ACID AND ALKALINE PHOSPHATASE ACTIVITIES

IN PLANTAIN FLOWER BRACT

BY

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LSC2003011

THE DEPARTMENT OF BIOCHEMISTRY

FACULTY OF LIFE SCIENCES

UNIVERSITY OF BENIN

BENIN CITY.

MARCH, 2025.

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A PROJECT SUBMITTED TO THE DEPARTMENT OF BIOCHEMISTRY IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF SCIENCES (B.Sc.) HONOUR DEGREE IN BIOCHEMISTRY, UNIVERSITY OF BENIN, BENIN CITY.

MARCH, 2025.

CERTIFICATION

This is to certify that the project topic titled “Acid and Alkaline Phosphatases of Plantain Flower Bract” has been approved by the department, and was carried out by Igbinovia Favour with matriculation number LSC2003011 from the department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Edo State.

Prof. (Mrs.) B.O. Agoreyo — Date

Project Supervisor

Dr. S.I Ojeaburu — Date

Project Coordinator

Prof. E.C. Onyeneke — Date

Head of Department

DEDICATION

This report is dedicate to God almighty for, the Author and Finisher of my life, for His unconditional love and mercy granted to me throughout the period of my project work..

ACKNOWLEDGEMENT

I want to specially acknowledge and express my profound gratitude to my project supervisor, the person of Prof. (Mrs.) B.O. Agoreyo for her contribution, encouragement and enlightenment.

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Lastly I want to specially acknowledge my parents, Mr. and Mrs. Igbinovia for their prayers, my brother Precious Igbinovia and my sister Kate Igbinovia for their financial support. And a profound gratitude to my Project Colleagues for the effort they put together to make this project a success.

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ABSTRACT

Phosphatase enzyme activities were investigated in plantain (Musa paradisiaca) bracts to determine the activities of ALP and ACP. Fresh bracts were collected and analyzed for both acid phosphatase (ACP) and alkaline phosphatase (ALP) activities using p-nitrophenyl phosphate as substrate. The study revealed that alkaline phosphatase activity was significantly higher than acid phosphatase activity in plantain bracts, with mean values of 0.265 ± 0 µmol/min/g fresh weight and 0.253 ± 0.008 µmol/min/g fresh weight, respectively. Maximum ACP activity was observed at pH 3.5, while ALP showed optimal activity at pH 9.5. Temperature optimization studies indicated peak activities at 45°C for ACP and 40°C for ALP. Mg2+ was used as a modulator and results gotten showed than it was a positive modulator for both ALP and ACP as their activity increased, While Pi was shown to inhibit the activities of both Enzymes. The presence of these phosphatases, particularly the predominant acid phosphatase, indicates their crucial role in phosphate metabolism during bract development and senescence. These findings provide valuable insights into the biochemical processes occurring in plantain bracts and may contribute to understanding the physiological changes during plantain flower development.

CHAPTER ONE: INTRODUCTION

1.1 Background of Study

Plantain (Musa paradisiaca L.) is a major staple food crop widely cultivated in tropical and subtropical regions of the world (Anand *et al.,* 2020). The plant produces large flower bracts that are often discarded as agricultural waste, despite containing valuable biochemical compounds including enzymes (Akinyemi *et al.,* 2010). Among these enzymes, phosphatases play crucial roles in plant metabolism and development.

Phosphatases are hydrolytic enzymes that catalyze the removal of phosphate groups from various organic phosphate compounds. They are classified into two main categories based on their pH optima: acid phosphatases (ACP) and alkaline phosphatases (ALP) (Tran *et al.,* 2010). These enzymes are widely distributed in nature and are found in various plant tissues where they participate in essential metabolic processes.

In plants, acid and alkaline phosphatases are involved in phosphate acquisition, metabolism, and recycling. They play vital roles in the mobilization and transport of phosphorus, especially during periods of phosphate deficiency (Hegeman and Grabau, 2001). These enzymes are particularly important in the hydrolysis of phosphate esters, energy transfer, and metabolic regulation (Wang *et al.,* 2011).

1.2 Statement of Problem

While phosphatases have been extensively studied in various plant tissues, there is limited information about their characteristics and properties in plantain flower bract. The potential enzymatic resources in these agricultural wastes remain largely unexplored, leading to missed opportunities for their biotechnological applications (Akinyemi *et al.,* 2010). Understanding the biochemical properties of these enzymes, including their optimal conditions and response to effectors, is crucial for their potential utilization.

1.3 Research Objectives

The objectives of this study are to:

1. Extract and characterize acid and alkaline phosphatases from plantain flower bract
2. Determine the optimum temperature and pH for the extracted enzymes

3. Investigate the effects of inorganic phosphate (Pi) and magnesium ions (Mg2+) on enzyme activity
4. Evaluate the potential applications of these enzymes in biotechnology

1.4 Significance of Study

This research contributes to the growing body of knowledge on plant phosphatases and provides valuable insights into the enzymatic properties of plantain flower bract. The findings could lead to the development of new applications in biotechnology, particularly in industries requiring phosphatase activity under specific conditions (Turner *et al.*, 2012). Additionally, this study promotes the utilization of agricultural waste products, contributing to sustainable resource management and waste reduction in the agricultural sector (Vincent *et al.*, 1992).

CHAPTER TWO: LITERATURE REVIEW

2.1 Plantain (Musa paradisiaca)

Plantain (Musa paradisiaca L.) belongs to the family Musaceae and is a hybrid between Musa acuminata and Musa balbisiana (Ploetz *et al.,* 2015). It is a large herbaceous plant reaching heights of 3-10 meters, characterized by a pseudostem formed by tightly packed leaf sheaths. The inflorescence emerges from the center of the pseudostem, bearing large purple-red bracts that protect the developing flowers (Heslop-Harrison and Schwarzacher, 2007).

Economically, plantain serves as a major staple food crop in tropical and subtropical regions, particularly in Africa where it contributes significantly to food security and income generation (Adeniji *et al.,* 2010). Global production of plantains reached approximately 39 million tonnes in recent years, with Africa accounting for about 73% of worldwide production.

The biochemical composition of plantain varies across different plant parts. The fruit pulp contains predominantly carbohydrates (31-32%), proteins (1.1-1.3%), lipids (0.2-0.3%), and various minerals including potassium, calcium, and iron (Anyasi *et al.,* 2013). The flower bracts contain significant amounts of proteins, phenolic compounds, and various enzymes including phosphatases, peroxidases, and polyphenol oxidases (Akinyemi *et al.,* 2010).

2.2 Phosphatase Enzymes

Classification and Types:

Phosphatases (EC 3.1.3) are hydrolytic enzymes that catalyze the removal of phosphate groups from various phosphate esters. They are broadly classified into acid phosphatases (ACP, EC 3.1.3.2) and alkaline phosphatases (ALP, EC 3.1.3.1) based on their pH optima (Tran *et al.,* 2010). Acid phosphatases show optimal activity below pH 7.0, while alkaline phosphatases function optimally above pH 7.0 (Lei *et al.,* 2013).

Structure and Function:

Plant phosphatases typically exist as metalloproteins containing metal ions crucial for their catalytic activity. Alkaline phosphatases are typically homodimeric enzymes with molecular weights ranging from 80-100 kDa, while acid phosphatases can exist in multiple molecular forms (Hegeman and Grabau, 2001). These enzymes play essential roles in phosphate metabolism, signal transduction, and stress responses in plants (Wang *et al.,* 2014).

Mechanism of Action:

The catalytic mechanism of phosphatases involves a two-step process. In the first step, a phosphoenzyme intermediate is formed through the nucleophilic attack on the phosphate ester substrate. The second step involves the hydrolysis of this intermediate, releasing inorganic phosphate and regenerating the free enzyme (Olczak *et al.,* 2003). This process is often regulated by various factors including metal ions, substrate concentration, and environmental conditions (Robinson *et al.,* 2012).

2.3 Alkaline Phosphatase

Properties and Characteristics:

Alkaline phosphatases (ALPs, EC 3.1.3.1) are homodimeric metalloenzymes that catalyze the hydrolysis of phosphomonoesters at alkaline pH. Each monomer typically contains two Zn2+ ions and one Mg2+ ion essential for catalytic activity (Coleman, 1992). Plant ALPs generally have molecular weights ranging from 80-120 kDa and exhibit optimal activity at pH 8.0-10.0 (Millán, 2006). These enzymes show broad substrate specificity, capable of hydrolyzing various phosphate esters including p-nitrophenyl phosphate, a commonly used synthetic substrate for activity measurements (Hoylaerts *et al.,* 2015).

Distribution in Plants:

ALPs are widely distributed throughout plant tissues, with particularly high concentrations in actively growing regions. They are found in cell walls, plasma membranes, and intracellular spaces (Bozzo *et al.,* 2004). Studies have shown significant ALP activity in root tissues, developing seeds, and young leaves. In Musa species, ALP activity has been detected in various tissues including fruits, pseudostem, and flower parts (Dosanjh *et al.,* 2020).

Applications:

Plant ALPs have found applications in various biotechnological processes. They are used as markers for stress responses and developmental stages in plants. Industrial applications include their use in phosphate removal from waste streams and as diagnostic tools in agriculture (Sebastian and Prasad, 2015).

2.4 Acid Phosphatase

Properties and Characteristics:

Acid phosphatases (ACPs, EC 3.1.3.2) are enzymes that optimally function at acidic pH ranges (4.0-6.0). Plant ACPs exist in multiple molecular forms, ranging from monomeric to oligomeric structures, with molecular masses varying from 18 to 84 kDa (Tran *et al.,* 2010). They are typically glycoproteins and can be classified into purple acid phosphatases (PAPs) and other ACPs based on their metal content and sequence characteristics (Olczak *et al.,* 2003).

Distribution in Plants:

ACPs are ubiquitously distributed in plant tissues, with particularly high expression during phosphate starvation. They are found in cell walls, vacuoles, and the apoplastic space. High ACP activity has been reported in senescing tissues, germinating seeds, and roots (Wang *et al.,* 2014). In particular, PAPs form a major group of plant ACPs, with multiple isoforms identified in various species (Hegeman and Grabau, 2001).

Applications:

ACPs play crucial roles in phosphate acquisition and recycling in plants. They have been utilized in phytoremediation processes and as indicators of soil phosphate availability. Their ability to function under acidic conditions makes them valuable for industrial applications requiring low pH environments (Robinson *et al.,* 2012).

2.5 Factors Affecting Enzyme Activity

Temperature Effects:

Temperature significantly influences phosphatase activity through its effects on enzyme structure and reaction kinetics. Most plant phosphatases show optimal activity between 37-50°C (Turner, 2010). Above the optimal temperature, enzyme activity decreases due to protein denaturation. Temperature stability studies of plant phosphatases have shown varying degrees of thermostability, with some enzymes retaining activity even after brief exposure to temperatures above 60°C (Lei *et al.,* 2013).

pH Effects:

The pH of the environment critically affects phosphatase activity by influencing the ionization state of amino acid residues at the active site and the overall protein structure. ALPs typically show maximum activity at pH 8.0-10.0, while ACPs function optimally at pH 4.0-6.0 (Millán, 2006). The pH optima can vary slightly depending on the source tissue and specific isoform. Some phosphatases exhibit broad pH ranges for activity, while others are more pH-sensitive (Bozzo *et al.*, 2004).

Metal Ion Effects:

Metal ions play crucial roles in phosphatase activity, either as essential cofactors or modulators. Mg^{2+} and Zn^{2+} are typically required for ALP activity, while Fe^{3+} is characteristic of PAPs (Olczak *et al.*, 2003). Other metal ions such as Ca^{2+}, Mn^{2+}, and Cu^{2+} can either activate or inhibit phosphatase activity depending on the concentration and specific enzyme type. Some metal ions may also protect enzymes against thermal denaturation (Hegeman and Grabau, 2001).

Substrate Concentration:

Phosphatase activity follows Michaelis-Menten kinetics, with activity increasing with substrate concentration until reaching saturation. The Km values for plant phosphatases vary depending on the substrate and enzyme source. Studies have shown that high phosphate concentrations can inhibit phosphatase activity through feedback inhibition mechanisms (Wang *et al.*, 2014).

CHAPTER THREE: MATERIALS AND METHODS

3.1 Materials

Plant Materials:

Fresh plantain (Musa paradisiaca L.) flower bracts were collected from a local farm. The bracts were immediately transported to the laboratory to maintain enzyme integrity. Only fresh, undamaged bracts were selected for the study.

Chemicals and Reagents:

All chemicals used were of analytical grade, with substrate and buffer preparations. The use of p-nitrophenyl phosphate (pNPP) as a substrate follows widely accepted protocols for phosphatase assays (Tran et al., 2010).

i. Sodium hydroxide (NaOH)
ii. Hydrochloric acid (HCl)
iii. Acetate buffer
iv. Sodium bicarbonate buffer
v. p-nitrophenyl phosphate (pNPP)
vi. 20mM Pi
vii. 20mM $MgSO_4$

Equipment:

i. Micropipettes
ii. Glass and plastic wares
iii. UV-Visible Spectrophotometer
iv. pH meter
v. Analytical balance
vi. Refrigerated centrifuge
vii. Water bath with temperature control
viii. Mortar and Pestle for Homogenization

3.2 Methods

Sample Collection and Preparation:

Fresh plantain flower bracts were washed thoroughly with distilled water to remove surface contaminants. The bracts were cut into small pieces and weighed.

Enzyme Extraction Procedure:

Following the method of Tran et al. (2010), with slight modifications, 10g of bract tissue was homogenized in 100mL of cold extraction buffer (acetate and Bicarbonate buffers, pH 3.6 and pH 10.2 respectively) using a chilled mortar and pestle. The homogenate was filtered through four layers of cheesecloth and centrifuged at 10,000 × g for 20 minutes. The supernatant was collected and used as the crude enzyme extract.

Enzyme Activity Assay:

Phosphatase activity was determined using pNPP as substrate according to the method described by Turner (2010). The reaction mixture contained:

i. 0.2mL enzyme extract
ii. 2.0mL substrate solution (5mM pNPP)
iii. 1.8mL appropriate buffer (0.1M CO_3 and acetate buffer respectively where appropriate).

The mixture was incubated at 37°C for 20 minutes. The reaction was terminated by adding 2mL of 0.1M NaOH. The released p-nitrophenol was measured spectrophotometrically at 405nm. One unit of enzyme activity was defined as the amount of enzyme that liberated 1μmol of p-nitrophenol per minute under the assay conditions.

Determination of Optimum pH:

The optimal pH was determined by measuring enzyme activity at various pH values (pH 1.0-5.0 and pH 7.0 – 12.0 for acid and alkaline phosphatase respectievely) using the following buffer systems:

i. 0.1M Acetate buffer
ii. 0.1M CO_3 buffer

Below is the experimental table for the determination of Optimum pH for both Alkaline and acid phosphatase respectively.

For Alkaline Phosphatase

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Substrate (PNPP) (ml)	__	2.0	2.0	2.0	2.0	2.0	2.0	2.0
0.1M CO_3 buffer (ml)	3.8 PH 10	1.8 PH 7	1.8 PH 8	1.8 PH 9	1.8 PH 9.5	1.8 PH 10	1.8 PH 11	1.8 PH 12
Enzyme (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes @ room temperature								
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

For Acid Phosphatase

Tube	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Substrate (PNPP) (ml)	__	2.0	2.0	2.0	2.0	2.0	2.0	2.0
0.1M Acetate buffer (ml)	3.8 PH 10	1.8 PH 7	1.8 PH 8	1.8 PH 9	1.8 PH 9.5	1.8 PH 10	1.8 PH 11	1.8 PH 12
Enzyme (ml)	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes @ room temperature								
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

Determination of Optimum Temperature:

The effect of temperature on enzyme activity was studied by conducting the standard assay at different temperatures ranging from 20°C to 65°C at 10°C intervals. For thermal stability studies, the enzyme was pre-incubated at various temperatures for 20 minutes before assaying for residual activity under standard conditions.

Below is the experimental table for the determination of Optimum Temperature for both Alkaline and acid phosphatase respectively.

For Alkaline Phosphatase

Tube	**1**	**2**	**3**	**4**	**5**	**6**
Substrate (PNPP) (ml)	__	2.0	2.0	2.0	2.0	2.0
0.1M CO_3 buffer (ml)	3.8	1.8	1.8	1.8	1.8	1.8
Temperature (^{0}C)	---	20	30	40	50	60
Enzyme (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes @ respective temperature						
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0

For Acid Phosphatase

Tube	**1**	**2**	**3**	**4**	**5**	**6**
Substrate (PNPP) (ml)	__	2.0	2.0	2.0	2.0	2.0
0.1M Acetate buffer (ml)	3.8	1.8	1.8	1.8	1.8	1.8
Temperature (^{0}C)	---	20	35	45	55	65
Enzyme (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes @ respective temperature						
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0

Study of Pi and Mg2+ Effects:

The effect of inorganic phosphate (Pi) and Mg^{2+} ion was studied by including a 1.0ml of 20mM Pi and 20mM $MgSO_4$ in the reaction mixture at varying substrate concentration. Below is the experimental table for the determination of Optimum Temperature for both Alkaline and acid phosphatase respectively

For Alkaline phosphatase with 20mM $MgSO_4$

Tube No.	**1**	**2**	**3**	**4**	**5**	**6**
1mM PNPP (ml)	0.0	0.4	0.8	1.2	1.6	2.0
20mM $MgSO_4$ (ml)	1.0	1.0	1.0	1.0	1.0	1.0
0.1M CO_3 buffer (ml)	3.8	3.4	3.0	2.6	2.2	1.8
Enzyme extract (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes at room temperature						
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0

With 20mM Pi

Tube No.	1	2	3	4	5	6
1mM PNPP (ml)	0.0	0.4	0.8	1.2	1.6	2.0
20mM Pi (ml)	1.0	1.0	1.0	1.0	1.0	1.0
0.1M CO_3 buffer (ml)	3.8	3.4	3.0	2.6	2.2	1.8
Enzyme extract (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes at room temperature						
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0

For Acid phosphatase with 20mM $MgSO_4$

Tube No.	1	2	3	4	5	6
1mM PNPP (ml)	0.0	0.4	0.8	1.2	1.6	2.0
20mM $MgSO_4$ (ml)	1.0	1.0	1.0	1.0	1.0	1.0
0.1M Acetate buffer (ml)	3.8	3.4	3.0	2.6	2.2	1.8
Enzyme extract (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes at room temperature						
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0

With 20mM Pi

Tube No.	1	2	3	4	5	6
1mM PNPP (ml)	0.0	0.4	0.8	1.2	1.6	2.0
20mM Pi (ml)	1.0	1.0	1.0	1.0	1.0	1.0
0.1M CO_3 buffer (ml)	3.8	3.4	3.0	2.6	2.2	1.8
Enzyme extract (ml)	0.2	0.2	0.2	0.2	0.2	0.2
Incubate for 20 minutes at room temperature						
0.1M NaOH (ml)	2.0	2.0	2.0	2.0	2.0	2.0

3.3 Statistical Analysis:

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA) followed by Tukey's test. P values < 0.05 were considered statistically significant. GraphPad Prism software version 8.0 was used for data analysis and graphical presentation.

CHAPTER FOUR

4.1 Result and Discussion

4.2 Temperature Studies

Optimum temperature determination

Upon analysis of the optimum temperature of both alkaline and acid phosphatase in the bracts of plantain flower, the following results were observed.

4.2.1 Effect of temperature on alkaline phosphatase activity

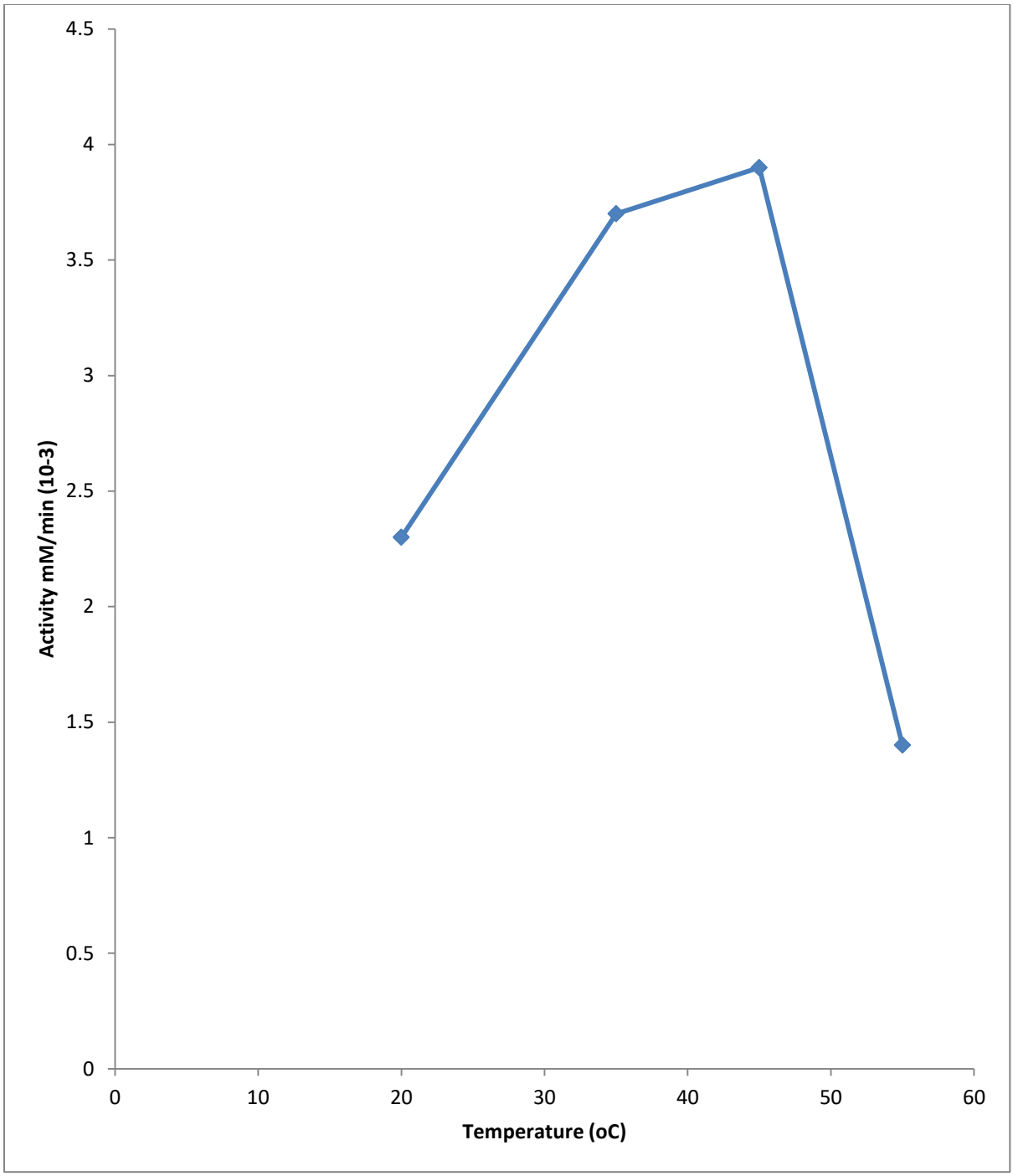
Triplicate Optical Densities.

Replicates	20 ^{0}C	30 ^{0}C	40 ^{0}C	50 ^{0}C	60 ^{0}C
1.	0.186	0.227	0.236	0.077	0.003
2.	0.185	0.227	0.232	0.075	0.009
3.	0.185	0.229	0.229	0.075	0.005
Mean	0.1853	0.228	0.223	0.076	0.007

PNP produce is extrapolated from the PNP Standard Calibration Curve Extrapolation table.

Tubes	Absorbance @ 405nm	PNP Produced (uM)	Activity (uMmin^{-1})	Standard form	Temperature
1.	0.000	0.000	0.00	0.000	
2.	0.1853	0.065	0.00325	3.25 x 10^{-3}	20
3.	0.228	0.08	0.004	4 x 10^{-3}	30
4.	0.232	0.082	0.0041	4.1 x 10^{-3}	40
5.	0.076	0.028	0.0014	1.4 x 10^{-3}	50
6.	0.007	0.003	0.00015	0.15 x 10^{-3}	60

A graph of Activity (uMmin^{-1} in standard form) is plotted against Temperature (^{0}C), and the Optimum temperature was observed to be 40 ^{0}C.


4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
Activity mM/min (10-3)
0
10
20
30
40
50
60
Temperature (oC)

4.2.2 Effect of temperature on Acid Phosphatase

Triplicate Values of Optical Densities

Replicates	20 degrees	35 degrees	45 degrees	55 degrees	65 degrees
1.	0.127	0.21	0.225	0.081	0.00
2.	0.129	0.22	0.227	0.081	0.00
3.	0.128	0.22	0.223	0.081	0.00
Mean	0.128	0.216	0.225	0.081	0.00

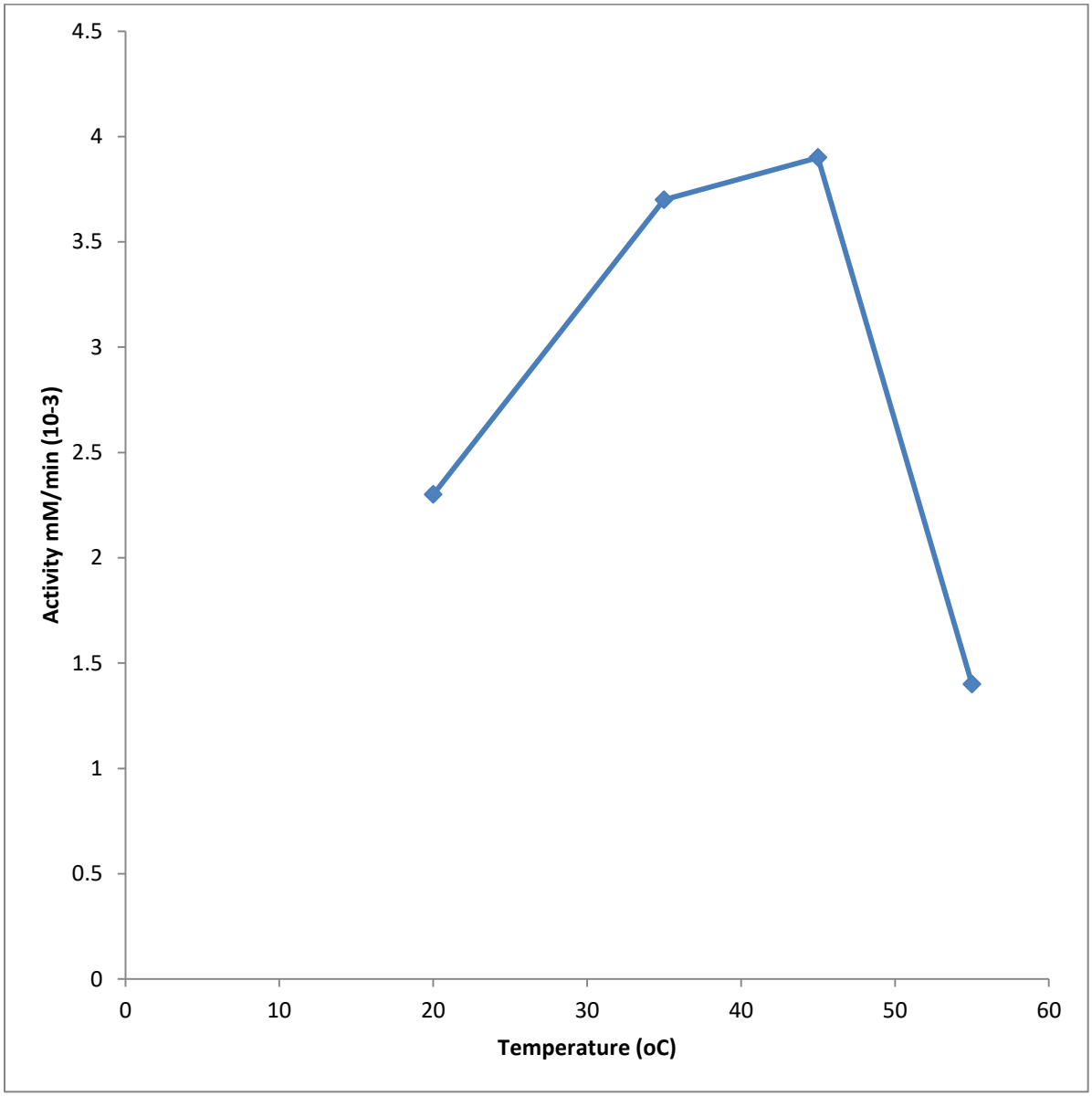
PNP produce is extrapolated from the PNP Standard Calibration Curve

Extrapolation table

Tubes	Absorbance @ 405nm	PNP Produced (uM)	Activity ($uMmin^{-1}$)	Standard form	Temperature
1.	0.000	0.000	0.00	0.000	
2.	0.128	0.046	0.0023	2.3×10^{-3}	20
3.	0.216	0.074	0.0037	3.7×10^{-3}	35
4.	0.225	0.078	0.0039	3.9×10^{-3}	45
5.	0.081	0.028	0.0014	1.4×10^{-3}	55
6.	0.000	0.0	0.000	0×10^{-3}	65

A graph of Activity ($uMmin^{-1}$ in standard form) is plotted against Temperature (^0C), and the Optimum temperature was observed to be 45 ^0C.

Graph of Temperature against activity



4.3 pH Studies

4.3.1 Effect of pH in Alkaline Phosphatase Activity

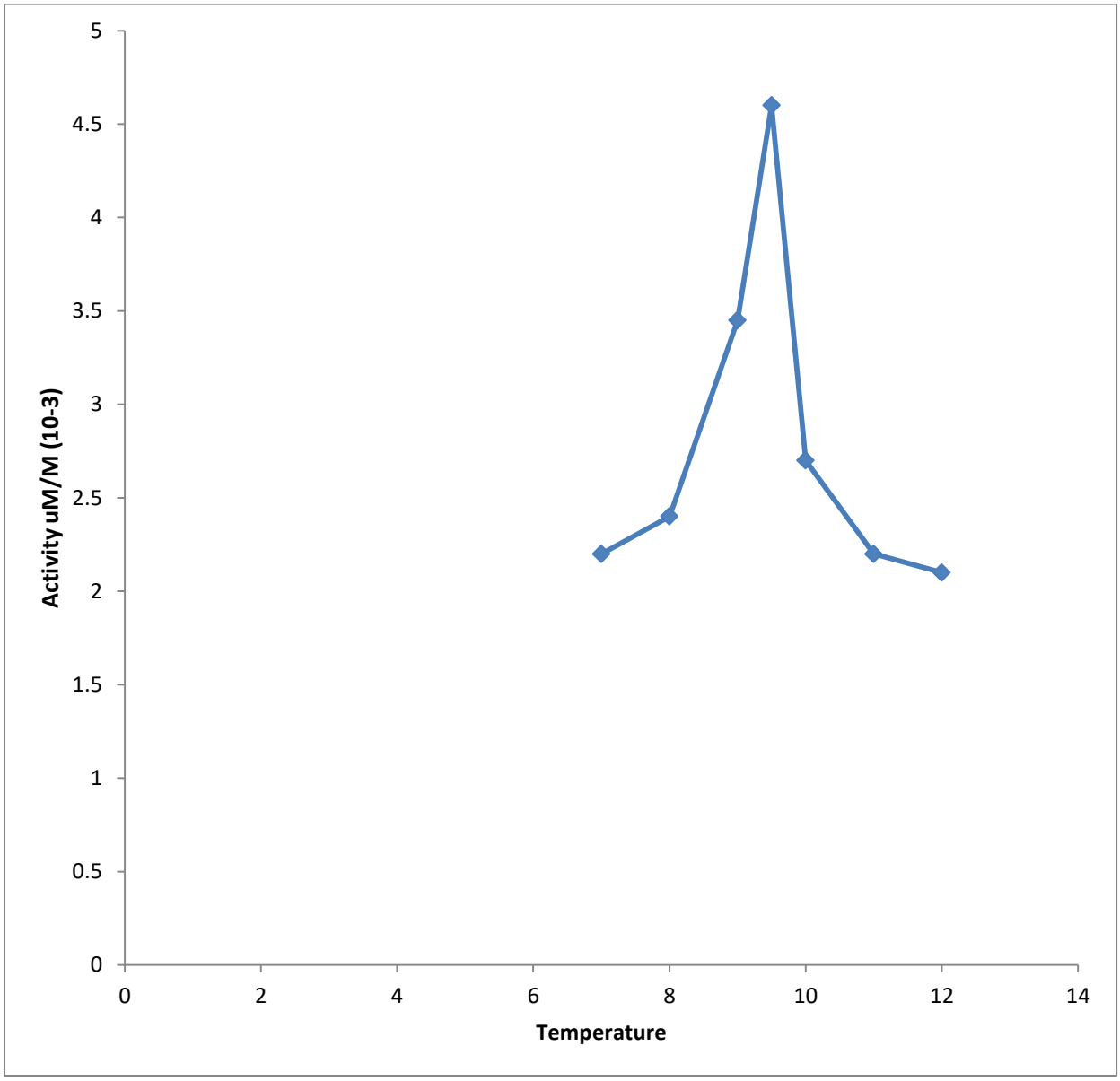
Replicate values

OPTICAL DENSITIES AT 405nM								
Replicate	**pH 10**	**pH 7**	**pH 8**	**pH 9**	**pH 9.5**	**pH 10**	**pH 11**	**pH 12**
1.	0.000	0.128	0.138	0.198	0.267	0.152	0.126	0.121
2.	0.000	0.127	0.137	0.196	0.264	0.151	0.127	0.121
3.	0.000	0.127	0.133	0.199	0.265	0.153	0.125	0.121
Mean	**0.000**	**0.127**	**0.136**	**0.1976**	**0.265**	**0.152**	**0.126**	**0.121**

Extrapolation table

Tubes	Absorbance @ 405nm	PNPP Product (uM)	Activity ($uMmin^{-1}$)	Standard form	PH
1.	**0**	0.000	0.000	0.000	10
2.	**0.127**	0.044	0.0022	2.2×10^{-3}	7
3.	**0.136**	0.048	0.0024	2.4×10^{-3}	8
4.	**0.1976**	0.069	0.00345	3.45×10^{-3}	9
5.	**0.265**	0.092	0.0046	4.6×10^{-3}	9.5
6.	**0.152**	0.054	0.0027	2.7×10^{-3}	10
7.	**0.126**	0.044	0.0022	2.2×10^{-3}	11
8.	**0.121**	0.042	0.0021	2.1×10^{-3}	12

GRAPH OF ACTIVITY AGAINST PH



Remark: From the graph, the optimum activity of alkaline phosphatase was observed at PH 9.5. Hence, PH 9.5 seems to be the best PH suited for alkaline phosphatase kinetic studies in freshly procured Plantain Flower Bract.

4.3.2 Effect of pH in Acid Phosphatase Activity

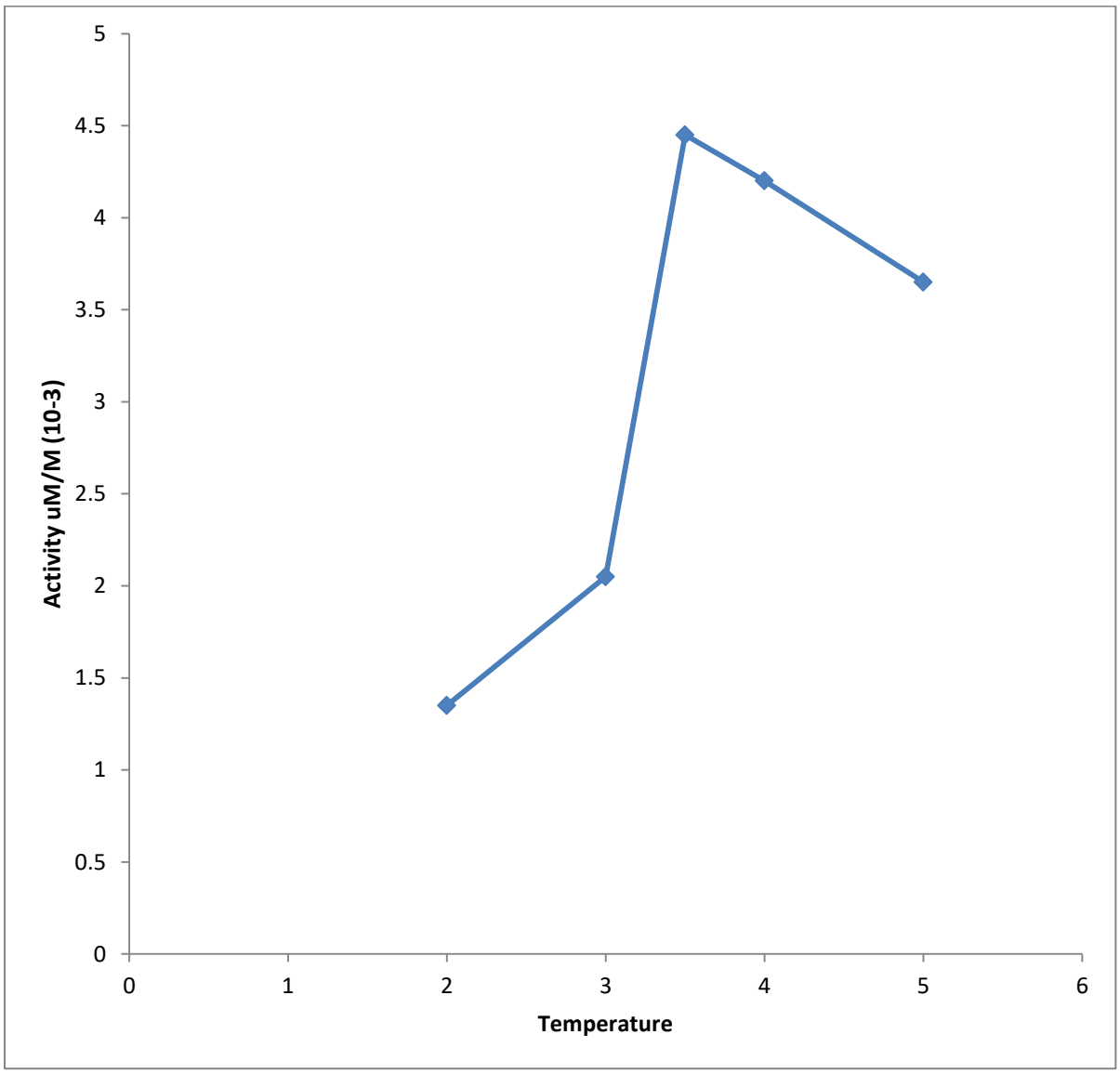
OPTICAL DENSITIES AT 405NM							
Replicate	**pH 4**	**pH 1**	**pH 2**	**pH 3**	**pH3.5**	**pH4**	**pH 5**
1.	0.000	0.003	0.11	0.119	0.255	0.243	0.212
2.	0.000	0.001	0.11	0.117	0.256	0.242	0.214
3.	0.000	0.002	0.11	0.118	0.254	0.241	0.212
MEAN	**0.000**	**0.002**	**0.11**	**0.118**	**0.253**	**0.242**	**0.213**

PNP produce is extrapolated from the PNP Standard Calibration Curve

Extrapolation table

Tubes	Absorbance @ 405nm	PNP Produced (uM)	Activity ($uMmin^{-1}$)	Standard form	PH
1.	0.000	0.000	0.000	0.000	4
2.	0.002	0.00	0.000	0.000	1
3.	0.075	0.027	0.00135	1.35×10^{-3}	2
4.	0.118	0.041	0.00205	2.05×10^{-3}	3
5.	0.255	0.089	0.00445	4.45×10^{-3}	3.5
6.	0.242	0.084	0.0042	4.2×10^{-3}	4
7.	0.213	0.073	0.00365	3.65×10^{-3}	5

GRAPH OF ACTIVITY AGAINST PH



Remark: From the graph, the optimum activity of acid phosphatase was observed at PH 3.5. Hence, PH 3.5 seems to be the best PH suited for acid phosphatase kinetic studies in freshly procured Plantain Flower Bract.

4.4 Effects of Pi and Mg2+ on ALP Activity

4.4.1 Normal (without Mg and Pi) Activity of ALP

Triplicate OD Readings

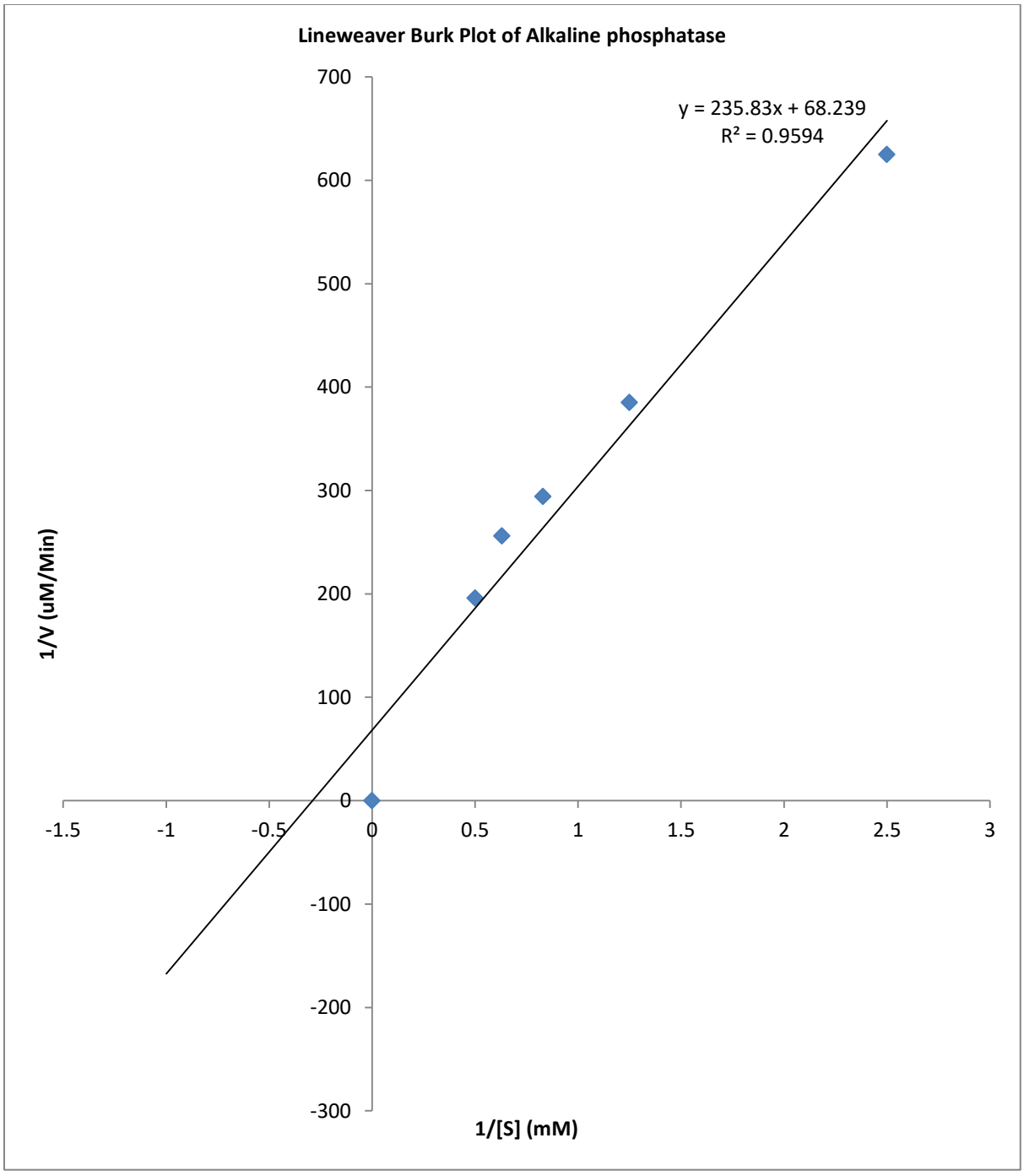
Exp. 1	0.09	0.145	0.198	0.225	0.295
Exp. 2	0.09	0.143	0.192	0.223	0.292
Exp. 3	0.09	0.147	0.195	0.227	0.298
Mean	0.09	0.145	0.195	0.225	0.295

Table 4.6

Extrapolation table (Normal)

Tubes	Absorbance @ 405nm	PNP (uM)	V (uM/Min)	1/V $(uM/Min)^{-1}$	S (uM)	1/S (uM^{-1})
1.	0.000	0.000	0.000	0.0	0.0	0.0
2.	0.090	0.032	0.0016	625	0.4	2.5
3.	0.145	0.052	0.0026	385	0.8	1.25
4.	0.195	0.068	0.0034	294	1.2	0.83
5.	0.225	0.078	0.0039	256	1.6	0.63
6.	0.295	0.102	0.0051	196	2.0	0.5

Table 4.7


Lineweaver Burk Plot of Alkaline phosphatase
y = 235.83x + 68.239
R² = 0.9594
700
600
500
400
300
200
100
0
-100
-200
-300
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3
1/V (uM/Min)
1/[S] (mM)

4.4.2 Effect of $MgSO_4$ on ALP

Triplicate Values

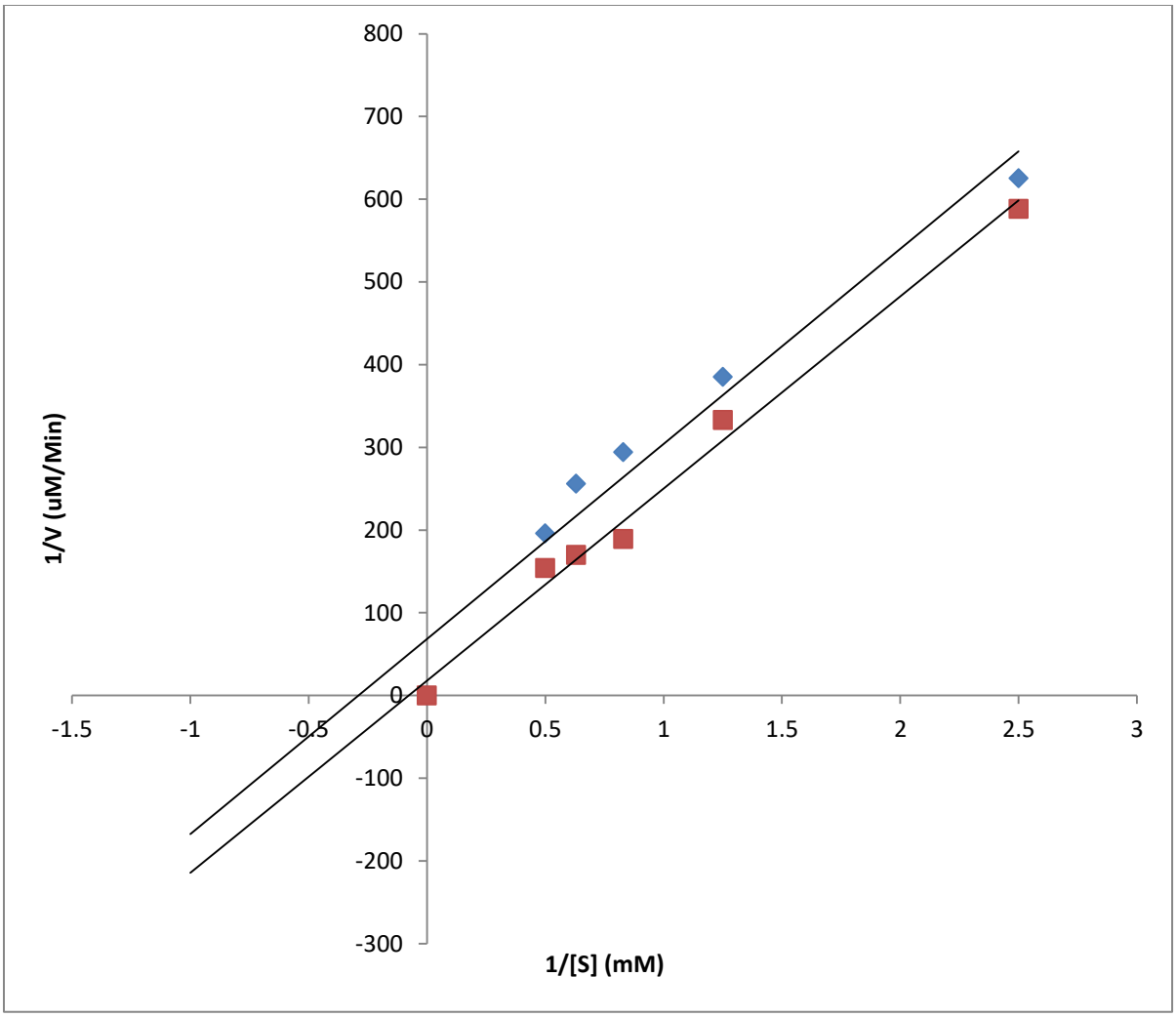
Absorbance Readings @ 420nm				
	Experiment 1	Experiment 2	Experiment 3	Mean
1.	0.098	0.098	0.098	0.098
2.	0.173	0.170	0.167	0.170
3.	0.305	0.3051	0.205	0.305
4.	0.340	0.342	0.338	0.340
5.	0.374	0.375	0.376	0.375

Table 4.8

Extrapolation Table 2.0 (With $Mgso_4$)

Tubes	Absorbance @ 405nm	PNP (uM)	V (uM/Min)	1/V $(uM/Min)^{-1}$	S (uM)	1/S (uM^{-1})
1.	0.000	0.000	0.000	0.0	0.0	0.0
2.	0.082	0.034	0.0017	588.23	0.4	2.5
3.	0.140	0.06	0.003	333.33	0.8	1.25
4.	0.256	0.106	0.0053	188.67	1.2	0.83
5.	0.298	0.118	0.0059	170	1.6	0.63
6.	0.365	0.13	0.0065	154	2.0	0.5

Table 4.9



Remark: Positive Modulator

4.4.3 Effect of Pi on ALP

Triplicate values

Absorbance Readings @ 420nm				
1.	Experiment 1	Experiment 2	Experiment 3	Mean
2.	0.07	0.09	0.08	0.08
3.	0.13	0.012	0.011	0.12
4.	0.17	0.15	0.19	0.17
5.	0.22	0.22	0.22	0.22
6.	0.257	0.259	0.258	0.258

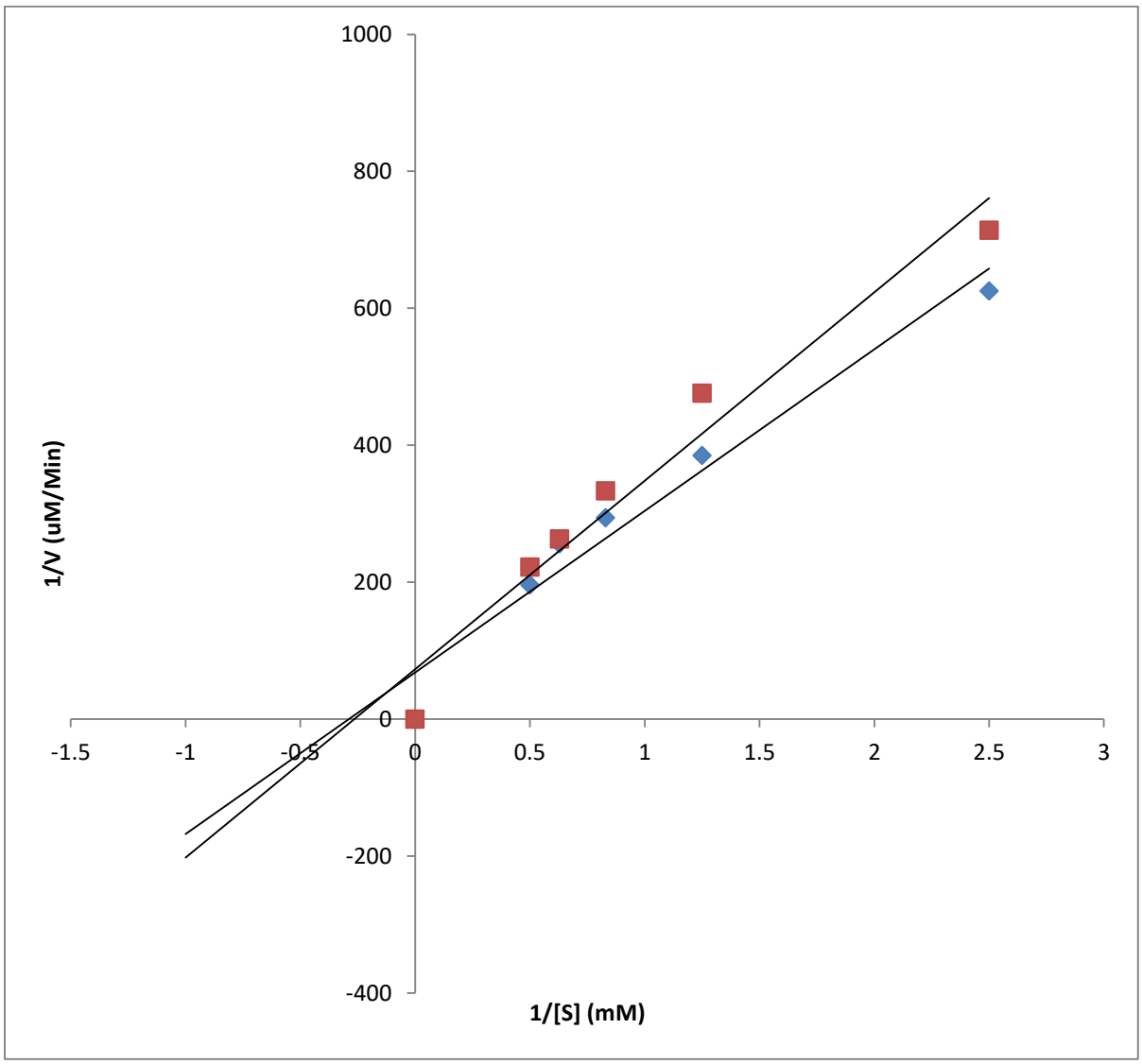
Table 4.10

Extrapolation Table Table 1.2 (With Pi)

Tubes	Absorbance @ 405nm	PNP (uM)	V (uM/Min)	1/V $(uM/Min)^{-1}$	S (uM)	1/S (uM^{-1})
1.	0.000	0.000	0.000	0.0	0.0	0.0
2.	0.08	0.028	0.0014	714.2	0.4	2.5
3.	0.12	0.048	0.0021	476	0.8	1.25
4.	0.17	0.06	0.003	333.33	1.2	0.83
5.	0.22	0.076	0.0038	263	1.6	0.63
6.	0.258	0.09	0.0045	222.22	2.0	0.5

Table 4.11

Graph of ALP Activities with and without Pi Against Substrate Concentration [S]



Remark: Competitive

4.5 Effects of Pi and Mg2+ on ACP Activity

4.5.1 Normal (without Mg and Pi) Activity of ALP

Triplicates

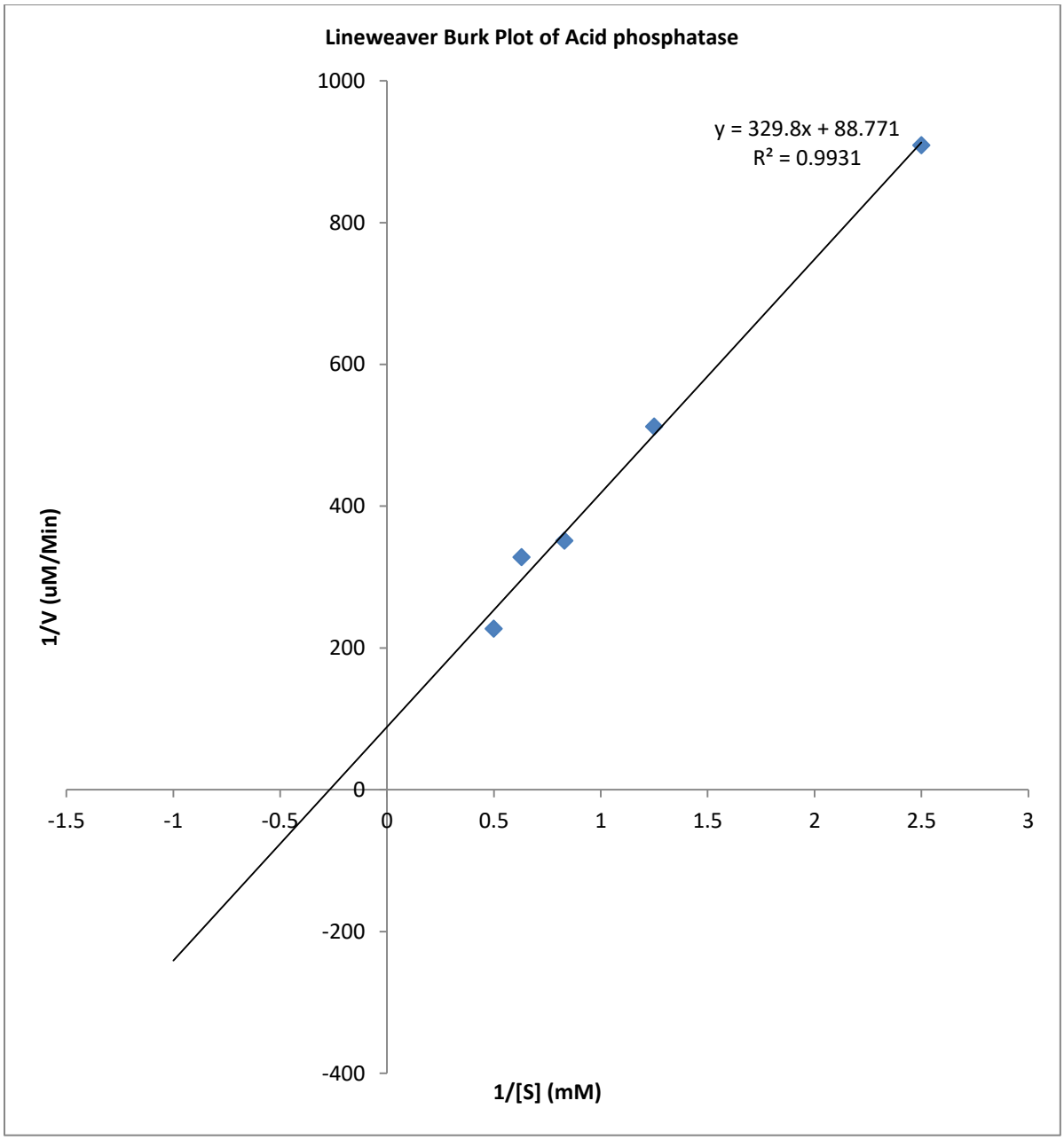
Exp. 1	0.06	0.13	0.164	0.175	0.250
Exp. 2	0.05	0.13	0.160	0.175	0.252
Exp. 3	0.07	0.09	0.162	0.175	0.248

Table 4.5.1

Extrapolation Table (Normal)

Tubes	Absorbance @ 405nm	PNP (uM)	V (uM/Min)	1/V $(uM/Min)^{-1}$	S (uM)	1/S (uM^{-1})
1.	0.000	0.000	0.000	0.0	0.0	0.0
2.	0.06	0.022	0.0011	909	0.4	2.5
3.	0.12	0.039	0.00195	512	0.8	1.25
4.	0.162	0.057	0.00285	351	1.2	0.83
5.	0.175	0.061	0.00305	328	1.6	0.63
6.	0.25	0.087	0.0044	227	2.0	0.5

Table 4.5.2


Lineweaver Burk Plot of Acid phosphatase
y = 329.8x + 88.771
R² = 0.9931
1/V (uM/Min)
1/[S] (mM)
1000
800
600
400
200
0
-200
-400
-1.5
-1
-0.5
0
0.5
1
1.5
2
2.5
3

4.5.2 Effect of $MgSO_4$ on ACP

Triplicate Values

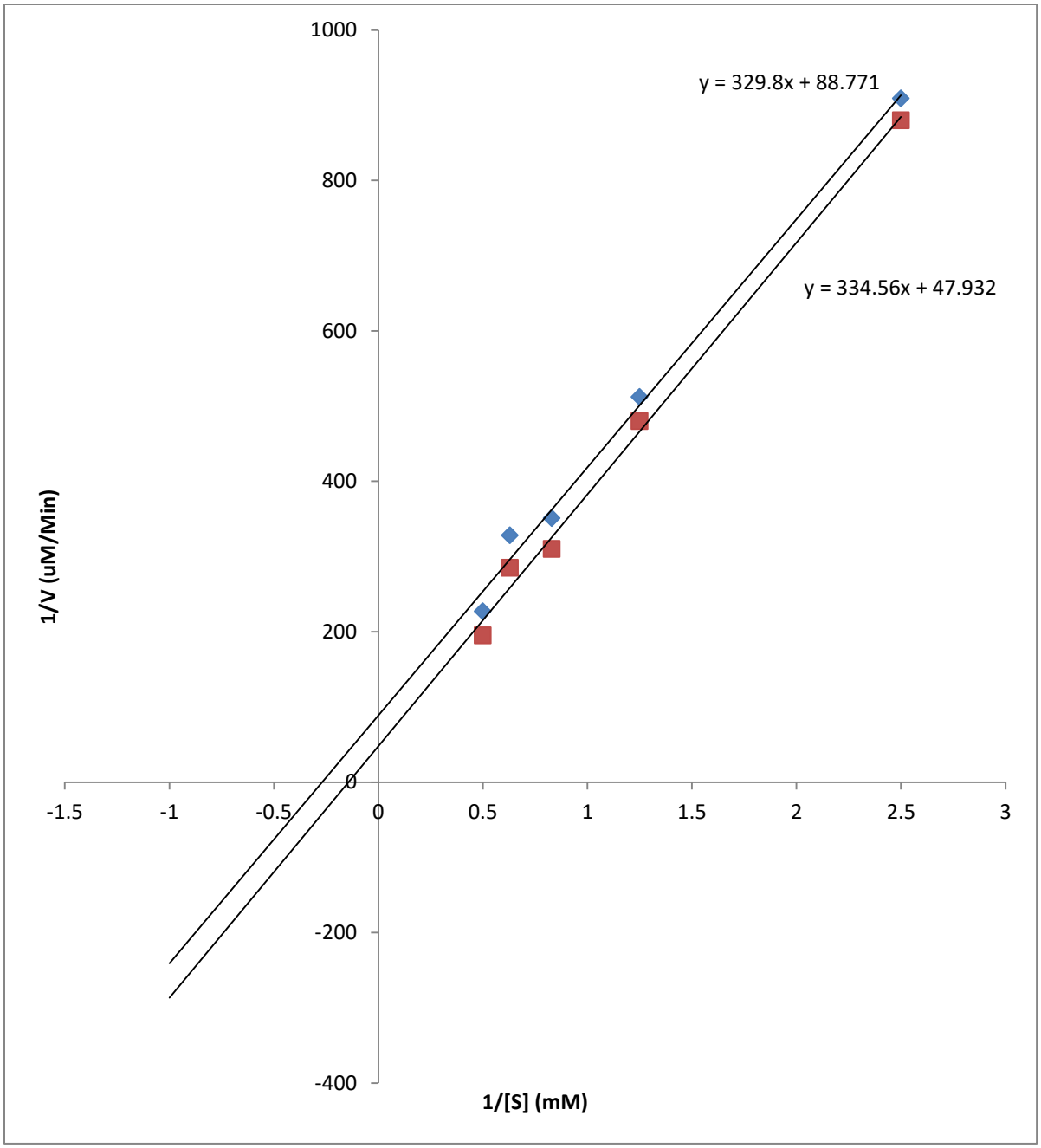
Absorbance Readings @ 420nm				
S/N	Experiment 1	Experiment 2	Experiment 3	Mean
1.	0.052	0.053	0.054	0.053
2.	0.107	0.115	0.12	0.111
3.	0.228	0.228	0.228	0.228
4.	0.269	0.271	0.27	0.27
5.	0.336	0.334	0.335	0.335

Table 4.5.3

Extrapolation Table 2.0 (With $MgSO_4$)

Tubes	Absorbance @ 405nm	PNP (uM)	V (uM/Min)	1/V $(uM/Min)^{-1}$	S (uM)	1/S (uM^{-1})
1.	0.000	0.000	0.00	0.000	0.0	0.0
2.	0.053	0.019	0.00095	1053	0.4	2.5
3.	0.111	0.036	0.0018	556	0.8	1.25
4.	0.227	0.079	0.00395	253	1.2	0.83
5.	0.269	0.093	0.00465	215	1.6	0.63
6.	0.336	0.116	0.0058	172.4	2.0	0.5

Table 4.5.4



Remark: Positive Modulator

4.5.3 Effect of Pi on ACP

Triplicate values

Taking the average Absorbance value:

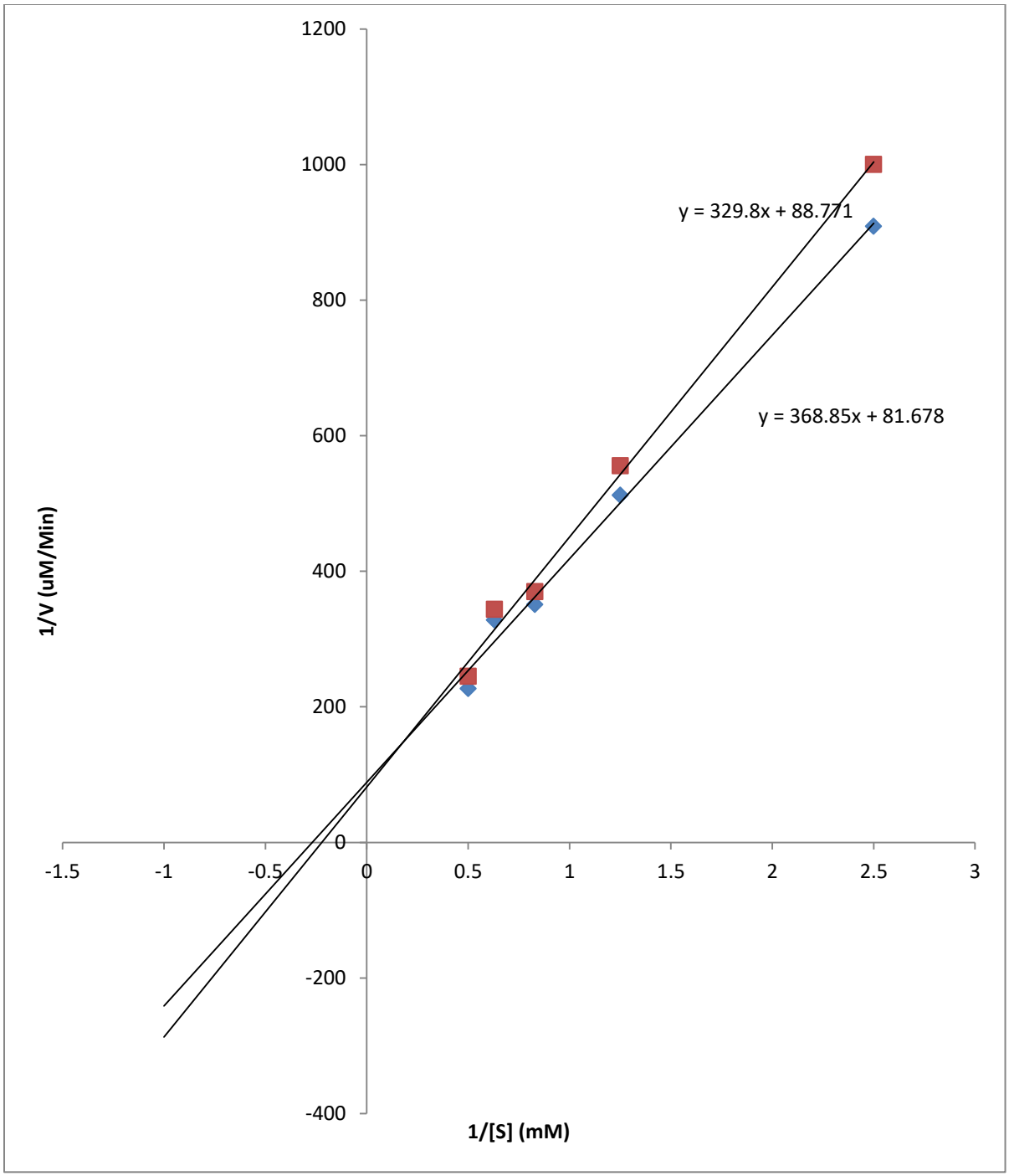
S/N	Experiment 1	Experiment 2	Experiment 3	Mean
1.	0.058	0.06	0.059	0.059
2.	0.104	0.106	0.105	0.105
3.	0.152	0.153	0.154	0.153
4.	0.164	0.166	0.165	0.165
5.	0.238	0.241	0.244	0.241

Table 4.5.5

Extrapolation Table (With PI)

Tubes	Absorbance @ 405nm	PNP (uM)	V (uM/Min)	1/V $(uM/Min)^{-1}$	S (uM)	1/S (uM^{-1})
1.	0.000	0.000	0.000	0.0	0.0	0.0
2.	0.059	0.021	0.00105	952	0.4	2.5
3.	0.11	0.039	0.00195	513	0.8	1.25
4.	0.153	0.054	0.0027	370	1.2	0.83
5.	0.165	0.058	0.0029	345	1.6	0.63
6.	0.241	0.084	0.0042	238	2.0	0.5

Table 4.5.6



Remark: Competitive

4.6 Discussion of Results

4.6.1 Effect of pH on both ALP and ACP

At the end of the experiment, the optimum pH at which ALP and ACP perform at their highest peak was observed to be at pH 9.5 and pH 3.5 respectively aligning with a study conducted (Millan, 2006), who observed similar results using plant tissues.

4.6.2 Effect of Temperature on both ALP and ACP

ALP and ACP were observed to perform maximum at the temperature of 40 ^{0}C and 45 ^{0}C respectively which also aligns with the study conducted by (Millan, 2006).

4.6.3 Effect of Mg^{2+} on ALP and ACP

Mg^{2+} are known activators of ALP, they play an important role in stabilizing the enzyme structure and facilitating the binding of the substrate to the active site. ALP requires metal ions, specifically divalent ions like Mg^{2+} and Zn^{2+} for optimal activity (McComb et al., 1979).

Mg^{2+} stabilizes the enzyme-substrate complex and help in positioning the phosphate group of the substrate for nucleophillic attack and also aids in the release of the phosphate product after hydrolysis. In the course of the experiment, it was observed that Mg^{2+} improved the activity of ALP, hence it can be considered a positive modulator or activator of ALP.

ACP on the other hand does not require Mg^{2+} for its activity and may even experience inhibition at high concentrations of Mg^{2+}. But in this study Mg^{2+} was observed to increase the activity of ACP so it can be considered a positive modulator and not an activator as it does not require Mg^{2+} for its activation.

4.6.4. Effect of Pi on ALP and ACP

Pi acts as a competitive or non-competitive inhibitor of ALP depending on its concentration and binding mode. Since ALP catalysis the hydrolysis of phosphate esters, the accumulation of free phosphate can cause product inhibition, reducing enzyme activity. Pi may compete with the substrate for binding at the active site or bind to an allosteric site, affecting enzyme conformation. Pi was observed to inhibit the activity of ALP in this study, it may be considered a competitive inhibitor (Coleman, 1992).

ACP on the other hand is strongly inhibited by Pi, primarily through competitive inhibition, Pi was observed to inhibit ACP activity more than ALP activity in this study. Pi is considered a competitive inhibitor to both ALP and ACP of Plantain Bract.

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APPENDIX

One Way Anova

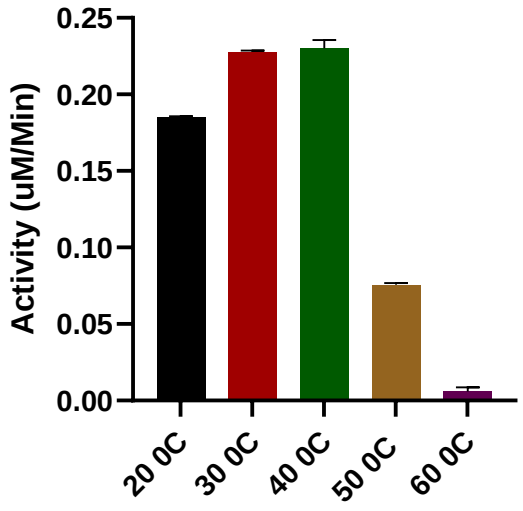
Effect of Temperature on Alkaline Phosphatase Activity

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.1593	4	0.03982	F (1.438, 4.314) = 4599	P<0.0001
Individual (between rows)	0.00001227	3	0.000004091	F (3, 12) = 0.4725	P=0.7071
Residual (random)	0.0001039	12	0.000008658		
Total	0.1594	19			

From the data analyzed, there is significant difference 0.1594 between pH values activity.

Descriptive statistics

Number of values	4	4	4	4	4
Number of missing values	0	0	0	0	0
Minimum	0.185	0.227	0.223	0.075	0.003
25% Percentile	0.185	0.227	0.2245	0.075	0.0035
Median	0.1852	0.2275	0.2305	0.0755	0.006
75% Percentile	0.1858	0.2288	0.235	0.07675	0.0085
Maximum	0.186	0.229	0.236	0.077	0.009
Mean	0.1853	0.2278	0.23	0.07575	0.006
Std. Deviation	0.0004717	0.0009574	0.005477	0.0009574	0.002582
Std. Error of Mean	0.0002358	0.0004787	0.002739	0.0004787	0.001291
Lower 95% CI	0.1846	0.2262	0.2213	0.07423	0.001891
Upper 95% CI	0.1861	0.2293	0.2387	0.07727	0.01011



According the chart obtain from the analyzed data, the optimum temperature of alkaline phosphatase is around 35 to 40 degrees

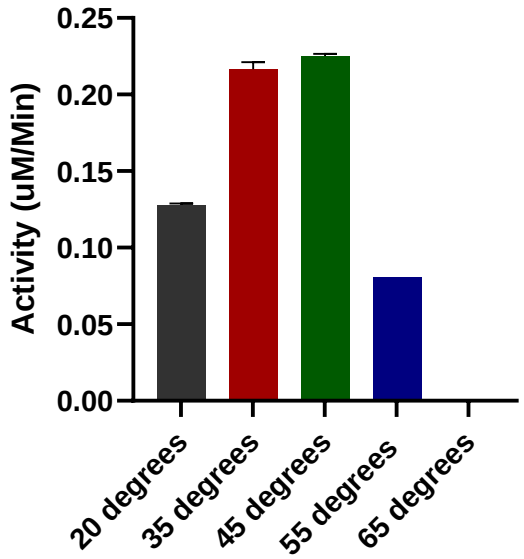
Effect of Temperature on Acid Phosphatase Activity

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.1432	4	0.03581	F (4, 15) = 6976	P<0.0001
Residual (within columns)	0.000077	15	0.000005133		
Total	0.1433	19			

From the data analyzed, there is significant difference 0.1594 between pH values activity.

Descriptive statistics

	20 degrees	35 degrees	45 degrees	55 degrees	65 degrees
Number of values	4	4	4	4	4
Minimum	0.127	0.21	0.223	0.081	0
25% Percentile	0.1273	0.2115	0.2235	0.081	0
Median	0.128	0.218	0.225	0.081	0
75% Percentile	0.1288	0.22	0.2265	0.081	0
Maximum	0.129	0.22	0.227	0.081	0
Mean	0.128	0.2165	0.225	0.081	0
Std. Deviation	0.0008165	0.004726	0.001633	0	0
Std. Error of Mean	0.0004082	0.002363	0.000817	0	0
Lower 95% CI	0.1267	0.209	0.2224	0.081	0
Upper 95% CI	0.1293	0.224	0.2276	0.081	0



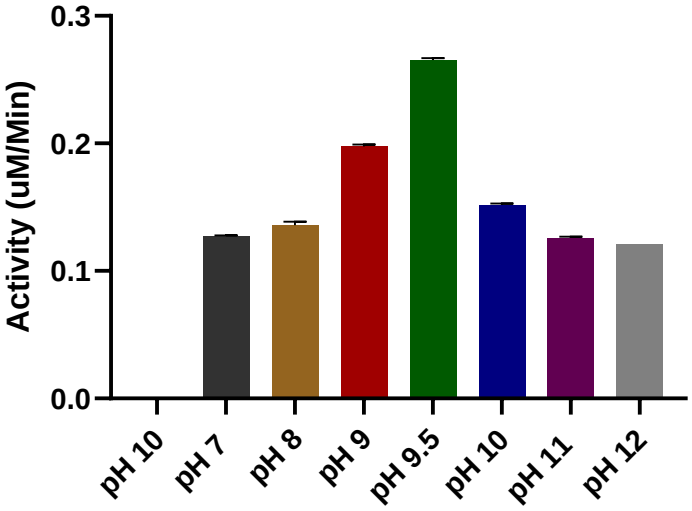
According to the analyzed data from the bar chart above, the optimum temperature of acid phosphatase in plantain flower bract is 40 to 45 degrees

Effect of pH on Alkaline Phosphatase Activity

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.1185	7	0.01693	F (7, 16) = 9675	P<0.0001
Residual (within columns)	0.000028	16	0.00000175		
Total	0.1186	23			

Descriptive statistics

	pH 10	pH 7	pH 8	pH 9	pH 9.5	pH 10	pH 11	pH 12
Number of values	3	3	3	3	3	3	3	3
Minimum	0	0.127	0.133	0.196	0.264	0.151	0.125	0.121
25% Percentile	0	0.127	0.133	0.196	0.264	0.151	0.125	0.121
Median	0	0.127	0.137	0.198	0.265	0.152	0.126	0.121
75% Percentile	0	0.128	0.138	0.199	0.267	0.153	0.127	0.121
Maximum	0	0.128	0.138	0.199	0.267	0.153	0.127	0.121
Mean	0	0.1273	0.136	0.1977	0.2653	0.152	0.126	0.121
Std. Deviation	0	0.000577	0.002646	0.001528	0.001528	0.001	0.001	0
Std. Error of Mean	0	0.000333	0.001528	0.000882	0.000882	0.000577	0.000577	0
Lower 95% CI	0	0.1259	0.1294	0.1939	0.2615	0.1495	0.1235	0.121
Upper 95% CI	0	0.1288	0.1426	0.2015	0.2691	0.1545	0.1285	0.121

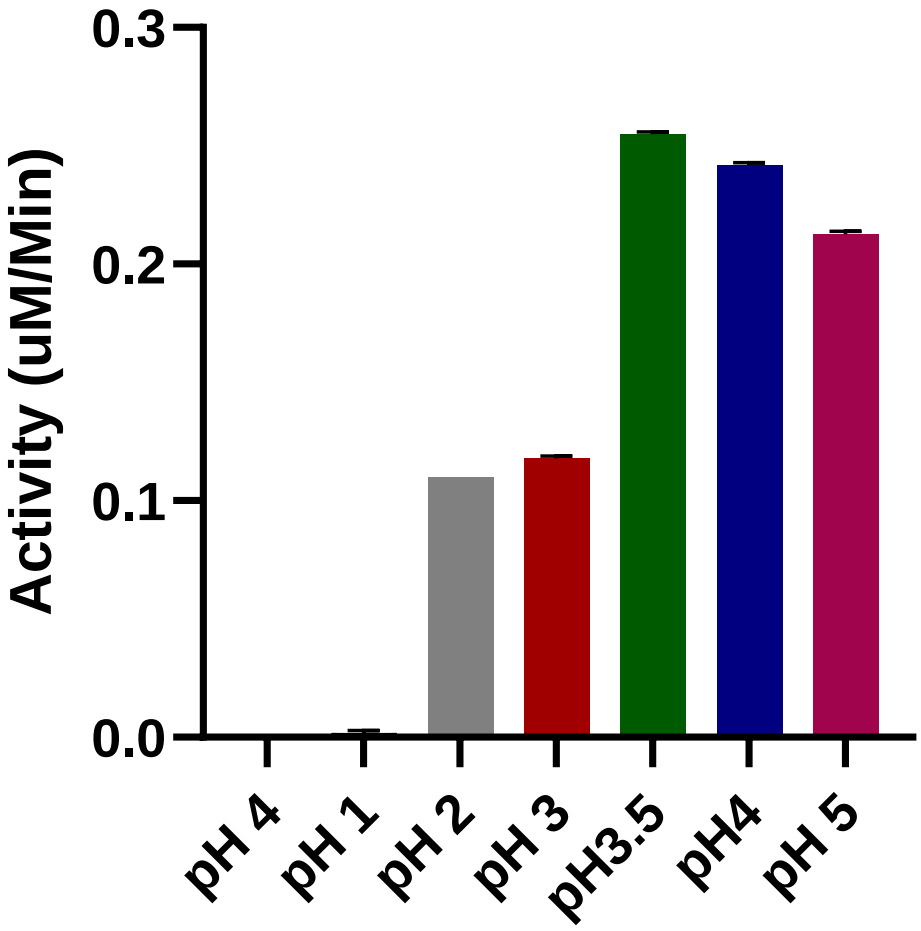

Activity (uM/Min)
0.3
0.2
0.1
0.0
pH 10
pH 7
pH 8
pH 9
pH 9.5
pH 10
pH 11
pH 12

Effect of pH on Acid Phosphatase Activity

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.2061	6	0.03435	F (6, 14) = 45088	P<0.0001
Residual (within columns)	0.00001067	14	7.619E-07		
Total	0.2061	20			

Descriptive statistics

	pH 4	pH 1	pH 2	pH 3	pH3.5	pH4	pH 5
Number of values	3	3	3	3	3	3	3
Minimum	0	0.001	0.11	0.117	0.254	0.241	0.212
25% Percentile	0	0.001	0.11	0.117	0.254	0.241	0.212
Median	0	0.002	0.11	0.118	0.255	0.242	0.212
75% Percentile	0	0.003	0.11	0.119	0.256	0.243	0.214
Maximum	0	0.003	0.11	0.119	0.256	0.243	0.214
Mean	0	0.002	0.11	0.118	0.255	0.242	0.2127
Std. Deviation	0	0.001	0	0.001	0.001	0.001	0.001155
Std. Error of Mean	0	0.000577	0	0.000577	0.000577	0.000577	0.000667
Lower 95% CI	0	-0.00048	0.11	0.1155	0.2525	0.2395	0.2098
Upper 95% CI	0	0.004484	0.11	0.1205	0.2575	0.2445	0.2155


Activity (uM/Min)
0.3
0.2
0.1
0.0
pH 4
pH 1
pH 2
pH 3
pH3.5
pH4
pH 5

Effect of Pi and $MgSO_4$ on Alkaline Phosphatase Activity

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.02039	8	0.002549	F (8, 30) = 4.468	P=0.0012
Row Factor	0.3081	4	0.07702	F (4, 30) = 135.0	P<0.0001
Column Factor	0.06967	2	0.03484	F (2, 30) = 61.06	P<0.0001
Residual	0.01712	30	0.0005706		

Descriptive statistics

Means	ALK Normal Without Mg and Pi	ALK With MgSO4	ALK With Pi	Row means
Row 1	0.09	0.098	0.08	0.08933
Row 2	0.145	0.17	0.051	0.122
Row 3	0.195	0.2717	0.17	0.2122
Row 4	0.225	0.34	0.22	0.2617
Row 5	0.295	0.375	0.258	0.3093
Column means	0.19	0.2509	0.1558	0.1989

Multiple Comparison

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	N 1	N 2	q	DF
Row 1:ALK Normal Without Mg and Pi vs. Row 1:ALK With MgSO4	0.09	0.098	-0.008	0.0195	3	3	0.4102	30
Row 1:ALK Normal Without Mg and Pi vs. Row 1:ALK With Pi	0.09	0.08	0.01	0.0195	3	3	0.5127	30
Row 1:ALK Normal Without Mg and Pi vs. Row 2:ALK Normal Without Mg and Pi	0.09	0.145	-0.055	0.0195	3	3	2.82	30
Row 1:ALK Normal Without Mg and Pi vs. Row 2:ALK With MgSO4	0.09	0.17	-0.08	0.0195	3	3	4.102	30
Row 1:ALK Normal Without Mg and Pi vs. Row 2:ALK With Pi	0.09	0.051	0.039	0.0195	3	3	2	30
Row 1:ALK Normal Without Mg and Pi vs. Row 3:ALK Normal Without Mg and Pi	0.09	0.195	-0.105	0.0195	3	3	5.384	30
Row 1:ALK Normal Without Mg and Pi vs. Row 3:ALK With MgSO4	0.09	0.2717	-0.1817	0.0195	3	3	9.316	30
Row 1:ALK Normal Without Mg and Pi vs. Row 3:ALK With Pi	0.09	0.17	-0.08	0.0195	3	3	4.102	30
Row 1:ALK Normal Without Mg and Pi vs. Row 4:ALK Normal Without Mg and Pi	0.09	0.225	-0.135	0.0195	3	3	6.922	30
Row 1:ALK Normal Without Mg and Pi vs. Row 4:ALK With MgSO4	0.09	0.34	-0.25	0.0195	3	3	12.82	30
Row 1:ALK Normal Without Mg and Pi vs. Row 4:ALK With Pi	0.09	0.22	-0.13	0.0195	3	3	6.665	30
Row 1:ALK Normal Without Mg and Pi vs. Row 5:ALK Normal Without Mg and Pi	0.09	0.295	-0.205	0.0195	3	3	10.51	30
Row 1:ALK Normal Without Mg and Pi vs. Row 5:ALK With MgSO4	0.09	0.375	-0.285	0.0195	3	3	14.61	30
Row 1:ALK Normal Without Mg and Pi vs. Row 5:ALK With Pi	0.09	0.258	-0.168	0.0195	3	3	8.614	30

Narrative Analysis

Data analyzed: Mg and Pi

Source of Variation	Degrees of Freedom	Sum of Squares	Mean square
Column Factor	2	0.06967	0.03484
Row Factor	4	0.3081	0.07702
Interaction	8	0.02039	0.002549
Residual (error)	30	0.01712	0.0005706
Total	44	0.4153	

Does Column Factor have the same effect at all values of Row Factor?

Interaction accounts for 4.911% of the total variance.

F = 4.47. DFn = 8, DFd = 30

The P value = 0.0012

If there is no interaction overall, there is a 0.12% chance of randomly observing so much interaction in an experiment of this size. The interaction is considered very significant.

Since the interaction is statistically significant, the P values that follow for the row and column effects are difficult to interpret.

Does Column Factor affect the result?

Column Factor accounts for 16.78% of the total variance.

F = 61.06. DFn = 2, DFd = 30

The P value is < 0.0001

If Column Factor has no effect overall, there is a less than 0.01% chance of randomly observing an effect this big (or bigger) in an experiment of this size. The effect is considered extremely significant.

Effect of Pi and $MgSO_4$ on Acid Phosphatase Activity

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Interaction	0.01982	8	0.002477	F (8, 30) = 53.06	P<0.0001
Row Factor	0.2548	4	0.06369	F (4, 30) = 1364	P<0.0001
Column Factor	0.02685	2	0.01342	F (2, 30) = 287.5	P<0.0001
Residual	0.001401	30	0.00004669		

Descriptive Statistics

Means	ACP Normal Without Mg and Pi	ACP WITH MgSO4	ACP With Pi	Row means
Row 1	0.06	0.053	0.059	0.05733
Row 2	0.1167	0.114	0.105	0.1119
Row 3	0.162	0.228	0.153	0.181
Row 4	0.175	0.27	0.165	0.2033
Row 5	0.25	0.335	0.241	0.2753
Column means	0.1527	0.2	0.1446	0.1658