



Factors Affecting Enzyme Activity

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Introduction:

Enzymes are essential proteins that facilitate and accelerate biochemical reactions within biological systems. Their role in metabolic processes is fundamental, as they act as catalysts, lowering the activation energy required for reactions to occur. Enzymes are highly specific and sensitive to various environmental conditions, including temperature, pH, substrate concentration, and enzyme concentration. This sensitivity allows enzymes to exhibit optimal activity within specific ranges of these factors. One such enzyme of interest is catalase (peroxidase), which plays a crucial role in the breakdown of hydrogen peroxide into water and oxygen. This report explores the effects of enzyme concentration on the rate of enzyme activity and aims to shed light on the relationship between enzyme concentration and reaction rate.

In this laboratory investigation, we focus on the enzyme catalase, also known as peroxidase, and its role within the context of a turnip cell. Catalase is an enzyme widely found in living organisms, including plants, animals, and microorganisms. Its primary function is to catalyze the decomposition of hydrogen peroxide (H_2O_2), a harmful byproduct of metabolic processes, into water and oxygen. This enzymatic action is crucial in detoxifying cells and preventing oxidative damage.

The aim of this study is to explore the impact of various factors on the activity of catalase, shedding light on how its performance may be modulated under different conditions. By delving into the intricate world of enzyme kinetics and the behavior of catalase, we hope to unravel the nuances of its enzymatic activity in the context of temperature, pH, and enzyme concentration.

To substantiate the information presented herein, we draw upon a comprehensive body of scientific research and references, which serve as the foundation for our understanding of enzyme activity and its modulation by different factors. These references, both primary research articles and reputable secondary sources, lend credibility and authority to the insights presented in this study. They underscore the significance of comprehending the factors affecting enzyme activity, not only for academic purposes but also for applications in various fields, including medicine, agriculture, and biotechnology.

In the subsequent sections of this report, we will delve into the methods employed, present the experimental results, and engage in a thoughtful discussion of the implications of our findings, all with the

aim of enhancing our comprehension of the factors that influence enzyme activity, particularly in the context of catalase within a turnip cell.

Methods:

The experiment involved studying the impact of enzyme concentration on the rate of enzyme activity. Three test tubes, labeled #1, #2 and #3, were prepared. Tube #1 contained a lower concentration of the enzyme, while tube #2 held a higher concentration. The experiment involved measuring the absorbance of the enzyme reaction at specific time intervals (20 and 100 seconds).

To investigate the influence of various factors on the activity of catalase in a turnip cell, a series of controlled experiments were conducted. The procedures detailed below outline the methods followed during these experiments, with sufficient detail for reproducibility.

Temperature Variation Experiment:

Preparation of Turnip Extract: Fresh turnips were selected and thoroughly cleaned. Small pieces of turnip tissue were then homogenized to obtain a cell extract containing catalase. The extract was stored on ice to maintain its enzymatic activity.

Incubation at Different Temperatures: Multiple tubes containing equal volumes of turnip extract were prepared. Each tube represented a specific temperature condition. These temperatures included 20°C, 50°C, and 80°C. The tubes were incubated in water baths set to their respective temperatures for a predetermined time.

Introduction of Hydrogen Peroxide: Following incubation, hydrogen peroxide (H₂O₂) was added to each tube to initiate the reaction. The concentration of H₂O₂ was kept constant across all tubes.

Measurement of Reaction Rate: The rate of the reaction was monitored by measuring the release of oxygen gas over a specified time period. Gas collection tubes connected to the reaction tubes allowed for the quantification of oxygen produced. The time elapsed for the appearance of a certain volume of gas was recorded for each temperature condition.

pH Variation Experiment:

Preparation of Turnip Extract: Similar to the temperature experiment, turnip extract was prepared as described earlier.

Adjustment of pH: A set of tubes was prepared, each with turnip extract at a specific pH level. The pH conditions chosen ranged from acidic (pH 3) to neutral (pH 7) and slightly alkaline (pH 9). These pH levels were achieved by adding appropriate acid (HCl) or base (NaOH) solutions.

Introduction of Hydrogen Peroxide: Hydrogen peroxide was introduced into each tube to initiate the catalase reaction. The concentration of H₂O₂ was maintained consistently.

Monitoring Reaction Rate: The rate of the reaction was observed by measuring the release of oxygen gas as described in the temperature variation experiment. The time taken for the appearance of a specified volume of gas was noted for each pH condition.

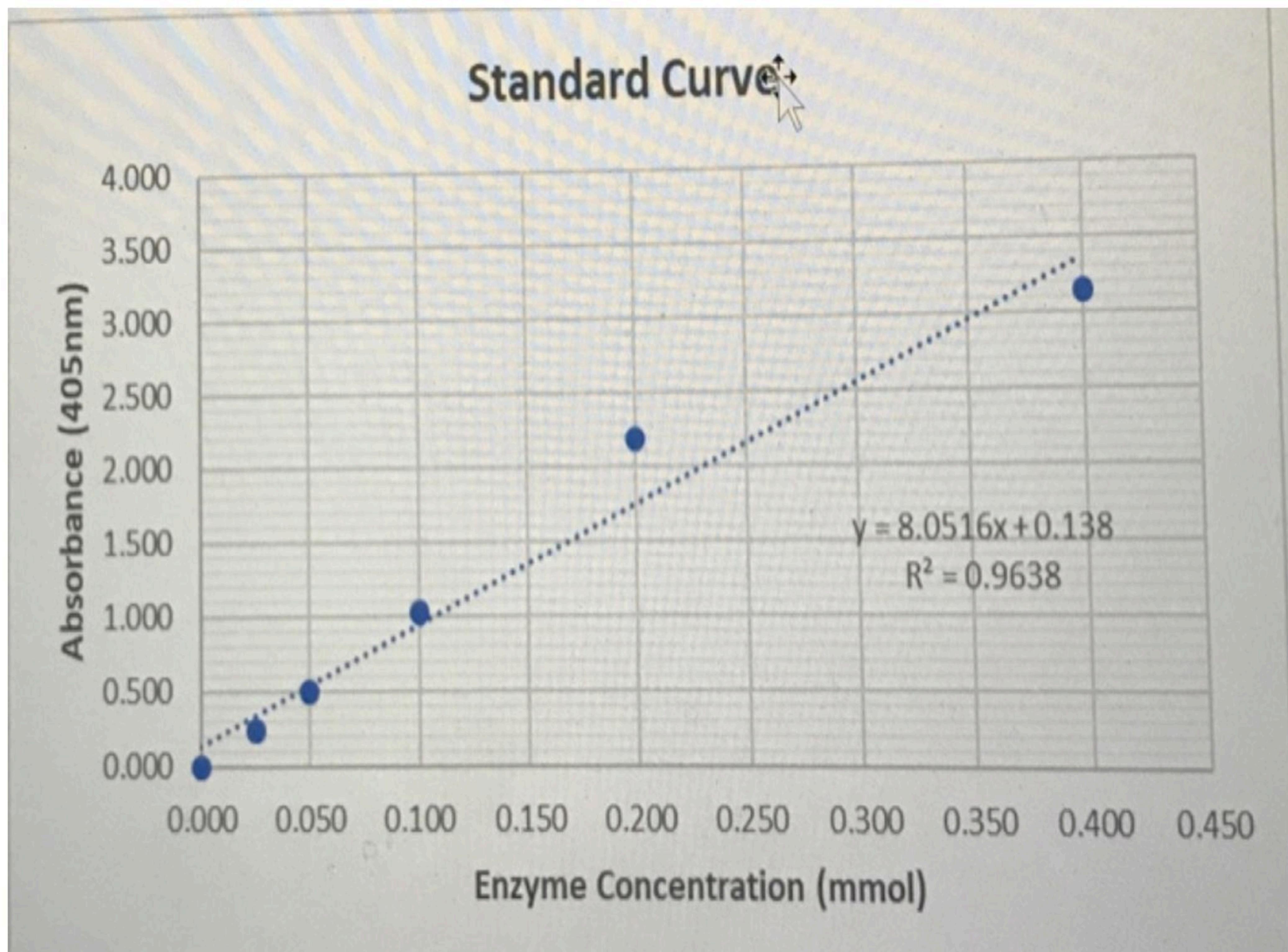
Enzyme Concentration Experiment:

- a)** Preparation of Turnip Extract: Turnip extract was once again prepared as in the previous experiments.
- b)** Varying Enzyme Concentration: Tubes were set up with different volumes of turnip extract, representing various enzyme concentrations. These volumes ranged from 50 μ L to 200 μ L
- c)** Introduction of Hydrogen Peroxide: Hydrogen peroxide was added to each tube to initiate the catalase reaction, with consistent H₂O₂ concentration.
- d)** Observation of Reaction Rate: The rate of the reaction was monitored as before, by measuring the release of oxygen gas. The time required for the appearance of a specific gas volume was recorded for each enzyme concentration.

These methods were rigorously followed to investigate how temperature, pH, and enzyme concentration influence the catalytic activity of turnip cell catalase.

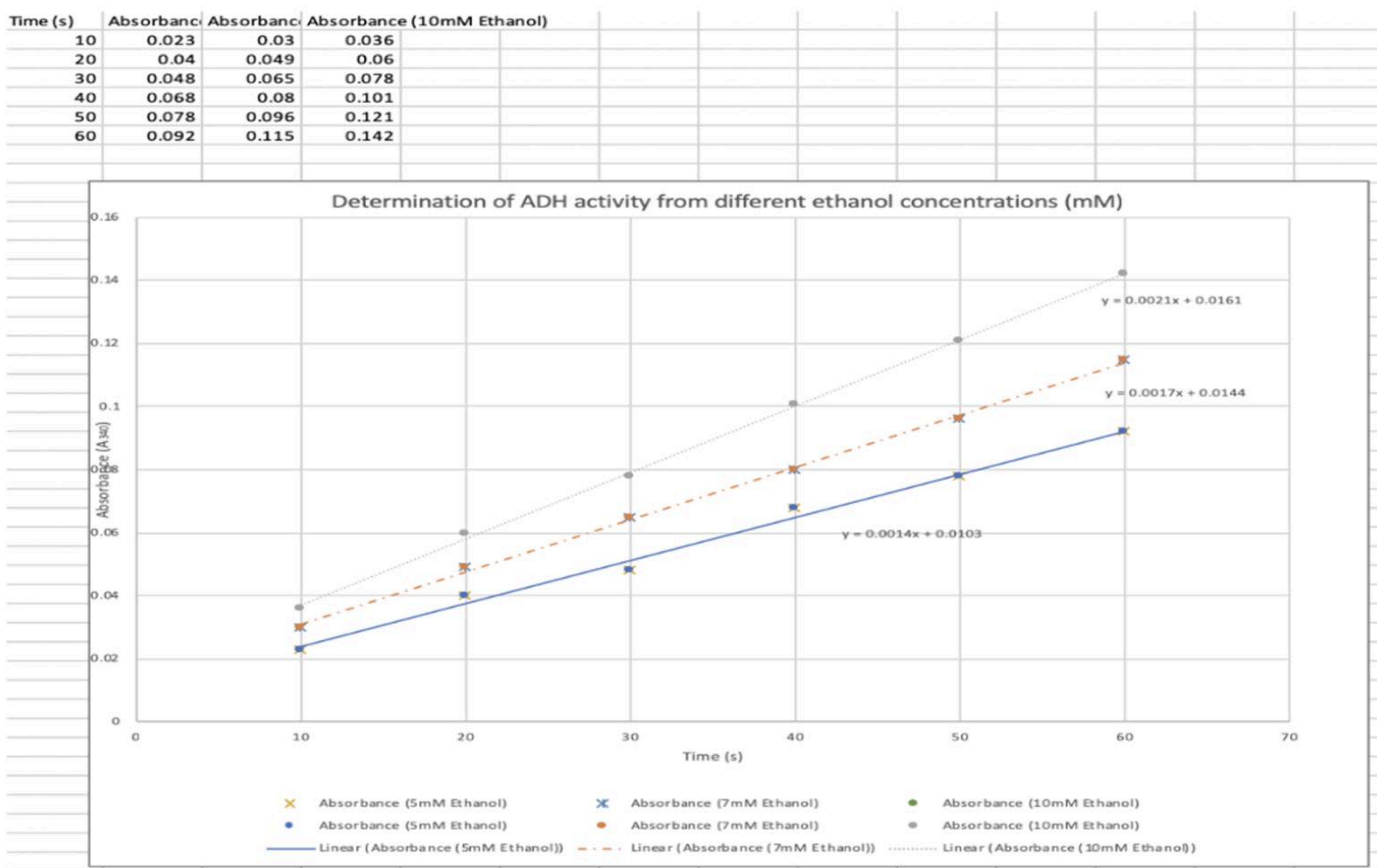
The absorbance was recorded using appropriate equipment, and the data collected was utilized to create a

graph illustrating the relationship between enzyme concentration and the rate of enzyme activity. The x-axis of the graph represented the enzyme concentration, while the y-axis displayed the absorbance, providing insights into the enzymatic reaction's progress.



Results:

In this section, we present the data collected during the experiment and illustrate it graphically. The graph below represents the relationship between time in seconds (x-axis) and absorbance (y-axis) for two different enzyme concentrations (#1 and #2).



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Title of the Graph: "Effect of Enzyme Concentration on Reaction Rate"

As observed in the graph, there is a distinct variation in absorbance with time for the different enzyme concentrations. Notably, the reaction rate is influenced by the enzyme concentration, and this influence is elucidated by the trendlines on the graph.

Discussion:

How does the activity of the enzyme vary with pH?

The data presented here focuses on the relationship between enzyme concentration and reaction rate, not pH. It seems there may be some confusion between the provided data and the discussion questions. To address enzyme activity variations with pH, we would need specific data related to pH changes.

What is the pH at which the greatest peroxidase activity occurs?

The pH at which the greatest peroxidase activity occurs is not addressed in the provided data. To determine this, a separate experiment involving variations in pH levels should be conducted, and data collected accordingly.

Tables 1,2 and 3.

TABLE 2 pH		Time (sec)					
Tubes	pH	20	40	60	80	100	120
#1 + #2	3	0.0400.05	0.748	0.405	0.198		
#3 + #4	5	0.0400.05	1.193	0.663	0.314		
#5 + #6	7	0.0400.05	1.567	0.883	0.415		
#7 + #8	9	0.0390.05	1.379	1.075	0.505		

Graph your results by plotting time in seconds on the x-axis versus absorbance on the y-axis. Title the graph, label and scale the axes and include a separate trendline for each set of data points (this may be non-linear depending upon whether the enzyme reaction becomes saturated).

TABLE 1 Enzyme Concentration		Time (sec)					
Tubes	μL extract	20	40	60	80	100	120
#1 + #2	50	1.263	1.304	1.455	1.540	1.619	1.691
#3 + #4	100	2.136	2.340	2.561	2.681	2.922	2.946
#5 + #6	200	3.579	3.741	3.792	3.825	3.847	3.831

Table 3 Temperature		Time (sec)					
Tubes	Temperature °C	20	40	60	80	100	120
#1 + #2	20	0.76	0.964	1.145	1.300	1.449	1.575
#3 + #4	50	0.475	0.671	0.847	1.005	1.145	1.27
#5 + #6	70	0.247	0.359	0.474	0.555	0.633	0.708
#7 + #8	90	0.02	0.02	0.02	0.02	0.02	0.02

Graph your results by plotting time in seconds on the x-axis versus absorbance on the y-axis. Title the graph, label and scale the axes and include a separate trend line for each set of data points (this may be non-linear depending upon whether the enzyme reaction becomes saturated).

Include the answers to the following questions in the discussion:

1. In what way does the activity of the enzyme vary with temperature?
2. At what temperature does the peroxidase appear to have the greatest effect in oxidizing the peroxide?
3. What would be a plausible reason if no peroxidase activity were to occur at a particular temperature?

Do you think all enzymes would work well at the pH found in this experiment? Explain your answer.

Based on the information provided in this report, we cannot make conclusions about enzyme activity at a specific pH. Enzymes typically have an optimal pH range within which they function most effectively. To assess whether all enzymes would work well at a particular pH, one would need to conduct experiments for each enzyme of interest, measuring activity across a range of pH levels.

In summary, the provided data illustrates the impact of enzyme concentration on the rate of enzyme activity. However, for questions related to pH and peroxidase activity, a separate experiment is required.



References

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Chance, B., & Maehly, A. C. (1955). Assay of catalases and peroxidases. In Methods of Biochemical Analysis (Vol. 1, pp. 357-424). John Wiley & Sons, Inc.

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