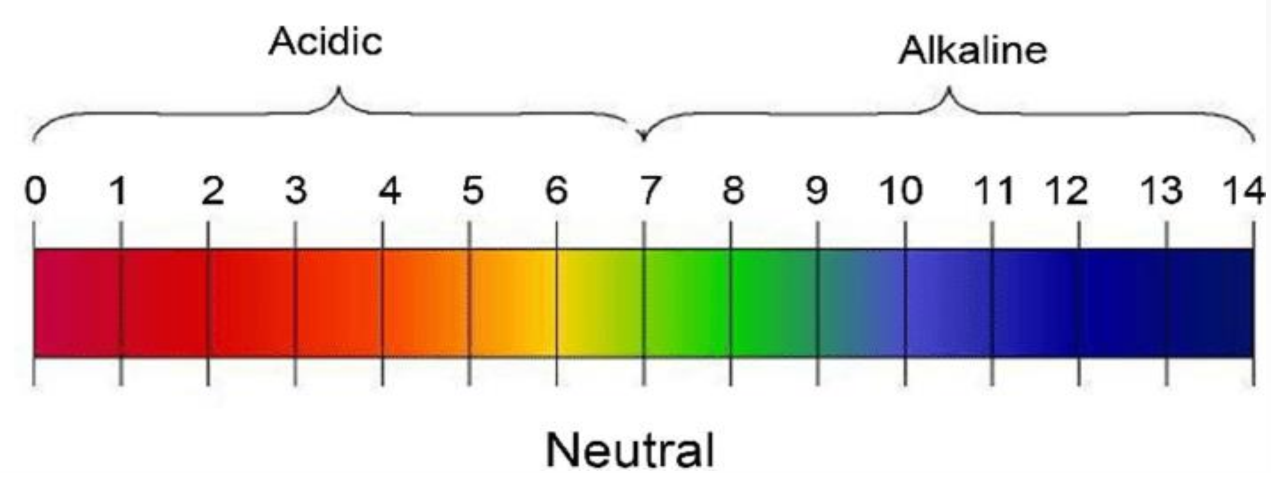
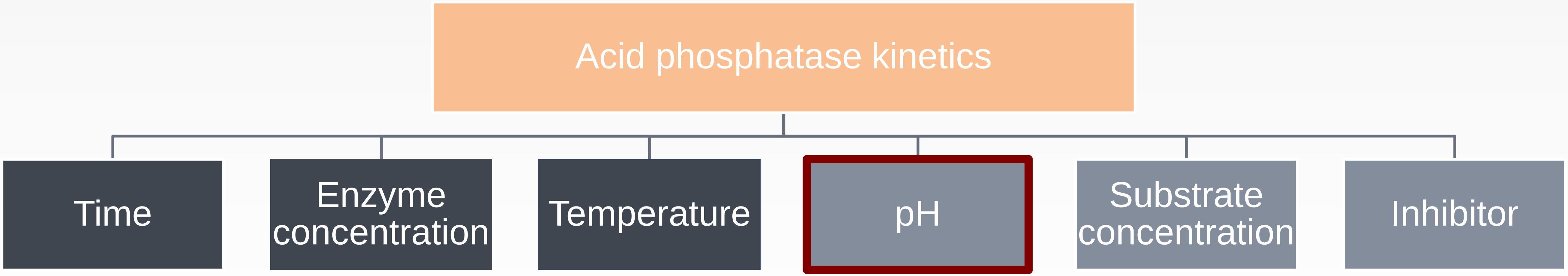
The effect of pH on the rate of an enzyme catalyzed reaction



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

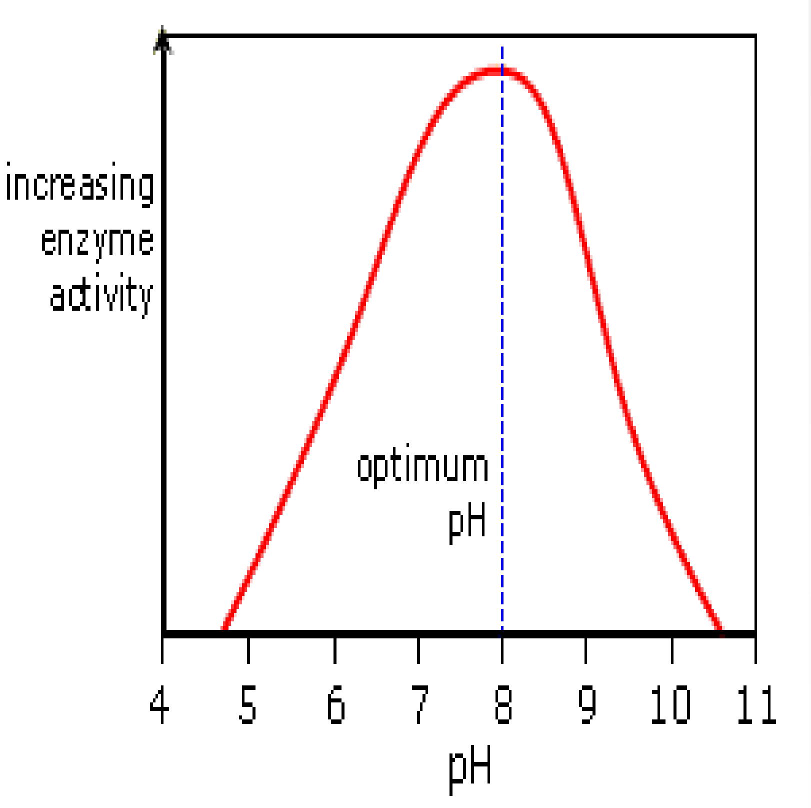


Objectives

- 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 enzyme activity

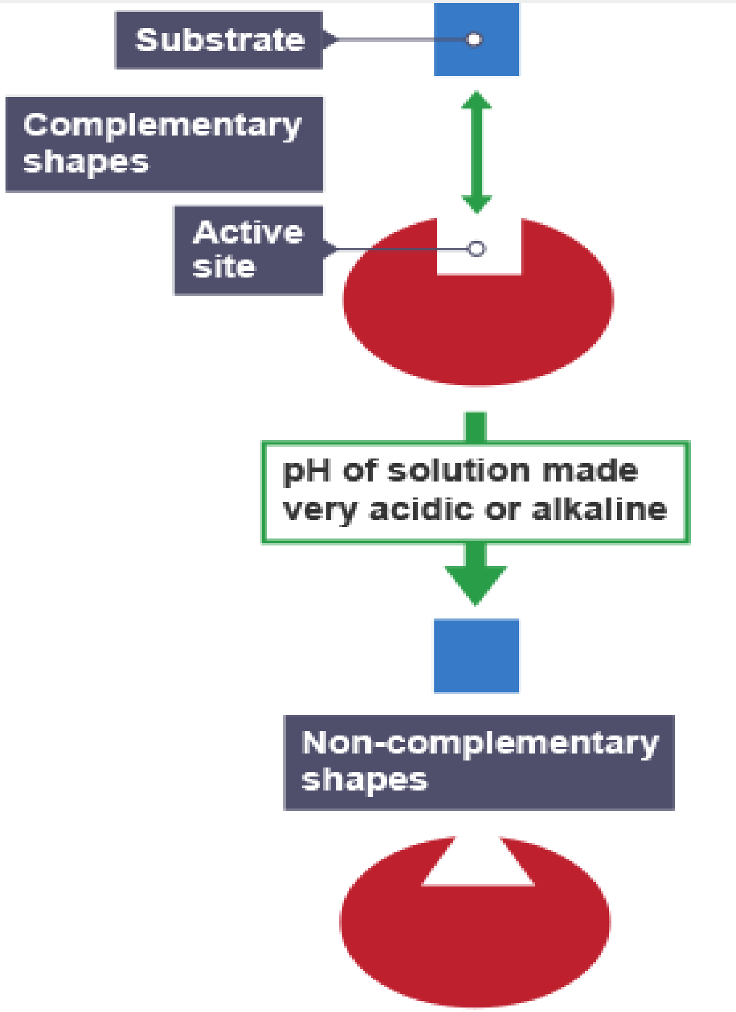
- The rate of enzymatic reaction depends on pH of the medium.
- Each enzyme have a pH where the enzyme is most active – which is known as the **optimum pH**.
- For most enzymes, the optimum pH lies in the range from pH 5 to pH 9.
- The optimum pH for an enzyme depends on where it normally works.
- **Extremely high or low pH** values generally result in complete loss of activity for most enzymes.



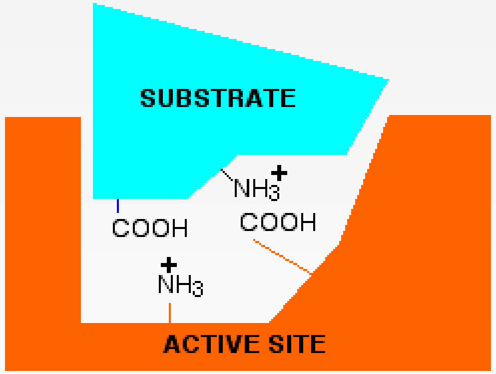
The effect of pH on enzyme activity

- Changes in pH **alter an enzyme's shape**. This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.

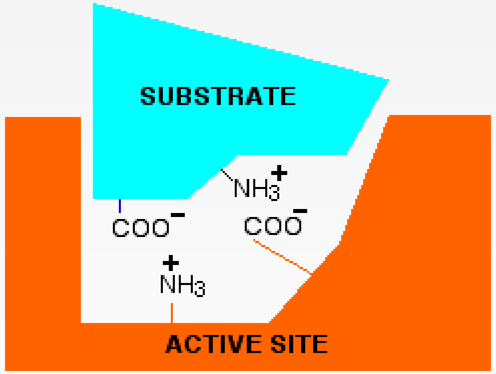
- pH can have an effect of the **state of ionization** of the ionization states of the amino acid residues involved in the catalytic activity of the enzyme(**active site**)



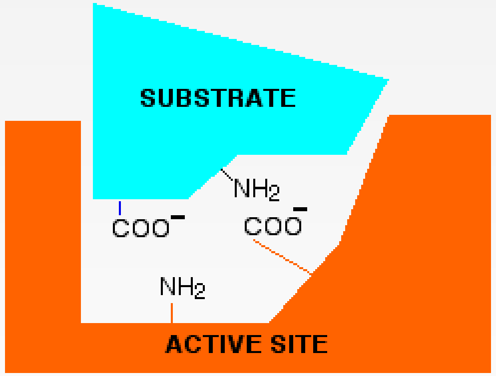
Changing pH will affect amino acid charges in active sites



Low pH



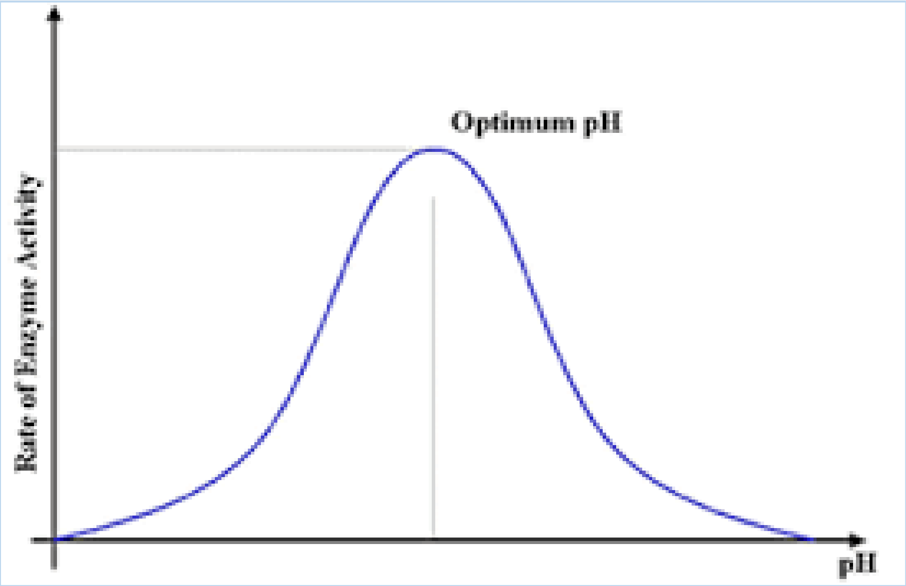
Neutral pH



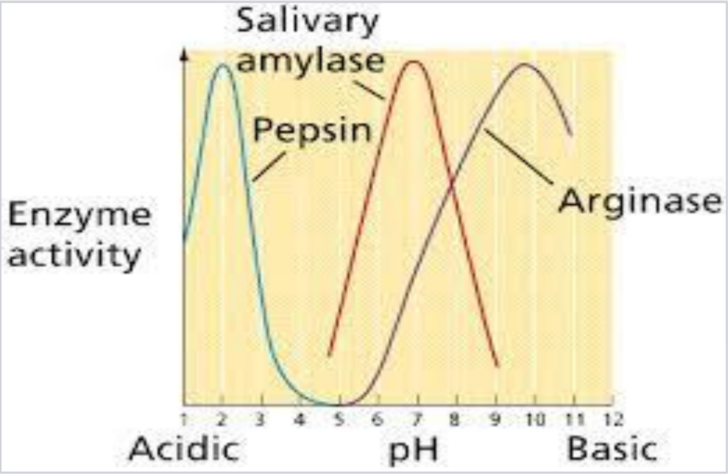
High pH

The shape of pH activity curve

- For the majority of enzymes, the relationship between the rate of an enzymatic reaction and pH takes form of a **bell-shape**.



The shape of pH activity curve



The shape of pH activity curve is determined by the following factors:

- *Enzyme denaturation at extremely high or low pH:*
  - With some exceptions, pepsin's optimum pH is extremely acidic.
  - And arginase's optimum pH is extremely basic.

- *Effects on the charged state of the substrate or enzyme:*
  - Most enzymatic reactions require both the substrate and the amino acid residues in the active site of the enzyme to have a **specific charge state**.
- → Changes in pH <u>change this charge state</u> and hence <u>affect the rate of the reaction</u>.

Principle

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

- If base (KOH) is added to the mixture after the completion of the reaction, the p-nitrophenol is converted to a **yellow colored** form which absorbs lights at 405 nm.

Method

In order to detect the effect of pH you must fix all the component except the 1 M sodium acetate buffer of different pH.	
Time (5 minutes)	constant
Enzyme concentration	constant
Substrate concentration (0.05M)	constant
Temperature (37˚C)	constant
pH	Variable **(pH 3, 4, 4.5, 5, 5.5, 6, 7, 8)**

- Prepare 16 tubes labeled as follows (note: there is a blank for each pH)
  - Blank: (B_3, B_4, $B_{4.5}$, B_5, $B_{5.5}$, B_6, B_7, $B_{8,}$ B_9)
  - Test: (T_3, T_4, $T_{4.5}$, T_5, $T_{5.5}$, T_6, T_7, $T_{8,}$ T_9)
- To each of these tubes add

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

- Place the tubes in a test tube rack situated in 37^{o}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
all Blanks	0 min	0 min
T_3	0 min	5 min
T_4	2 min	7 min
$T_{4.5}$	4 min	9min
T_5	6 min	11 min
$T_{5.5}$	8 min	13 min
T_6	10 min	15 min
T_7	12 min	17 min
T_8	14 min	19 min
T9	16 min	21 min

- ***Notes:***
- In blank tube add KOH first then the enzyme, to prevent the reaction from happening.

Results

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

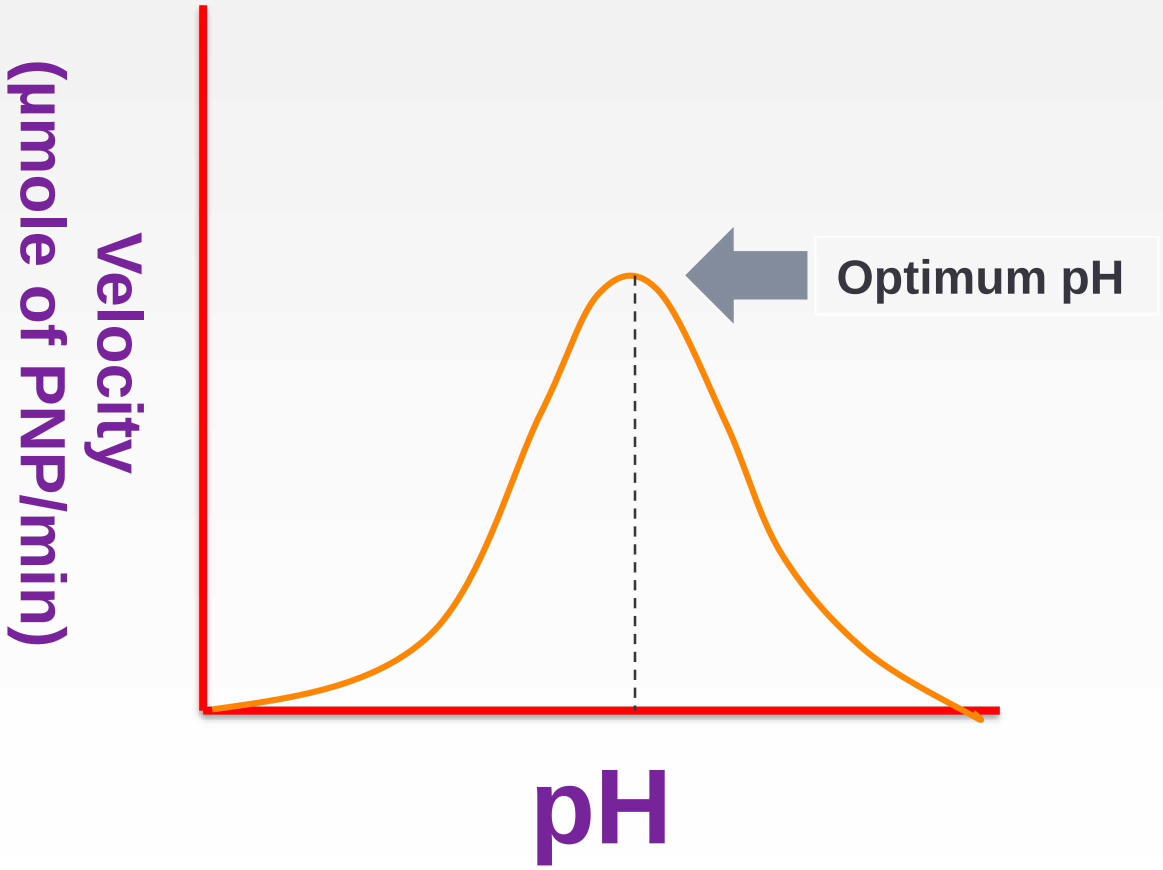
Plot a graph illustrating the effect of different pHs on the rate of the reaction.

Calculations:

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

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

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



Discussion

- An introductory statement
- Principle
- From the curve, explain and discuss the shape of the curve and the relationship between the activity of acid phosphatase and pH.
- Define the optimum pH and determine which buffer is the best from the curve.