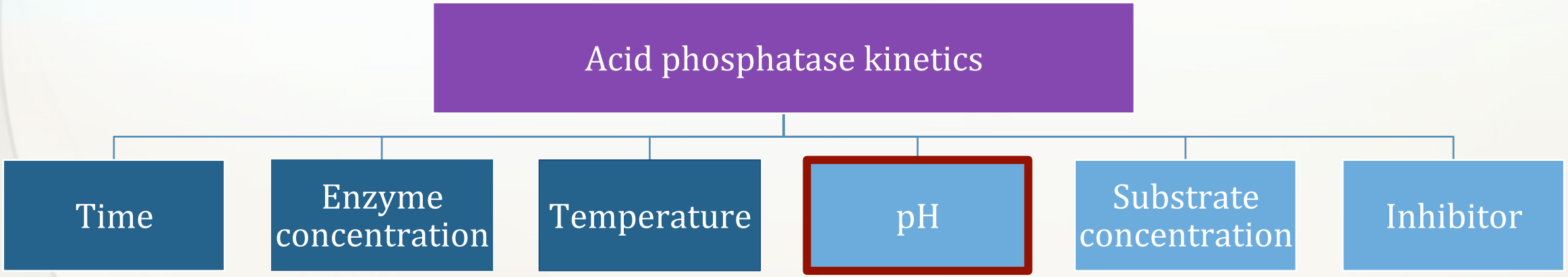
322 BCH

Exp (6)

The effect of pH on the rate of an enzyme catalyzed reaction



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



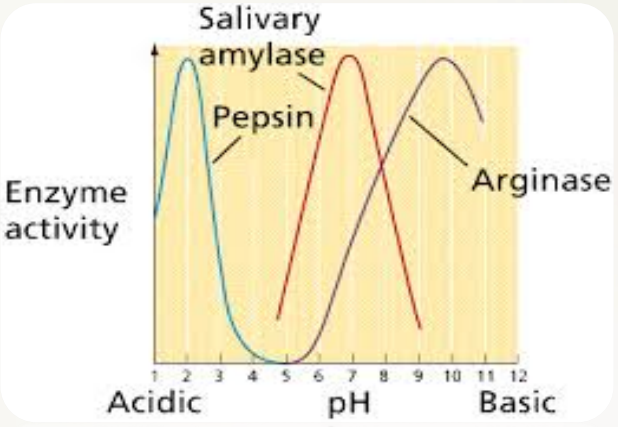
Objectives

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

- To determine the optimum pH for such a reaction.

The Effect of pH on Enzymes

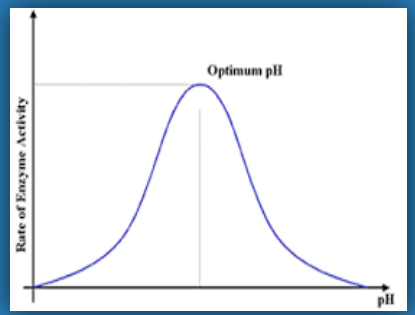
- The rate of enzymatic reaction depends on pH of the medium.
- Each enzyme has an **optimum pH**, where the rate of enzymatic reaction is maximum.
- At **higher** or **lower** pH, the rate of an enzymatic reaction *decreases.*
- For most enzymes, the optimum pH lies in the range from ***pH 5 to pH 9.***
  - With some exceptions, pepsin's optimum pH is extremely acidic.
  - And arginase's optimum pH is extremely basic.



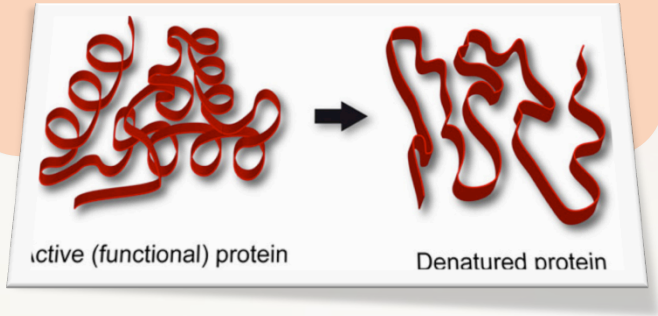
The relationship between the rate of an enzymatic reaction and pH takes form of a *bell-shape.*

Change in pH effects the charged state of the substrate or amino acid residues in the active site of the enzyme, hence affecting the rate of the reaction

Bell-Shaped curve

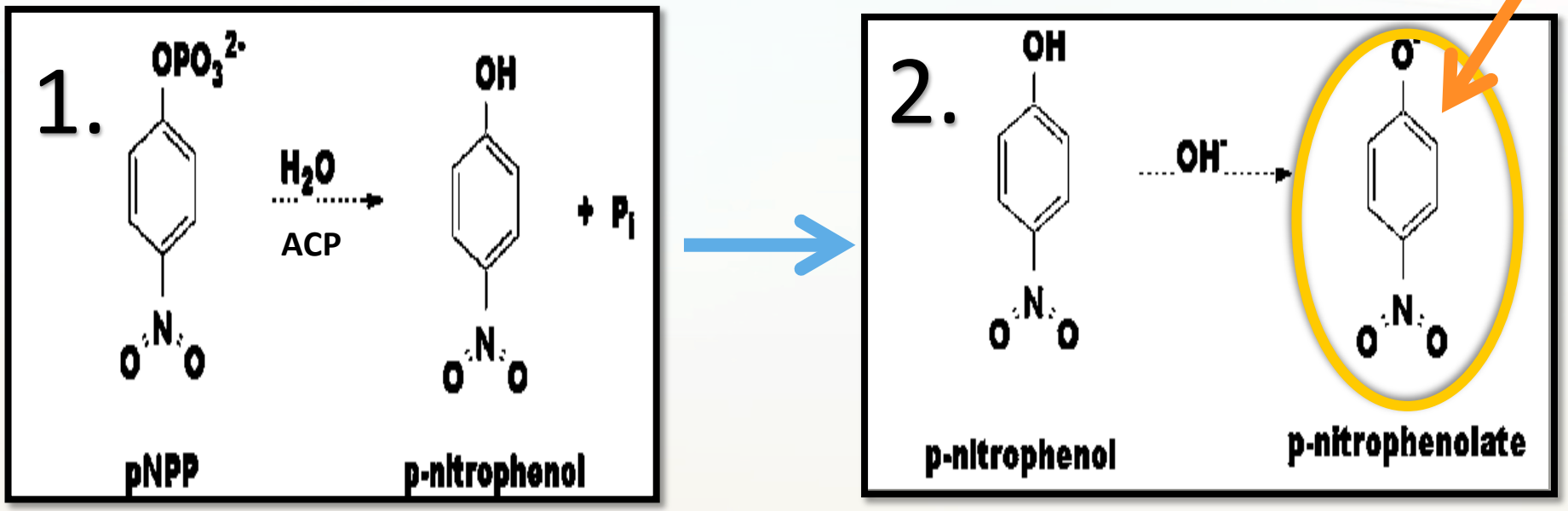


Enzyme denaturation at extremely high or low pH



Principal

Its concentration can be measured at **405 nm.**

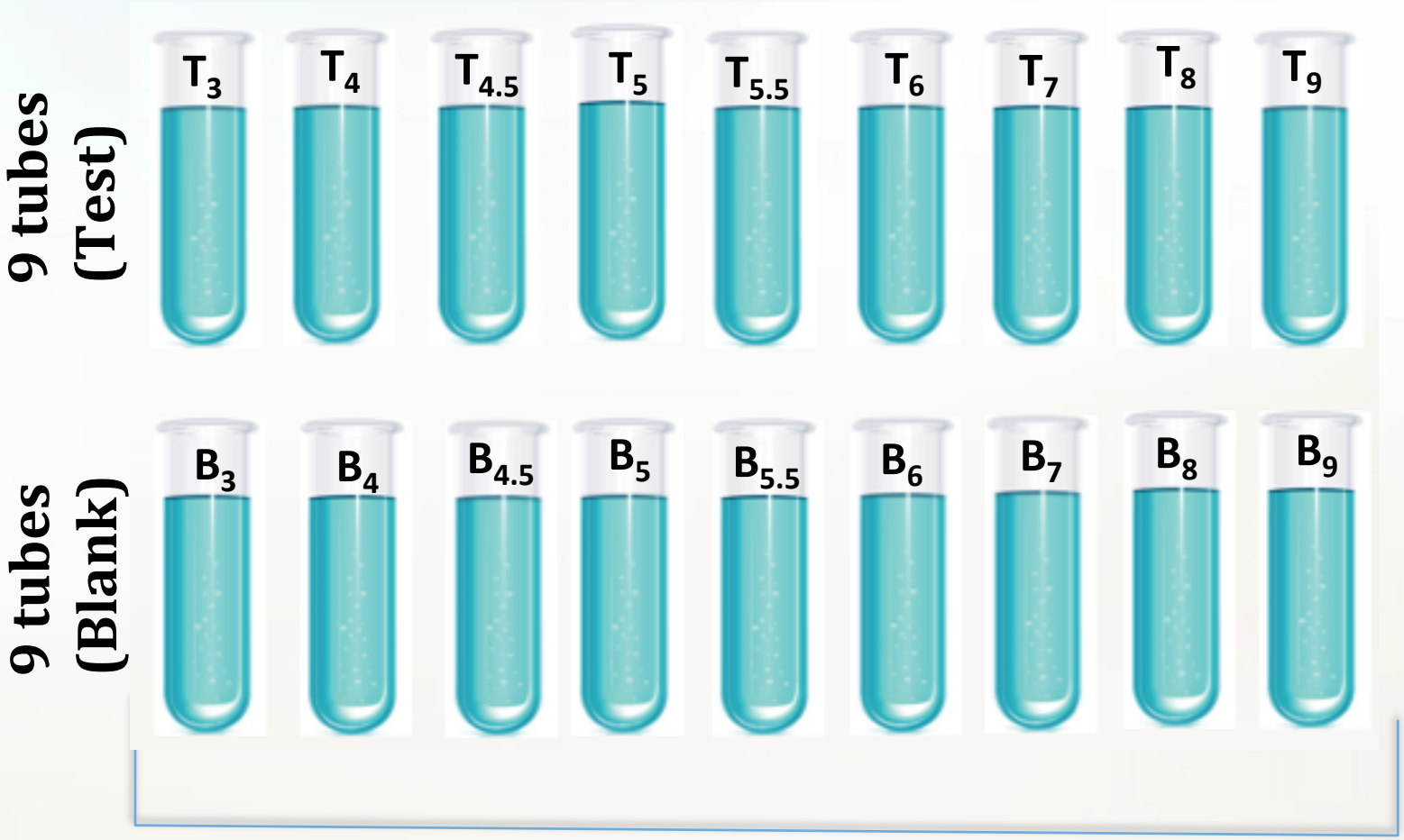


Method:

Add to each tube (Test and Blank):

- 0.5 ml of Corresponding pH buffer
- 0.5 ml of pNPP
- 0.5 ml $MgCl_2$
- 5 ml water

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

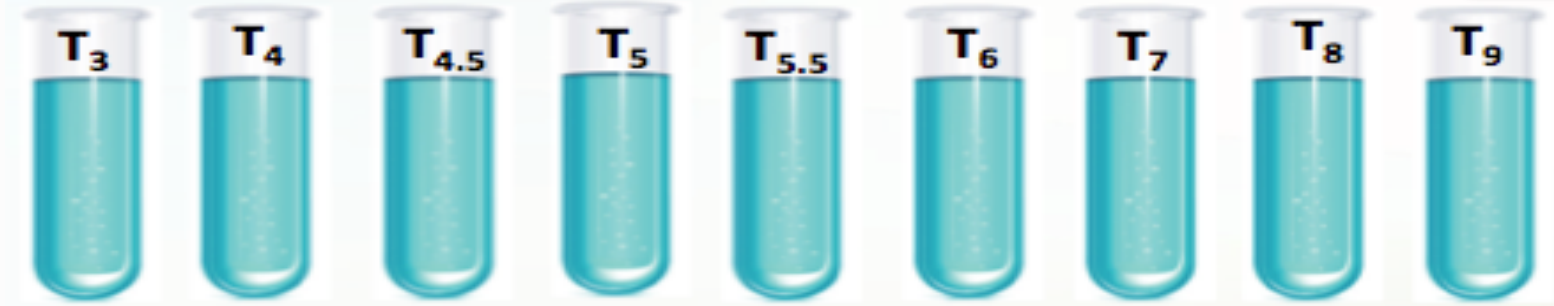


All the factors that affect enzyme kinetics are constant except ***pH*** where it varies in each tube

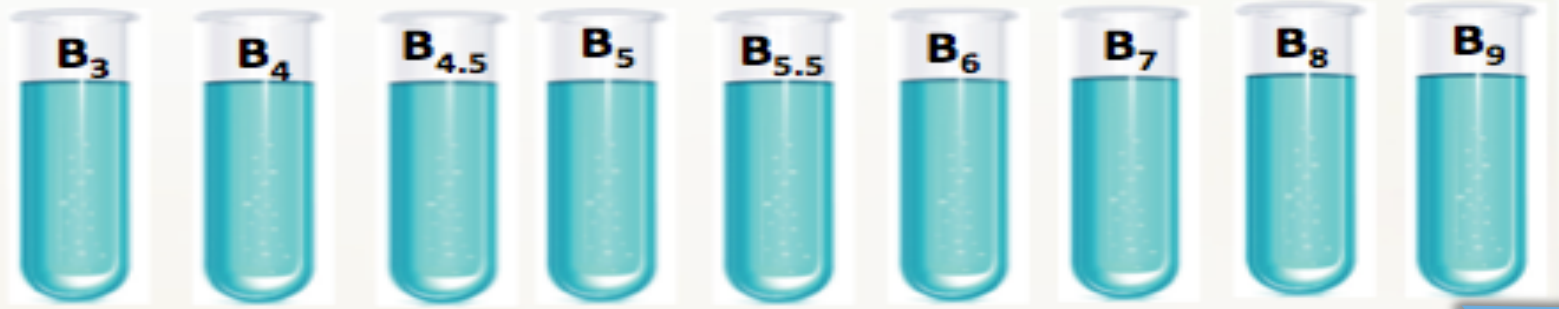
Time = 5 min Temp= 37 °C [S] = 0.05M

To start the reaction add add **0.5ml of enzyme**

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



Start at	0	2	4	6	8	10	12	14	16
Stop at	5	7	9	11	13	15	17	19	21



Keep in a water bath maintained at 37 ºC for other 5 minutes.

For Blank tubes: Add KOH ((FIRST)) then E, **to prevent the reaction from happening.**

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

Results

pH	Absorbance 405 nm	Velocity (µmole of PNP/min)
3		
4		
4.5		
5		
5.5		
6		
7		
8		
9		

Plot a graph illustrating the effect of different pHs on the rate of the reaction.

Calculations:

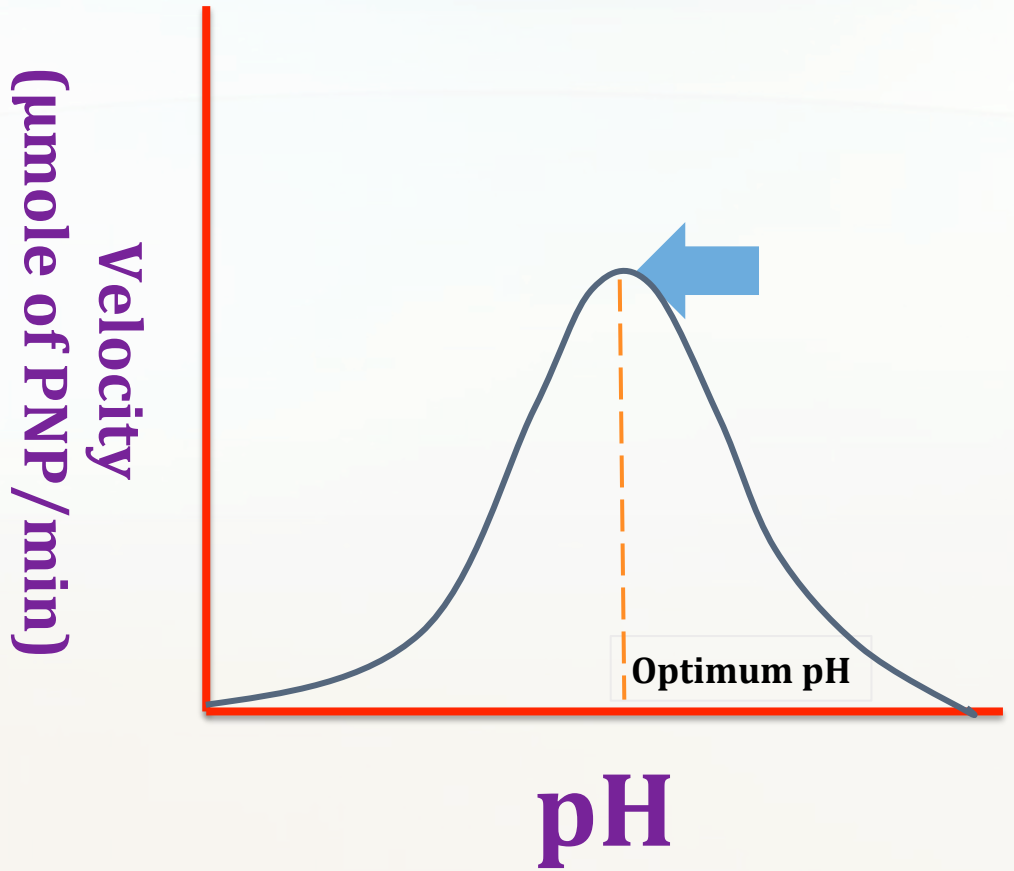
Velocity (V) = $(A \times 10^6) / (E \times \text{time}) =$ **µmole of PNP/min**

A= absorbance

E= extension coefficient=18.8×10^3

Time = 5 min

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



Bell- shape curve

Discussion

- An introductory statement (In this experiment, we studied the effect of different pH on the rate of acid phosphatase catalyzed reaction.)
- Principle
- From the curve, explain and discuss the relationship between the activity of acid phosphatase and pH.
- Define the optimum pH and determine which buffer is the best from the curve.