THE EFFECT OF PH ON THE RATE OF AN ENZYME CATALYZED REACTION

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Enzymes have an optimum pH or pH range in which their activity is maximal.

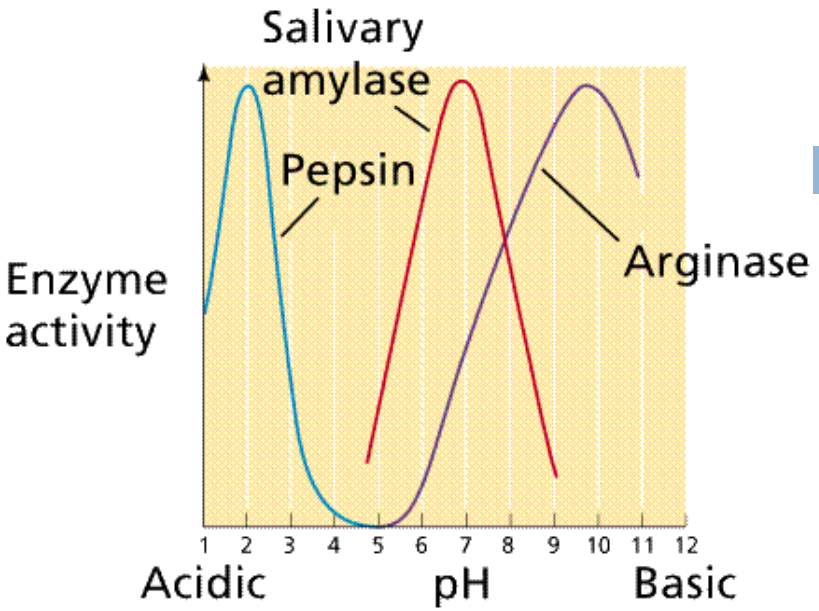
Optimum pH: **is the pH at which the rate of reaction is maximum.**

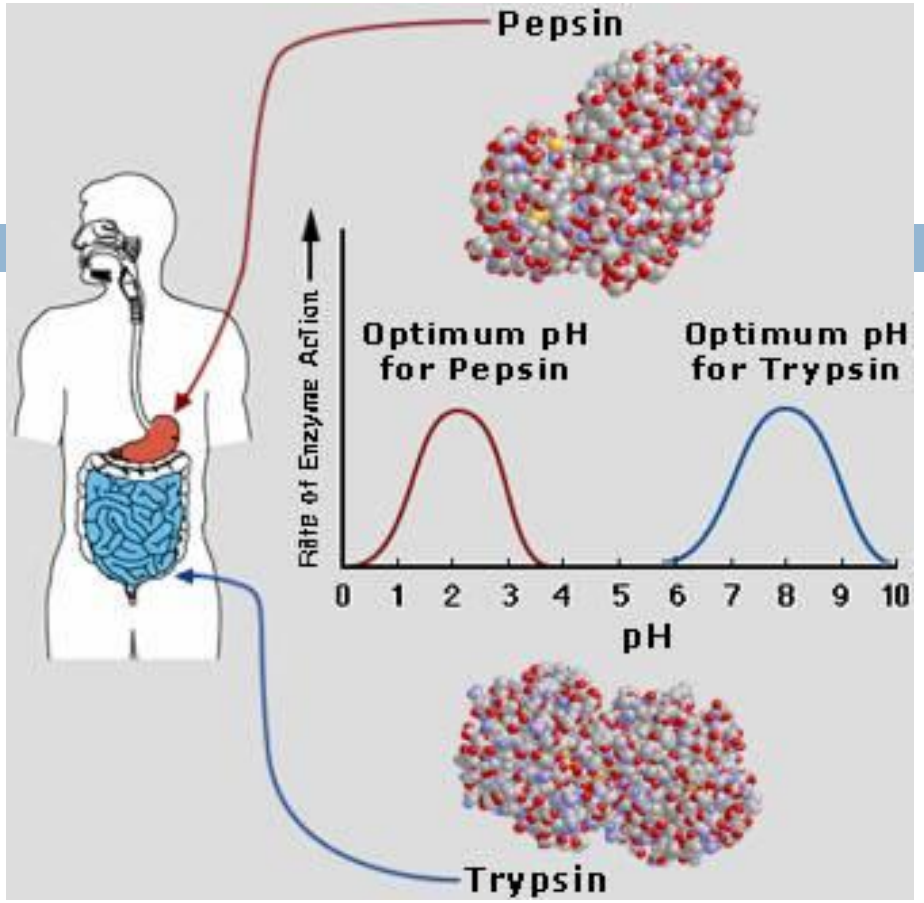
At **higher or lower** pH, the rate of an enzymatic reaction **decrease**.

For most enzymes, the optimum pH lies in the range from pH 5 to pH 9

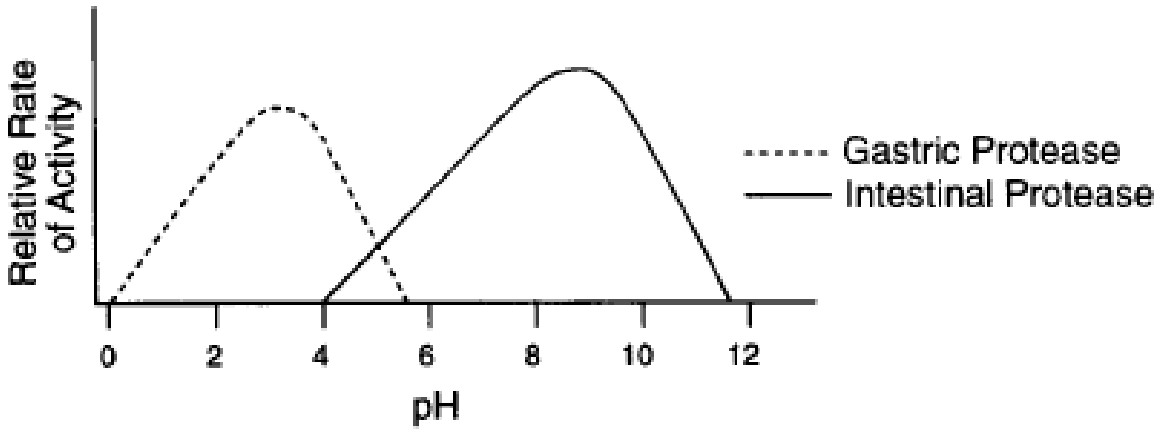
The shape of pH activity curve is determined by the following factors:

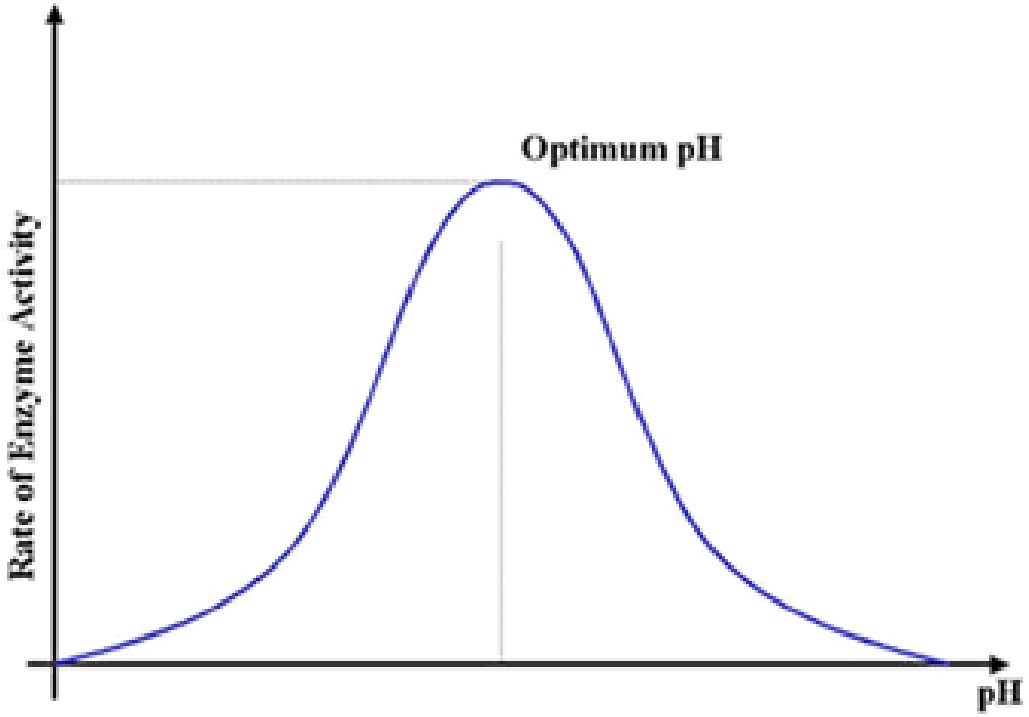
- Enzyme denaturation at extremely high or low pH
- Effects on the charged state of the substrate or enzyme.





Different Enzymes with Different optimum PH





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

Objectives:

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

b) To determine the optimum pH for such a reaction.

Materials:

- Test tubes
- Pipettes
- Cuvettes
- Water bath
- Stopwatch
- Spectrophotometer
- Acid phosphatase enzyme
- 0.05 M PNPP
- 1 M sodium acetate buffer of different PH **(3,4,4.5,5,5.5,6,7,8)**
- 0.1 M MgCl2
- 0.1M KOH

Method:

- Prepare 16 tubes labeled as follows:
- (A_3 (blank), A_4, $A_{4.5}$, A_5, $A_{5.5}$, A_6, A_7, A_8)
- ($B_{3,}$ B_4, $B_{4.5}$, B_5, $B_{5.5}$, B_6, B_7, B_8).
- To each of these tubes add:
- 0.5ml of correspondingly pH sodium acetate buffer (note: there is a blank for each pH)
- 0.5ml of MgCl2
- 0.5ml of p-nitrophenolphosphate
- 5ml of distilled water

- Place the tube in a test tube rack situated in 37°C water bath and let stand for 5 minutes.
- Initiate each assay at **2-minute intervals** by adding 0.5ml of the enzyme,
- run each reaction for 5 minutes, and stop it by adding 0.5ml of KOH. (Note: for the blank, the substrate is not added)
- 5. Determine the absorbance at 405 nm of each experimental tube against its own blank (tube A).

2-minute intervals adding

Tube no.	Start the reaction (min.) by Enzyme	Stop the reaction (min.) by add KOH 0.5 ml
all Blanks	0 min	0 min
B3	0 min	5 min
B4	2 min	7 min
B4.5	4 min	9min
B5	6 min	11 min
B5.5	8 min	13 min
B6	10 min	15 min
B7	12 min	17 min
B8	14 min	19 min

Results:

pH	Absorbance at 405 nm	Velocity (μ moles of P-NP/minute)
3		
4		
4.5		
5		
5.5		
6		
7		
8		

- Convert the absorbance data to velocity data.
- Plot a graph illustrating the effect of different pH on the rate of the reaction. (PH against rate of reaction)

Discussion

Establish the relationship between pH and the rate of an enzyme catalyzed reaction, and determine which buffer is the best (the optimum pH)for acid phosphatase enzyme

Thank You