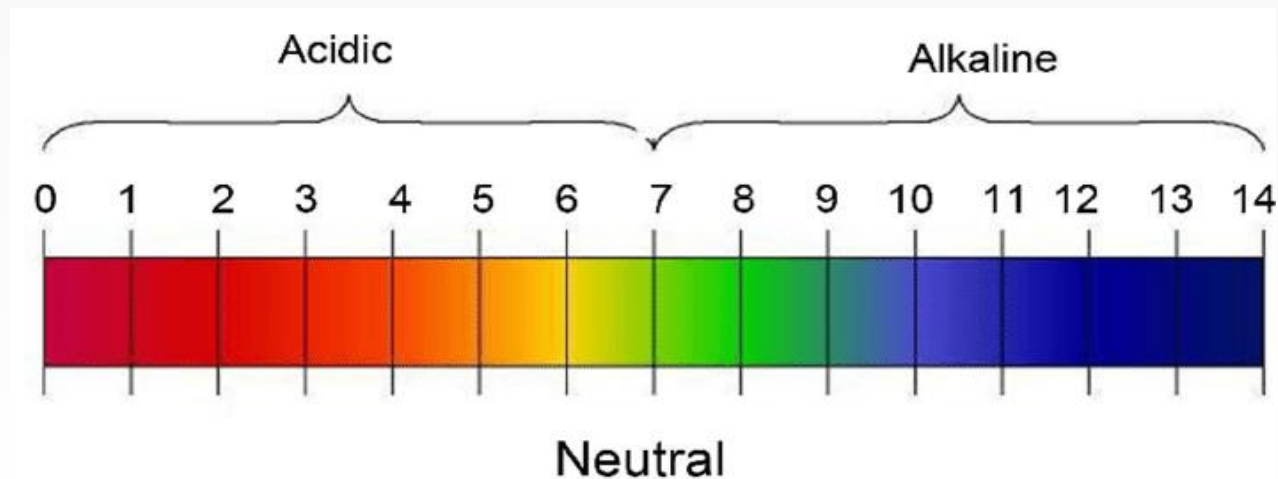
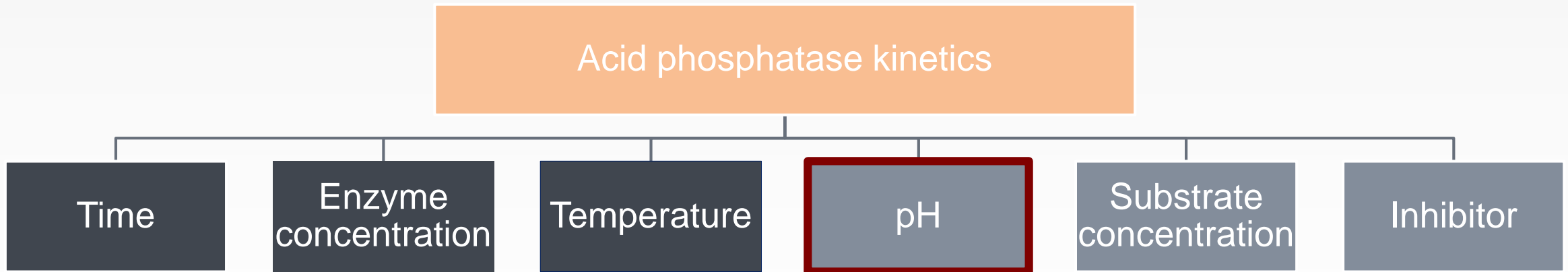


# The effect of pH on the rate of an enzyme catalyzed reaction



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



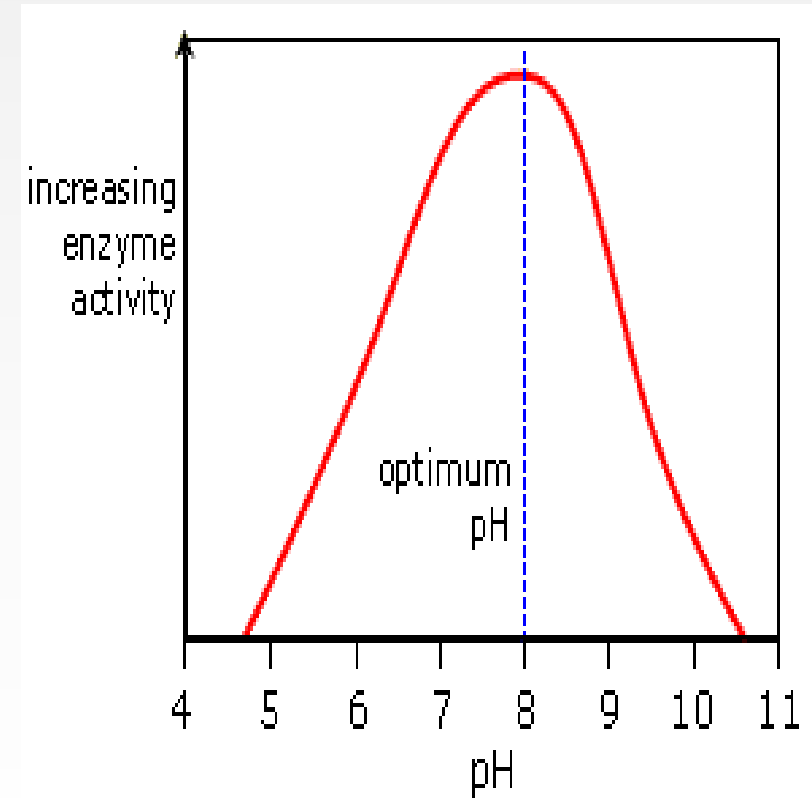


# Objectives

- To establish the relationship between pH and the rate of an enzyme catalyzed reaction.
- To determine the optimum pH for such a reaction.

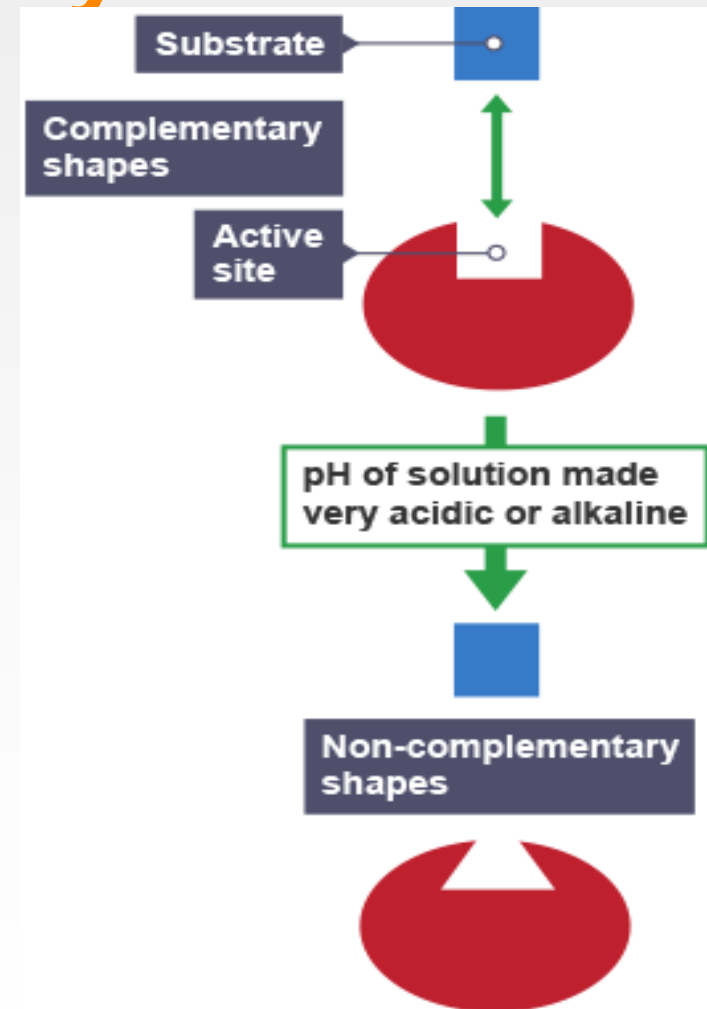
# The effect of pH on enzyme activity

- 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.

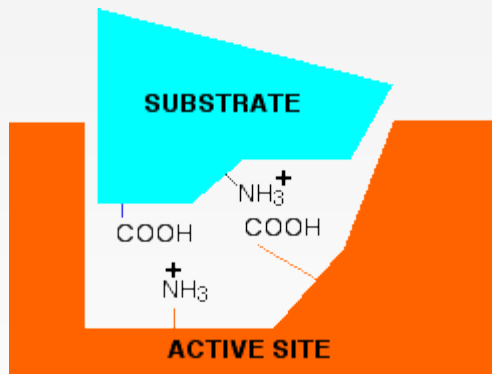


# The effect of pH on enzyme activity

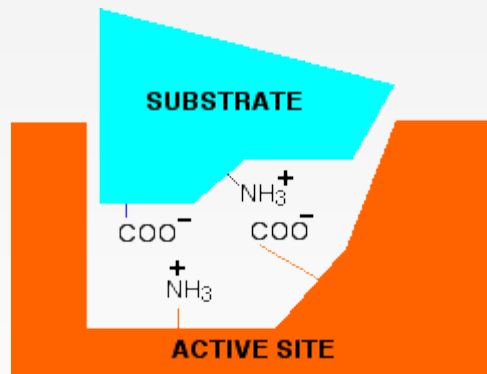
- Changes in pH **alter an enzyme's shape**. This is because changes in pH can make and break intra- and intermolecular bonds, changing the shape of the enzyme and, therefore, its effectiveness.
- pH can have an effect of the **state of ionization** of the ionization states of the amino acid residues involved in the catalytic activity of the enzyme (**active site**)



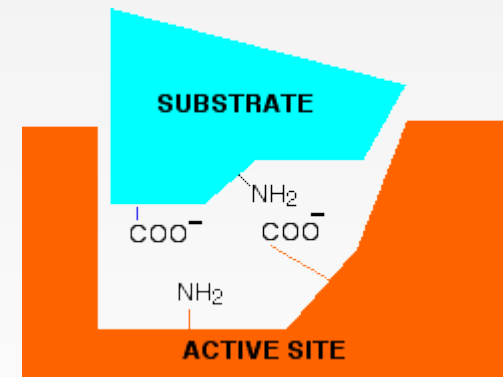
# Changing pH will affect amino acid charges in active sites



Low pH



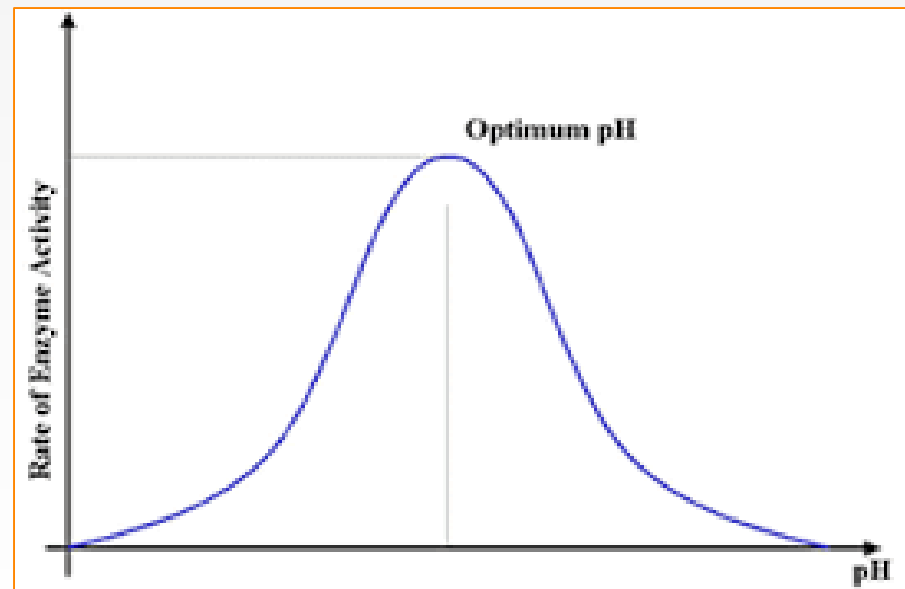
Neutral pH



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**.



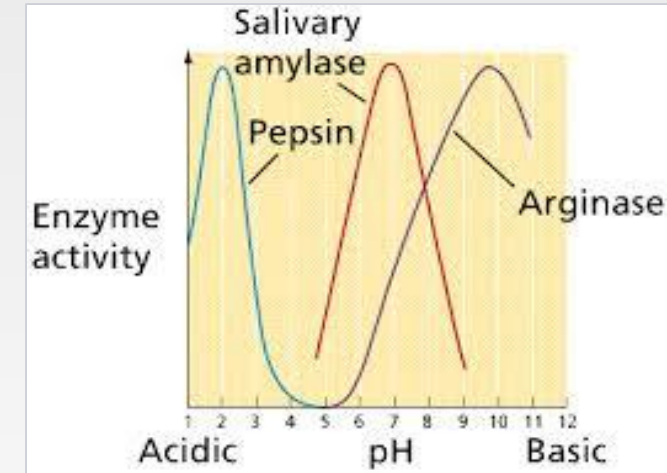
The shape of pH activity curve is determined by the following factors:

- *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:*

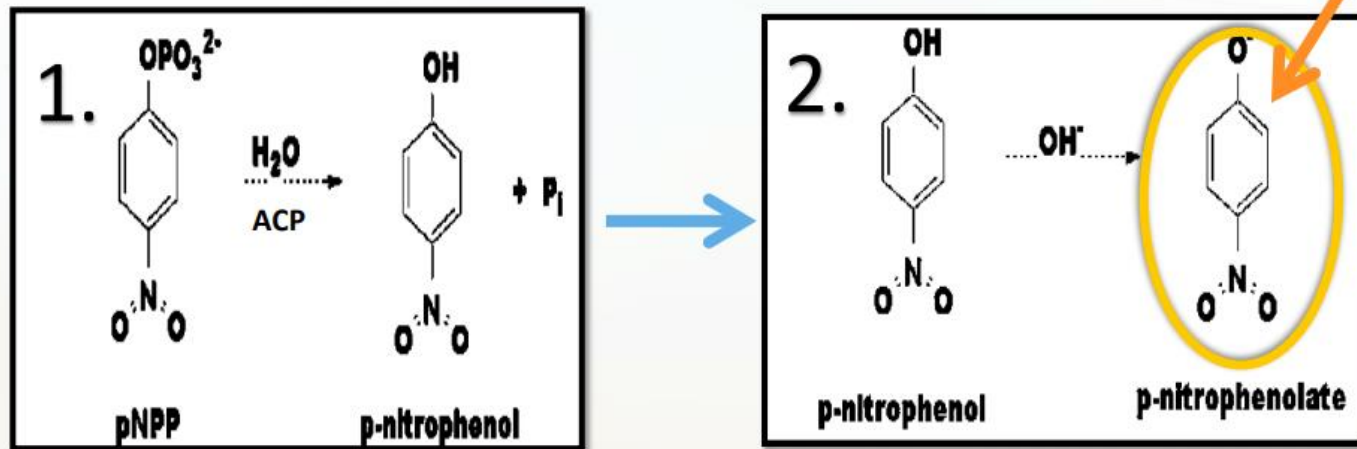
- Most enzymatic reactions require both the substrate and the amino acid residues in the active site of the enzyme to have a **specific charge state**.
- → Changes in pH change this charge state and hence affect the rate of the reaction.





# Principal

Its concentration can be measured 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, 4.5, 5.5, 7, 9)

# Method

- Prepare 16 tubes labeled as follows (note: there is a blank for each pH)
  - Blank: (B<sub>3</sub>, B<sub>4.5</sub>, B<sub>5.5</sub>, B<sub>7</sub>, B<sub>9</sub>)
  - Test: (T<sub>3</sub>, T<sub>4.5</sub>, T<sub>5.5</sub>, T<sub>7</sub>, T<sub>9</sub>)
- To each of these tubes add

<b>Chemical</b>	<b>Volume (ml)</b>
Corresponding pH sodium acetate buffer	0.5
0.1M MgCl <sub>2</sub>	0.5
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
all Blanks	0 min	0 min
T <sub>3</sub>	0 min	5 min
T <sub>4.5</sub>	2 min	7 min
T <sub>5.5</sub>	4 min	9min
T <sub>7</sub>	6 min	11 min
T <sub>9</sub>	8 min	13 min

- Notes:**

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

# Results

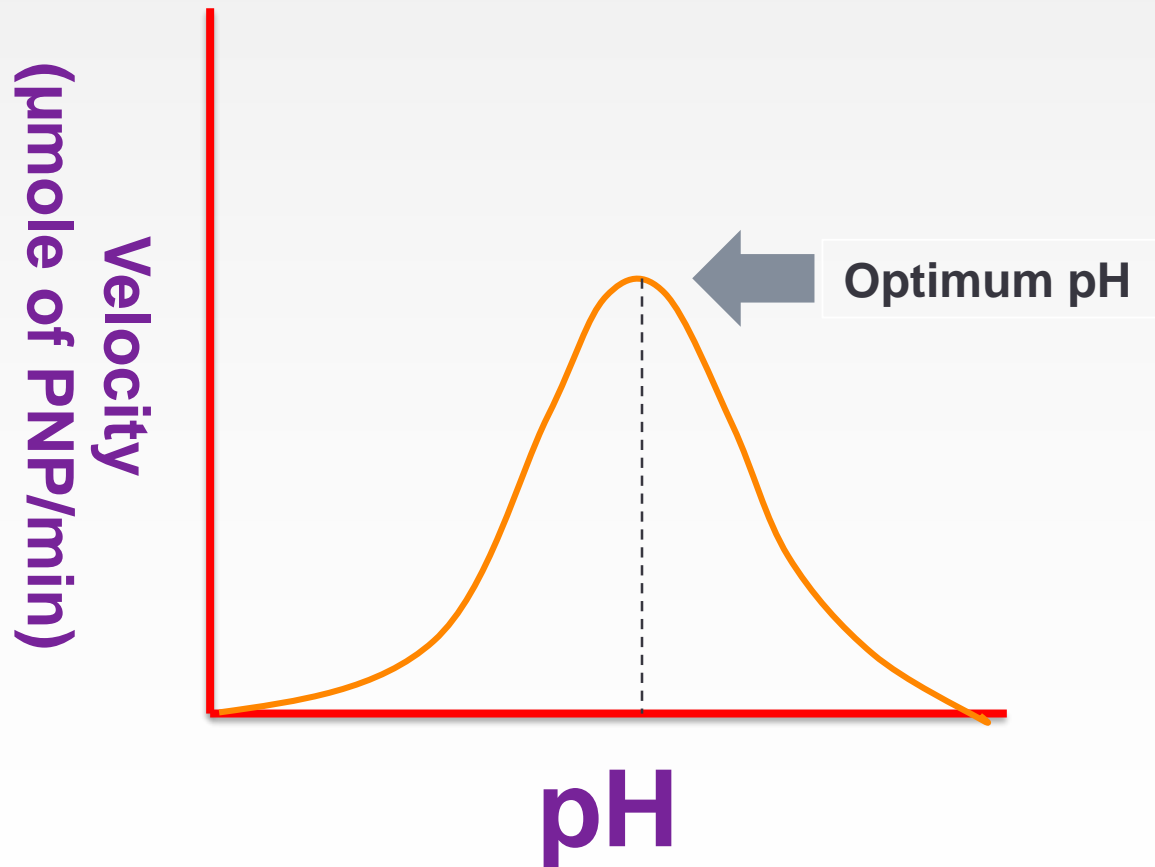
pH	Absorbance 405 nm	Velocity ( $\mu$ mole of PNP/min)
3		
4.5		
5.5		
7		
9		

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

# Calculations:

- **Velocity (V) =  $(A \times 10^6) / (E \times \text{time}) =$   $\mu\text{mole of PNP/min}$**
- A= absorbance
- E= extension coefficient= $18.8 \times 10^3$
- 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 pH is the best from the curve.