

KINETICS STUDY OF ALKALINE PHOSPHATASE EXTRACTED FROM
Agama agama **LIVER IN THE PRESENCE OF MAGNESIUM IONS**



PROJECT REPORT

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Biochemistry

BY

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CERTIFICATION

This is to certify that the research project have been conducted by OCHEPO,MarthaOjumeysi(2010304024) and has been approved as meeting part of the requirements for the award of Bachelor of Science (B.Sc) degree in Biochemistry of Usmanu Danfodiyo University, Sokoto.

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DEDICATION

This research project is dedicated to God Almighty, whose grace, wisdom, unconditional love, mercy and strength have guided me throughout my academics journey.

I also dedicate this research project to my beloved parents and siblings, whose love, sacrifice, and unwavering support have been my greatest source of inspiration and to my family and friends, who encouraged me through every challenge in my academics journey, this accomplishment is as much as yours as it is mine.

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ABSTRACT

Alkaline phosphatase (ALP; EC 3.1.3.1) is a metalloenzyme that catalyzes the hydrolysis of phosphate esters under alkaline conditions, playing a vital role in phosphate metabolism, signal transduction, and bone mineralization and is influenced by divalent metal ions such as magnesium (Mg^{2+}). It is widely expressed in tissues such as bone, liver, kidney, and intestine, with distinct isoforms including tissue-nonspecific ALP, intestinal ALP, placental ALP, and germ cell ALP and widely used as a biomarker in clinical diagnostics, particularly for liver and bone disorders, due to its tissue-specific isoforms and sensitivity to physiological changes. Kinetics study of alkaline phosphatase (ALP) extracted from *Agama agama* liver in the presence of magnesium ions investigated the kinetic effects of magnesium ions (Mg^{2+}) on ALP extracted from the liver of *Agama agama*. ALP activity was measured using para-nitrophenyl phosphate (p-NPP) as substrate, monitoring the product para-nitrophenol spectrophotometrically at 405nm. ALP kinetics were assessed at varying p-NPP concentrations in the absence and presence of different Mg^{2+} concentrations (0.0312, 0.1560, and 0.7800)mg/L. Results showed that in the absence of Mg^{2+} , ALP exhibited moderate activity with a K_m of 0.3923mM and V_{max} of 0.0237 μ mol/min. Addition of Mg^{2+} at low concentration (0.0312mg/L) significantly increased enzyme activity, with V_{max} rising to 0.0992 μ mol/min despite an increased K_m of 1.6416mM, indicating enhanced catalytic efficiency but reduced substrate affinity. Higher Mg^{2+} concentrations (0.1560mg/L, 0.7800mg/L) gradually inhibited ALP activity, with decreases in both V_{max} and K_m , suggesting mixed activation and inhibition effects. The activation constant (K_a) was determined as 1.8359mM. Lineweaver-Burk plots confirmed these kinetic changes. These findings highlight Mg^{2+} as a critical modulator of ALP activity in reptiles, acting as an activator at low concentrations and an inhibitor at higher levels, consistent with its cofactor role observed in other species, highlighting its dual regulatory role. This study provides insight into reptilian enzyme regulation and contributes to comparative biochemical understanding of metal ion enzyme interactions.

Keywords: Alkaline Phosphatase (ALP), *Agama agama*, Para-nitrophenyl phosphate, Magnesium (Mg^{2+}) ions, UV-Vis spectrophotometer, and Lineweaver-Burk plot.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF THE STUDY.

Alkaline phosphatase (ALP; EC 3.1.3.1) is a ubiquitous enzyme found in various tissues across vertebrates, including the liver, bone, kidney, and intestine. It is a hydrolytic enzyme that catalyzes the hydrolysis of phosphate esters under alkaline conditions, playing a vital role in phosphate metabolism, signal transduction, and bone mineralization (Harroun and Vallée-Bélisle, 2023). The activity of ALP is known to be influenced by several factors, including pH, temperature, substrate concentration, and the presence of metal ion such as Mg^{2+} . It is widely expressed in tissues such as bone, liver, kidney, and intestine, with distinct isoforms including tissue-nonspecific ALP, intestinal ALP, placental ALP, and germ cell ALP and widely used as a biomarker in clinical diagnostics, particularly for liver and bone disorders, due to its tissue-specific isoforms and sensitivity to physiological changes (Cannalireet *al.*, 2023).

In reptiles, particularly *Agama agama*, a common lizard species in West Africa, the liver serves as a central organ for metabolic regulation and detoxification. The study of ALP in this species offers a unique opportunity to explore enzymatic behavior in ectothermic vertebrates, which are often underrepresented in biochemical research. Reptilian enzymes may exhibit distinct kinetic properties due to evolutionary adaptations, environmental pressures, and metabolic strategies that differ from those of mammals (Abubakar *et al.*, 2013). ALP activity is modulated by divalent cations, notably magnesium and zinc, and may itself influence magnesium homeostasis by altering phosphate gradients and interacting with magnesium transporters (Nieet *al.*, 2020).

Magnesium ions (Mg^{2+}) are essential cofactors for numerous enzymatic reactions, including those catalyzed by ALP. As divalent cations, magnesium functions as an indispensable

cofactor in hundreds of intracellular enzymatic reactions (Fiorentini *et al.*, 2021). They influence enzyme conformation, substrate binding, and catalytic efficiency by stabilizing the enzyme-substrate complex and facilitating nucleophilic attack on phosphate groups (Nie *et al.*, 2020). The interaction between Mg^{2+} and ALP is particularly important in understanding enzyme regulation and optimizing assay conditions for both research and clinical applications.

Previous studies have demonstrated that the presence of magnesium ions can significantly enhance ALP activity in mammalian systems (Rej, 1977). However, limited data exist on the effect of Mg^{2+} on ALP activity in reptiles, especially in *Agama agama*. This gap in knowledge presents an opportunity to investigate the kinetic parameters Michaelis-Menten constant (K_m) and maximum velocity (V_{max}) of ALP in the presence of magnesium ions, thereby contributing to the broader field of comparative enzymology.

1.2 STATEMENT OF THE PROBLEM.

Despite the biological significance of alkaline phosphatase and its widespread use in diagnostics, there is a notable lack of research on its kinetic behavior in reptiles. Most existing studies focus on mammalian systems, leaving a gap in our understanding of enzyme function in ectothermic vertebrates. Specifically, the effect of magnesium ions on ALP activity in *Agama agama* has not been thoroughly investigated.

This gap limits the applicability of current enzymatic models to reptiles and hinders the development of species specific diagnostic and therapeutic approaches. Without detailed kinetic data, it is difficult to assess the physiological role of ALP in reptilian metabolism or to compare its activity across taxa. Therefore, there is a pressing need to study the kinetics of ALP from *Agama agama* liver in the presence of magnesium ions.

1.3 JUSTIFICATION OF THE STUDY.

Reptiles, such as *Agama agama*, offer valuable insights into enzyme function under different physiological and environmental conditions. Understanding the kinetics of ALP in *Agama agama* can inform ecological physiology, evolutionary biology, and veterinary diagnostics.

Moreover, magnesium ions are known to modulate enzyme activity, but their specific effects on reptilian ALP remain poorly understood. By characterizing the influence of Mg^{2+} on ALP kinetics, this study can contribute to the optimization of enzymatic assays and the development of more accurate diagnostic tools. It also provides foundational data for future research on enzyme cofactor interactions in non-mammalian species.

The study is particularly relevant in the context of increasing interest in biodiversity and the biochemical uniqueness of lesser studied organisms. As reptiles face environmental challenges and habitat loss, understanding their physiology at the molecular level becomes crucial for conservation and health monitoring.

1.4 AIM AND OBJECTIVES OF THE STUDY

1.4.1 AIM

The aim of this study was to evaluate the kinetic parameters of alkaline phosphatase isolated from the liver of *Agama agama* in the presence of magnesium ions.

1.4.2 OBJECTIVES

The specific objectives were;

- I. To isolate alkaline phosphatase from the liver tissue of *Agama agama*.

- II. To determine the kinetic parameters (K_m and V_{max}) of the enzyme using para-nitrophenyl phosphate (pNPP) as a substrate.
- III. To evaluate the effect of varying concentrations of magnesium ions on the alkaline phosphatase's activity.

CHAPTER TWO

2.0 LITERATURE REVIEW.

Enzymes are highly specialized protein molecules that serve as catalysts in nearly all biological processes. From cellular respiration to DNA replication, enzymes accelerate chemical reactions by reducing the activation energy required for those reactions to proceed. Unlike inorganic catalysts, enzymes operate under mild conditions of temperature and pH and exhibit remarkable specificity for their substrates. This specificity and efficiency have made them central to biochemical studies, industrial bioprocesses, and pharmaceutical development (Nelson and Cox, 2017).

The study of enzyme kinetics focuses on the rates of enzymatic reactions and the factors that influence these rates, such as substrate concentration, enzyme concentration, temperature, and pH. The Michaelis-Menten model, a cornerstone of enzyme kinetics, provides a quantitative framework for understanding enzyme substrate interactions (Widmer *et al.*, 2013).

Enzymes and the reactions that they catalyse are critical to a wide range of fields. Beyond their important functions in biology, domains such as organic synthesis, nanotechnology, medicine, and industries including food and biofuel production are just a few of the many applications that utilise enzymes (Buller *et al.*, 2023). Among the various types of enzymes, the alkaline phosphatase family (ALP; EC 3.1.3.1) has attracted enormous research interest. Investigation of this enzyme ranges from employing microbial ALPs in industrial biotechnology, to exploring its biological role in animals, and studying human ALPs for health applications (Le-Vinh *et al.*, 2022). Substantial efforts have been devoted to characterising the four iso-enzymes of ALP present in humans: intestinal alkaline phosphatase (IALP), placental alkaline phosphatase (PLALP), placental-like or germ cell alkaline phosphatase (GCALP), and tissue-nonspecific alkaline phosphatase (TNALP) found

throughout the body but mainly in the liver, kidney, and bones (Sharma *et al.*, 2014). These and other ALPs have a conserved active site that can dephosphorylate phosphate monoesters and other phosphate-containing substrate molecules to yield inorganic phosphate (Pi) and an alcohol or a phenol.

Enzyme kinetics is a fundamental branch of biochemistry that explores the rates at which enzyme-catalyzed reactions proceed and the factors influencing these rates. Enzymes, as biological catalysts, accelerate chemical reactions critical for metabolic processes by lowering the activation energy required for substrate conversion (StudySmarter, 2024).

Enzyme kinetics is the concept of enzyme activity, which quantitatively describes the catalytic capability of an enzyme under defined conditions. Factors such as substrate concentration, enzyme concentration, pH, temperature, and the presence of inhibitors or activators modulate enzyme activity (Robinson, 2015). These parameters not only govern catalytic efficiency but also determine enzyme functionality in physiological and industrial settings.

Enzyme specificity is another key aspect of enzyme kinetics, referring to the selective interaction between enzyme and substrate. This specificity arises from the unique structural complementarity between an enzyme's active site and its substrate, guiding the enzyme to catalyze only particular reactions or substrates (Lewis and Stone, 2023). Understanding specificity is vital for designing enzyme inhibitors and modifying enzyme selectivity to suit various applications.

The Michaelis-Menten kinetic model remains central to interpreting enzyme behavior, describing how reaction velocity depends on substrate concentration. The parameters K_m (Michaelis-Menten constant) and V_{max} (maximum velocity) are crucial quantifying enzyme efficiency and substrate affinity (Berg *et al.*, 2023).

2.1 STRUCTURE AND FUNCTION OF ALKALINE PHOSPHATASE.

Alkaline phosphatase (ALP, EC 3.1.3.1) is a widely distributed membrane-bound glycoprotein enzyme that catalyzes the hydrolysis of phosphate monoesters under alkaline conditions, releasing inorganic phosphate. It exists in different isoforms, including tissue specific types intestinal, placental, germ cell and a tissue-nonspecific isoform predominantly found in the liver, bone, and kidney (L/B/K ALP) (Sharma *et al.*, 2014).

Structurally, ALP is typically a dimeric enzyme with each subunit containing around 428-429 amino acids, stabilized by intra-chain disulfide bonds and coordinated metal ions such as zinc and magnesium at its active site (Makris *et al.*, 2023). These metal ions are critical for catalytic activity and structural stability. Recent studies on bacterial and mammalian isoforms have detailed the enzyme's tertiary and quaternary structures, elucidating the mechanism of substrate binding and catalysis through a phosphoseryl intermediate (Le-Vinh *et al.*, 2022).

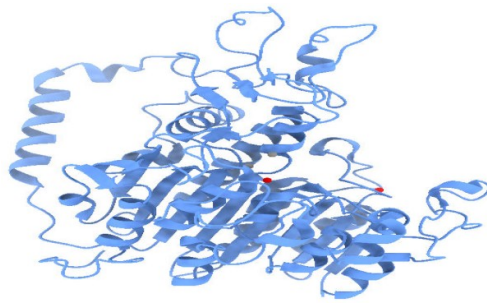


Figure1: Three dimensional structure of alkaline phosphatase (Le-Vinh *et al.*, 2022).

Functionally, ALP plays a crucial role in physiological processes, particularly in bone mineralization, membrane transport, biomolecule dephosphorylation and phosphate metabolism (Sharma *et al.*, 2014). The tissue-nonspecific isoform regulates the local concentration of inorganic phosphate by hydrolyzing pyrophosphate, a mineralization inhibitor, thereby promoting hydroxyapatite crystal formation essential for skeletal

development and maintenance (Vimalraj, 2020). Intestinal ALP contributes to phosphate absorption and intestinal health, while placental ALP has roles in fetal development (Sharma *et al.*, 2014). TNAP deficiency caused by genetic mutations can thus result in hypophosphatasia of bones/teeth and elevated levels of PP_i in plasma and urine (Kühn *et al.*, 2021) TNAP also converts pyridoxal 5'-phosphate (PLP), the active form of Vitamin B₆ and a vital coenzyme, to pyridoxal to enable its diffusion across cellular membranes. After this, it can be phosphorylated once again. An absence of TNAP in the brain can impede neurotransmitter synthesis, thereby causing epileptic seizures in infants with hypophosphatasia (Bartlett *et al.*, 2022). Phosphoethanolamine (PEA) is also a putative natural substrate of TNAP, but its purpose remains unknown. Recently, phosphocholine has been proposed too as a natural substrate of liver TNAP (Bessueille *et al.*, 2023).

ALP is also notable in clinical diagnostics as a biomarker for liver and bone diseases due to its differential tissue expression and elevated serum levels in pathological conditions (Rader, 2017). Emerging research highlights ALP's broader biological functions, including immune modulation and involvement in pathological calcification, inviting further exploration into its molecular mechanisms and therapeutic potential (Rader, 2017; Shaban *et al.*, 2022).

2.2 SOURCES AND BIOCHEMICAL PROPERTIES OF ALP IN REPTILES.

Alkaline phosphatase (ALP) is a ubiquitous enzyme involved in phosphate metabolism, with key physiological roles including mineralization and membrane transport (Sharma *et al.*, 2014). While extensively studied in mammals, insights into ALP in reptiles remain comparatively limited but are growing through recent biochemical and comparative studies.

In reptiles, ALP exists as multiple iso-enzymes expressed across various tissues, including liver, bone, kidney, and intestine, mirroring the distribution seen in other vertebrates (Sharma *et al.*, 2014). These iso-enzymes contribute to critical physiological processes such as skeletal development and phosphate homeostasis, essential due to the reptiles' reliance on calcium

and phosphate for bone mineralization and shell formation in species like turtles (Vimalraj, 2020). Tissue-specific ALP isoenzymes in reptiles show distinct electrophoretic patterns and differential regulation based on developmental stage and environmental conditions, reflecting adaptation to ectothermic metabolism.

Biochemically, reptilian ALP shares core properties with mammalian counterparts, including optimal activity in alkaline pH ranges (8-10) and dependence on divalent metal ions such as zinc and magnesium at the active site for catalysis and structural stability (Makris *et al.*, 2023). Reptile ALP generally exhibits heat stability and kinetic parameters similar to mammalian ALP, but some studies suggest slight variations in substrate specificity and inhibitor sensitivity, possibly related to species-specific adaptations (Le-Vinh *et al.*, 2022).

The enzyme's molecular weight in reptiles approximates 86 kDa per subunit as seen in mammals, consistent with a dimeric structure stabilized by disulfide bridges and metal cofactors (Makris *et al.*, 2023). Functional assays confirm that reptilian ALP hydrolyzes a broad range of phosphate esters, releasing inorganic phosphate, vital for energy metabolism and bone mineralization processes (Vimalraj, 2020).

2.3MECHANISM OF ACTION OF ALKALINE PHOSPHATASE.

Alkaline phosphatase (ALP) catalyzes the hydrolysis of phosphate monoesters, releasing inorganic phosphate and an alcohol, and is a prototypical metalloenzyme that employs a multi-metal ion catalytic center for its function (Bobyry *et al.*, 2012). The enzyme is structurally characterized by a homodimeric configuration, each subunit harboring active sites containing two zinc ions and one magnesium ion essential for catalysis and structural integrity (Bobyry *et al.*, 2012).

The catalytic mechanism proceeds via a two-step process involving the formation of a covalent phosphoseryl intermediate. Initially, the nucleophilic attack on the substrate's phosphorus atom is performed by a serine residue in the active site, facilitated by the

coordinated metal ions that stabilize the transition state and polarize the phosphate group (Borosky *et al.*, 2011). This serine nucleophile displacement leads to the release of the alcohol product and formation of the phosphoseryl enzyme intermediate.

Subsequently, the intermediate undergoes hydrolysis as a water molecule, activated by the metal ions, attacks the phosphoserine bond, releasing inorganic phosphate and regenerating the free enzyme (Zhang *et al.*, 2021). The enzymatic preference for phosphate monoester hydrolysis over similar substrates is governed not solely by metal ion electrostatics but also by precise positioning of hydrogen bonding and dipolar interactions in the active site, which confer high substrate specificity and catalytic efficiency (Andrews *et al.*, 2014).

This double-displacement mechanism facilitated by a trinuclear metal center and a covalent intermediate is supported by high-resolution crystallographic data and mutagenesis studies, making ALP a paradigm for understanding metalloenzyme catalysis (Zhang *et al.*, 2021).

2.4 METAL ION ACTIVATION OF ALKALINE PHOSPHATASE.

Alkaline phosphatase (ALP) is a metalloenzyme whose catalytic efficiency critically depends on the presence and coordination of metal ions at its active site. Primarily, ALP harbors two zinc ions and one magnesium ion per catalytic site, which are indispensable for enzymatic function (Le-Vinh *et al.*, 2022). These metal ions play distinct yet cooperative roles in substrate binding, catalysis, and structural stabilization.

The two zinc ions function directly in catalysis by stabilizing the transition state and coordinating the phosphate group of the substrate. Zinc ion 1 (Zn1) polarizes the phosphate ester bond and stabilizes the leaving group, whereas zinc ion 2 (Zn2) stabilizes the pentacoordinate transition state phosphorus atom (Zhang *et al.*, 2021). Meanwhile, the magnesium ion (Mg^{2+}) plays both catalytic and structural roles by activating the nucleophilic

water molecule for the hydrolysis step and stabilizing enzyme conformation (Le-Vinh *et al.*, 2022).

Recent kinetic and structural analyses emphasize that Mg^{2+} binding facilitates conformational changes in the enzyme, enhancing substrate turnover and catalytic rates (Le-Vinh *et al.*, 2022). Furthermore, other divalent metal ions such as Mn^{2+} , Co^{2+} , and Ni^{2+} can partially substitute for Mg^{2+} , activating ALP through similar conformational modulation, though with variations in catalytic efficiency (Le-Vinh *et al.*, 2022). Chelating agents like EDTA inhibit ALP by removing essential metal cofactors, thus confirming their crucial role in enzyme activity.

The interplay of these metal ions orchestrates a finely tuned mechanism, enabling ALP to achieve efficient phosphate ester hydrolysis under physiological conditions. Understanding this metal ion activation has implications for enzyme engineering, inhibitor design, and therapeutic targeting in diseases where ALP activity is dysregulated.

2.5 FACTORS INFLUENCING ENZYME KINETICS.

Factors influencing enzyme kinetics include pH, temperature, substrate availability, enzyme-substrate affinity and other factors such as inhibitor or activator.

2.5.1 pH INFLUENCE

Enzymes have an optimal pH at which their catalytic activity is maximal. Deviations from this optimal pH can alter the ionization state of amino acid residues at the active site or substrate, affecting binding and reaction rate. Extreme pH values can permanently change enzyme conformation or cause denaturation, reducing or abolishing activity (Robinson, 2015).

2.5.2 TEMPERATURE EFFECTS

Temperature strongly affects enzyme kinetics by influencing enzyme stability and molecular motion. Each enzyme has an optimal temperature where the reaction rate is highest. Increasing temperature enhances reaction rates up to a point but excessive heat can denature the enzyme, reducing activity. Temperature sensitivity can also interact with substrate levels; for some enzymes, the optimal temperature shifts with substrate availability (Tang and Riley, 2024).

2.5.3 SUBSTRATE AVAILABILITY AND BINDING AFFINITY

The concentration of substrate has a direct impact on enzyme kinetics, affecting the rate at which reactions proceed. High substrate availability tends to increase the reaction rate until enzyme saturation occurs. Additionally, the binding affinity between enzyme and substrate is a critical determinant of enzyme activity. Recent research highlights not only this affinity but also other factors like the Arrhenius prefactor, which relates to the frequency of successful collisions between enzyme and substrate, as important to enzyme function (Chiba *et al.*, 2024).

2.5.4 OTHER FACTORS

Additional influences include molecular structure changes induced by environmental conditions and factors such as inhibitors or activators that affect enzyme kinetics by interfering with substrate binding or catalytic activity (VanDee *et al.*, 2024)

2.6 KINETICS PARAMETERS OF ALKALINE PHOSPHATASE

Alkaline phosphatase (ALP) is an extensively studied enzyme with well-characterized kinetic parameters that are critical for understanding its catalytic efficiency and substrate affinity. The kinetic behavior of ALP is commonly described by Michaelis-Menten parameters,

including the maximum velocity ($V_{\max}$) and the Michaelis-Menten constant (K_m), which reflects the substrate concentration at half-maximal velocity (Hui *et al.*, 2013).

The maximum velocity ($V_{\max}$) denotes the catalytic turnover number of the enzyme under conditions of substrate saturation, reflecting the rate at which the enzyme converts substrate to product when all active sites are occupied. The Michaelis constant (K_m) represents the substrate concentration at which the reaction velocity is half of $V_{\max}$ and serves as an indicator of the enzyme's affinity for its substrate; a lower K_m corresponds to a higher affinity and vice versa. These kinetic parameters were determined to characterize enzyme efficiency and substrate binding under the specified assay conditions (Mohammed and Khalaf, 2023). For ALP, typical K_m values for substrates like p-nitrophenyl phosphate (pNPP) range from micromolar (μM) to low millimolar (mM) concentrations, varying with the source organism and assay conditions such as pH and temperature (Mohammed and Khalaf, 2023).

The enzyme exhibits optimal activity at alkaline pH, usually between pH 8 and 10, with activity sensitive to factors like temperature and ionic strength. Arrhenius type behavior has been observed, with activation energies that affect the velocity of phosphate ester hydrolysis (Hui *et al.*, 2013).

Recent kinetic studies have also integrated the effects of nanomaterials on ALP activity, reporting modulations in K_m and $V_{\max}$, which have implications for biosensor design and biotechnological applications (Mohammed and Khalaf, 2023). Standardized kinetic assays using spectrophotometric detection continue to be essential for defining ALP activity under various physiological and experimental conditions (Harroun & Vallée-Bélisle, 2023).

2.7 INFLUENCE OF MAGNESIUM IONS ON ENZYME ACTIVITY

Magnesium ions (Mg^{2+}) are critical cofactors for a broad range of enzymes, with estimates suggesting over 600 enzymes require Mg^{2+} for proper catalytic function (Barbagallo *et al.*,

2023). Mg^{2+} plays a multifaceted role in enzymology by stabilizing substrate binding, activating nucleophiles, and inducing conformational changes that optimize enzyme catalysis. Mg^{2+} involves electrostatic stabilization of negatively charged substrates and transition states, particularly in phosphorylation and hydrolysis reactions ubiquitous in metabolic pathways (Nam *et al.*, 2024). For example, Mg^{2+} coordinates phosphate groups in nucleotides like ATP, enhancing their susceptibility to enzymatic attack and facilitating phosphoryl transfer reactions essential for energy metabolism and signal transduction (Barbagallo *et al.*, 2023; Nam *et al.*, 2024).

Magnesium (Mg^{2+}) serves as a crucial cofactor in the activation of various enzymes, facilitating biochemical reactions essential for cellular function. Its role in enzyme activation is primarily attributed to its ability to stabilize negative charges on substrates and enzyme active sites, thereby enhancing catalytic efficiency (Berg *et al.*, 2023). For instance, magnesium ions are integral to the function of kinases, which transfer phosphate groups to substrates, a process vital for energy metabolism and signal transduction (Liet *al.*, 2022). Additionally, magnesium is involved in the structural stabilization of nucleic acids, influencing enzymes such as DNA and RNA polymerases, which are critical for genetic expression and replication (Bisswanger, 2017). Recent research has also indicated that magnesium's interaction with other metal ions can modulate enzyme activity, highlighting its multifaceted role in enzymatic regulation (Li *et al.*, 2020).

Recent structural and computational studies have shown that Mg^{2+} can induce conformational rearrangements within enzyme active sites that precisely position substrates and catalytic residues, thereby lowering activation energy barriers and increasing reaction rates (Nam *et al.*, 2024). Molecular dynamics and quantum mechanical calculations comparing Mg^{2+} and Mn^{2+} reveal that Mg^{2+} often provides a more stable catalytic environment, while Mn^{2+} may

enhance catalytic rates through increased active site polarization, highlighting the nuanced roles of metal cofactors (Roy & Cisneros, 2025).

Mg²⁺ also acts as a crucial regulator of enzyme structural integrity and function beyond catalysis, influencing enzyme folding, stability, and interactions with other biomolecules (Barbagallo *et al.*, 2023). Given its abundance and essentiality in cellular metabolism, magnesium deficiency can impair enzymatic activities and metabolic functions, underscoring its physiological significance.

2.7.1 Mg²⁺ OSCILLATIONS DURING ACTIVITY

During physical activity, intracellular magnesium ion (Mg²⁺) concentrations exhibit dynamic oscillations that are crucial for muscle function and energy metabolism. Mg²⁺ serves as an essential cofactor for ATP synthesis, regulating enzyme activities involved in energy production, muscle contraction, and relaxation. Oscillations in Mg²⁺ levels influence calcium homeostasis by modulating calcium influx into muscle cells, which is fundamental to muscle contraction cycles (Yu *et al.*, 2023). Adequate Mg²⁺ availability ensures efficient ATP utilization during muscle contraction and contributes to muscle recovery post-exercise. Recent research highlights that Mg²⁺ oscillations are associated with neuromuscular excitability, muscle performance, and the prevention of muscle fatigue and cramps (Liguori *et al.*, 2024). Furthermore, magnesium deficiency has been linked to impaired muscle function, increased susceptibility to injury, and delayed recovery, emphasizing the importance of maintaining Mg²⁺ homeostasis during periods of increased physical demand. Magnesium's role extends beyond bioenergetics to include anti-inflammatory effects that mitigate exercise-induced muscle damage and support muscle repair (Tarsitano *et al.*, 2024). These oscillatory dynamics reflect an adaptive physiological

response that optimizes muscle performance and energy metabolism during physical activity.

2.7.2 INTEGRATION OF ALKALINE PHOSPHATASE AND Mg^{2+} OSCILLATIONS

Alkaline phosphatase (ALP) is a metalloenzyme that contains zinc and magnesium ions at its active site, essential for its catalytic activity. Magnesium binds to a specific site (referred to as the M3 site) on ALP, playing a crucial role in enzyme activation by stabilizing the enzyme's conformation and facilitating catalysis. Unlike zinc ions, which directly participate in catalytic chemistry, magnesium modulates the structural integrity and the enzyme's dynamic properties, allowing the enzyme to function efficiently under physiological conditions without causing major conformational shifts (Hung *et al.*, 2001).

The process of magnesium binding to ALP is characterized by a slow-binding mechanism, with magnesium concentrations within physiological ranges (approximately 0.5–1.0 mM free Mg^{2+}) being sufficient to sustain optimal enzyme activity. This magnesium interaction is dynamic and can be influenced by oscillations in cellular magnesium levels, which may modulate ALP's activity in vivo through periods of enhanced or reduced catalytic efficiency (Hung *et al.*, 2001).

Magnesium's role in ALP function integrates through its ability to stabilize metal coordination geometry involving zinc ions, indirectly controlling catalytic site occupancy and enzyme kinetics. This interplay ensures that ALP activity can respond to fluctuating magnesium concentrations in tissues, contributing to processes such as phosphate metabolism, bone mineralization, and liver function regulation (Lowe *et al.*, 2023).

Structural studies of ATPase enzymes highlight that Mg^{2+} coordinates the β and γ phosphate groups of ATP, stabilizing the transition state of hydrolysis (Nam *et al.*, 2024). The liberated Mg^{2+} ions thus act as critical modulators for subsequent metabolic reactions and help coordinate energy-dependent cellular activities. ATP hydrolysis releases magnesium ions

because Mg^{2+} is initially bound to ATP, stabilizing it; when ATP breaks down into ADP and phosphate, this complex dissociates, increasing the levels of free Mg^{2+} in the cell. This surge in free Mg^{2+} is crucial for regulating various enzymatic processes and cellular activities that depend on magnesium as a cofactor (Lowe *et al.*, 2023).

During ATP re-synthesis, magnesium ions (Mg^{2+}) are re-sequestered as Mg^{2+} binds tightly to the newly formed ATP molecule, stabilizing it and restoring its biologically active form. This sequestration of Mg^{2+} is essential for maintaining cellular energy homeostasis and enabling efficient catalytic processes involving ATP-dependent enzymes (Nam *et al.*, 2024).

The re-synthesis of ATP re-sequesters Mg^{2+} as newly formed ATP molecules bind Mg^{2+} tightly, restoring its biologically active and stable form. This continuous release of Mg^{2+} during ATP hydrolysis and its recapture during ATP synthesis create oscillatory Mg^{2+} signals within the cell, effectively functioning as a biochemical capacitor that buffers cellular energy dynamics and coordinates metabolic reactions (Nam *et al.*, 2024).

2.8 COMPARATIVE STUDIES OF LIVER ALKALINE PHOSPHATASE IN SOME SPECIES

Liver alkaline phosphatase (ALP) is a critical enzyme involved in phosphate metabolism, widely distributed across vertebrates with notable interspecies variability in activity and isozyme composition. Mammals generally exhibit higher liver ALP activity, reflecting their endothermic metabolism and increased tissue turnover rates (Sharma *et al.*, 2014). Birds often show enzyme activities comparable to mammals but with distinct isozyme patterns adapted to their high metabolic demands and flight physiology (Goldman *et al.*, 2025). Reptiles typically present an intermediate ALP activity profile, lower than mammals but sometimes comparable to birds depending on species and environmental context. This intermediate catalytic activity is accompanied by greater thermal stability and resistance to

inhibitors, consistent with ectothermy and fluctuating environmental temperatures (Lakhani and Kanwar, 2025).

Isozyme complexity also varies; mammalian liver ALP is characterized by tissue-nonspecific isoforms with extensive genetic regulation, whereas birds and reptiles display greater isozyme heterogeneity, requiring species-specific reference ranges for clinical interpretation (Goldman *et al.*, 2025). Such biochemical diversity underlines evolutionary adaptations to differing metabolic and environmental pressures.

In clinical and physiological contexts, liver ALP in reptiles is interpreted with caution due to variable basal levels and isozyme forms, often requiring correlation with other biomarkers to assess hepatic or systemic disease states accurately (Sharma *et al.*, 2014).

2.8.1 Mammals

In mammals, liver ALP is one of the most prominent isoforms and is widely used as a biomarker for hepatic and bone disorders. It is typically present in moderate to high concentrations in the liver and is highly sensitive to inhibitors such as L-homoarginine and L-phenylalanine. Mammalian ALP is thermolabile, with denaturation occurring at 56°C for 5 minutes at pH 7.0, making it suitable for heat-inactivation studies. The enzyme's activity is influenced by physiological conditions such as age, diet, and disease states. Elevated liver ALP levels are commonly associated with cholestasis, hepatocellular damage, and bone growth or remodeling (Malissiova *et al.*, 2022).

2.8.2 Birds

Birds exhibit a distinct pattern of ALP expression compared to mammals. Hepatic ALP activity in avian species is generally lower, with bone and intestinal tissues showing higher levels of expression. This reduced hepatic activity limits the enzyme's utility in avian liver diagnostics, where other enzymes like aspartate aminotransferase (AST) and bile acids are preferred. Avian ALP is also more heat-sensitive than its mammalian counterpart, and its

isozyme diversity is less pronounced. These differences reflect the unique metabolic and physiological adaptations of birds, including their high metabolic rate and specialized digestive systems (Wilkanowska, 2017).

2.8.3 Reptiles

Reptilian hepatic alkaline phosphatase (ALP) activity exhibits an intermediate kinetic profile, with catalytic rates generally lower than those observed in mammalian counterparts but occasionally aligning with avian species, contingent on phylogeny and environmental parameters. The reptilian ALP isoforms display enhanced thermal resilience and reduced susceptibility to chemical inhibitors, congruent with their ectothermic physiology and thermoregulatory adaptations to variable ambient temperatures (Little and Seebacher, 2017). Isoenzyme heterogeneity within reptilian ALP is pronounced, necessitating species-specific baseline values for accurate enzymatic activity assessment. Clinically, reptilian ALP measurements are interpreted with caution, emphasizing integrative analysis alongside other biochemical and physiological biomarkers (Miriouni *et al.*, 2025).

2.8.4 Amphibians

Amphibians display moderate liver ALP activity, which varies with developmental stage and habitat. Their ALP enzymes are adapted to aquatic environments and show intermediate thermal stability. Limited data exist on amphibian ALP isoforms, and its diagnostic use remains minimal (Irving *et al.*, 2024).

2.8.5 Fish

Fish generally have low liver ALP activity, with intestinal isoforms being more dominant. Their ALP enzymes are highly stable in cold aquatic environments and show limited sensitivity to common inhibitors. Liver ALP is not widely used in aquaculture diagnostics due to its low expression and variability (Irving *et al.*, 2024)

CHAPTER THREE

3.0 MATERIALS AND METHOD

3.1 MATERIALS

Fresh *Agama agama* liver tissue

Table 1: List of equipment

Equipment	Model	Manufacturer
Centrifuge	MF-80	Hanil scientific industry, China
Spectrophotometer	Model SP-300	Optima Tokyo Japan, made in Japan
Weighing balance	Model FEJ-300	
Grant Water bath	WB-500	United Kingdom
pH meter	Model 3518N	
Freezer	Thermal cool c1202	China
Homogenizer		

Table 2: List of glass wares

Glass ware	Grade	Manufacturer
Pyrex test tubes	20ml	S.Pyrex, England
Pyrex conical flask	250ml	S.Pyrex, England
Pipette	10ml	S.Pyrex, England
Measuring cylinder	(10ml, 50ml and 100ml)	S.Pyrex, England
Reagent bottles	500ml	S.Pyrex, England
Volumetric flask	(250ml, 500ml, 1000ml)	S.Pyrex, England

Table 3: List of reagents

Reagent	Grade	Manufacturer
Glycine	Laboratory	Sigma chemical company. ST Loius Mo53175 USA
Sodium hydroxide (NaOH)	ACS reagent	Thermo Electron Lls India Pvt. Ltd Godrej Coliseum, 101A-101B, Sion(East), Mumbai-400 022
Sodium chloride (NaCl)	Laboratory reagent	Loba Chemie Pvt.Ltd.
Para-nitrophenyl phosphate (p-NPP)	Laboratory	Titan biotech Ltd India
Hydrochloric acid (HCL)	Analytical	BDH Chemical Ltd Poole England. Repackage in Nigeria by NAAFCO (scientific supplier) Ltd
Magnesium chloride (MgCl ₂)	Laboratory	Thermo Electron Lls India Pvt. Ltd Godrej Coliseum, 101A-101B, Sion(East), Mumbai-400 022

3.2 METHODS

3.2.1 SAMPLE COLLECTION AND PREPARATION

The *Agama agama* was caught at the back of Biochemistry Laboratory in Usmanu Danfodiyo University Sokoto, Sokoto state. The *Agama agama* was then taken to Zoology unit in Biological science department of Usmanu Danfodiyo University Sokoto for identification, after the identification standard procedure was employed to collect the liver, and was immediately chilled in ice-cold normal saline and homogenized in glycine-NaOH buffer to obtain the homogenate, after which the homogenate was centrifuged at 4000rpm for 50minutes and the supernatant was collected as the crude enzyme extract (alkaline phosphatase).

3.2.2 ENZYME ASSAY

A set of four test tubes were labeled, 0.45ml dilutions of p-nitrophenylphosphate (p-NPP) solution were introduced into a set of four corresponding test tubes containing 0.45ml of 0.1M glycine-NaOH buffer (pH =9.9). The mixture was incubated at 37°C for 5 minutes. The enzyme reaction was started by adding 0.1ml of crude liver extract into various test tubes at regular time interval of 5minutes for 20minutes and this make the total reaction volume to be 1ml. 5ml of 0.2M NaOH solution was added to the mixture to terminate the reaction. The mixture was transferred into a cuvette and absorbance was measured with a spectrophotometer at wavelength 405nm.

3.2.3 EFFECT OF MAGNESIUM IONS ON THE RATE OF ENZYME ACTIVITY

A set of four test tubes were labeled. To each of the test tubes, 0.40ml of the Glycine-NaOH (0.1M pH 9.9) buffer, 0.27ml of the different p-nitrophenyl phosphate concentration (0mMol/L, 0.5mMol/L, 0.25mMol/L, 0.125mMol/L) and 0.23ml of 0.0312mg/L concentration of MgCl₂ was added respectively and to the blank 0.00ml of the substrate and 0.9ml of the buffer were added. The test tubes were then incubated for 5minutes at 37°C. The crude enzyme extract

0.1ml solution was added to various test tubes to start the reaction at an interval of 5minutes for 20minutes and this make the total reaction volume to be 1ml. 5ml of 0.2M NaOH solution was added to terminate and the absorbance was taken spectrophotometrically at 405nm. This assay was repeated with 0.1560mg/L, 0.7800mg/L concentration of MgCl_2 respectively.

CHAPTER FOUR

4.0 RESULTS, DISCUSSION, CONCLUSION, AND RECOMMENDATIONS

4.1 RESULTS

4.1.1 ENZYME ACTIVITY ASSAY

The activity of alkaline phosphatase was measured using para-nitrophenyl phosphate (p-NPP) as the substrate. The release of para-nitrophenol was monitored spectrophotometrically at wavelength 405nm.

In the absence of Mg^{2+} , alkaline phosphatase showed moderate activity as seen in table 4 and in the presence of Mg^{2+} ions enzyme activity increased significantly, indicating its role as an activator.

4.1.2 EFFECT OF MAGNESIUM IONS CONCENTRATION

Varying concentration of Mg^{2+} (0.0312, 0.1560, 0.7800)mg/L were used to determine their effect on alkaline phosphatase activity and the maximum activity was observed at the lowest concentration of Mg^{2+} used (0.0312mg/L) as seen in table 4.

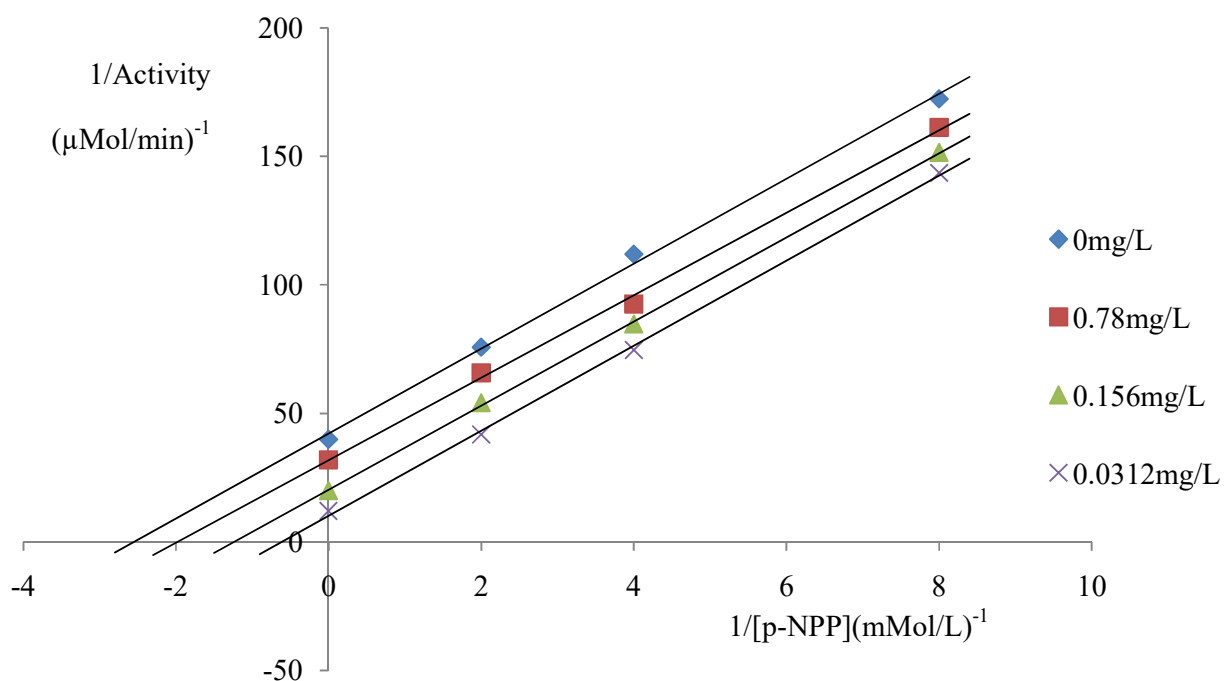


Figure 2: Primary Lineweaver Burk plot of ALP assay in the presence and absence of Mg^{2+} ions.

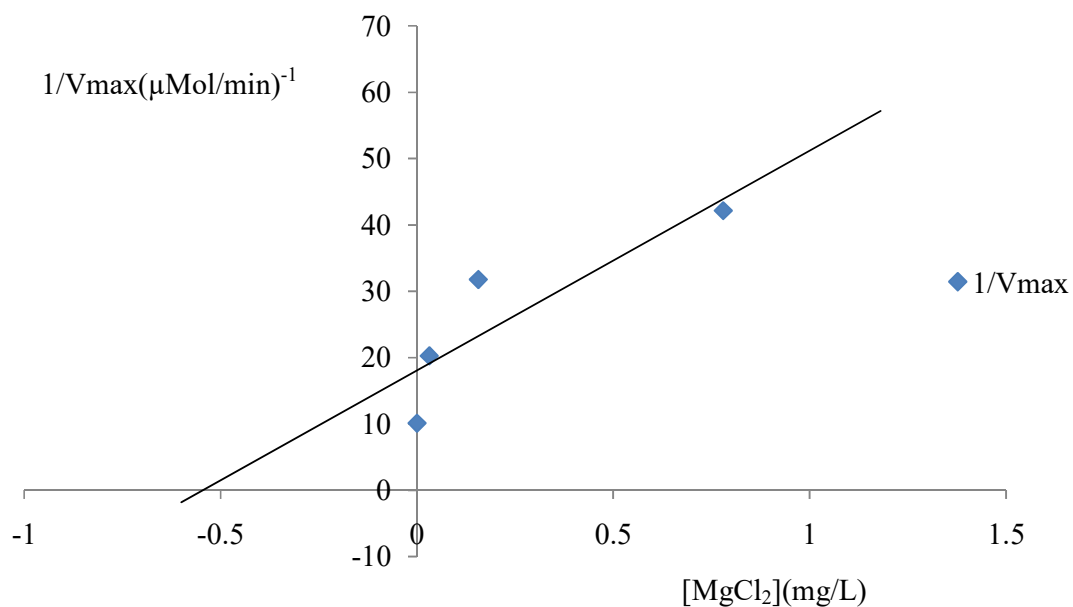


Figure 3: Secondary Line-Weaver Burk plot of ALP assay in the presence and absence of Mg^{2+} ions.

4.2 DISCUSSION

The findings of this study demonstrate that magnesium ions, supplied as MgCl_2 , exert a concentration dependent regulatory effect on alkaline phosphatase (ALP) extracted from the liver of *Agama agama*. In the absence of Mg^{2+} , the enzyme displayed moderate catalytic efficiency, as reflected by its K_m (0.3923mM) and $V_{\max}$ (0.0237 $\mu\text{mol}/\text{min}$). These values align with previous reports on reptilian ALP, which generally exhibits lower catalytic rates than mammalian counterparts due to ectothermic metabolic adaptations (Lakhani and Kanwar, 2025).

At low Mg^{2+} concentration (0.0312 mg/L), ALP activity increased significantly, with $V_{\max}$ rising to 0.0992 $\mu\text{mol}/\text{min}$. This enhancement is consistent with the established role of Mg^{2+} as a cofactor that stabilizes the active site, promotes proper substrate orientation, and facilitates nucleophilic attack during phosphate ester hydrolysis. The observed increase in K_m (1.6416mM) at this concentration suggests that while catalytic turnover improved, substrate affinity decreased. This pattern is characteristic of metal ion induced conformational changes that enhance catalytic rate but alter binding dynamics (Sharma *et al.*, 2014).

As Mg^{2+} concentration increased to 0.1560 mg/L and 0.7800 mg/L, both K_m (0.8086mM and 0.5055mM) and $V_{\max}$ (0.0494 $\mu\text{mol}/\text{min}$ and 0.0315 $\mu\text{mol}/\text{min}$) respectively declined relative to the optimal concentration. This indicates that excess Mg^{2+} exert inhibitory effects, due to oversaturation of metal binding sites or disruption of the optimal metal coordination geometry required for catalysis. Similar inhibitory trends at high metal ion concentrations have been reported in mammalian and microbial ALP studies, supporting the notion of a biphasic activation inhibition response (Ghudhaib *et al.*, 2016).

The activation constant ($K_a = 1.8359 \text{ mM}$) further confirms that ALP from *Agama agama* requires only minimal Mg^{2+} for optimal activation, and higher concentrations shift the enzyme toward reduced catalytic efficiency. This dual behavior may reflect physiological adaptations in reptiles, where fluctuating environmental ion availability necessitates enzymes capable of functioning across a broad range of metal ion concentrations (Bravo *et al.*, 2023). The results highlight the importance of Mg^{2+} as a modulator of ALP activity in reptiles and provide valuable comparative biochemical insights. The concentration dependent activation and inhibition observed in this study underscore the need to consider metal ion levels when interpreting ALP activity in reptilian physiology and diagnostics.

4.3 CONCLUSION

Kinetics study of alkaline phosphatase(ALP) extracted from *Agama agama* liver in the presence of magnesium ions demonstrates that magnesium ions significantly influence the kinetic behavior of alkaline phosphatase extracted from the liver of *Agama agama*. Low Mg^{2+} concentration significantly enhanced ALP activity by increasing V_{max} , indicating strong activation. However, higher Mg^{2+} concentrations progressively reduced both K_m and V_{max} , suggesting partial inhibition. These findings confirm that Mg^{2+} acts as a dual modulator serving as an activator at optimal levels and an inhibitor when present in excess. The results contribute to a deeper understanding of reptilian enzyme regulation and highlight the importance of metal ion balance in biochemical processes. Further research involving purified enzyme fractions and structural analyses is recommended to elucidate the precise mechanisms underlying Mg^{2+} mediated modulation of ALP activity.

4.4 RECOMMENDATIONS

1. It is recommended to conduct the kinetics using partially or fully purified *Agama agama* liver ALP to eliminate interference from other proteins and improve accuracy of kinetics parameters.
2. Additional studies should explore the combined effects of temperature, pH, and Mg^{2+} ions to better understand environmental regulation of *Agama agama* ALP activity.
3. Further studies should investigate the precise mechanism by which Mg^{2+} influences ALP conformation and catalytic efficiency, possibly using structural biology techniques such as X-ray crystallography or molecular dynamics simulation.
4. The effect of other divalent metal ions such as Zn^{2+} , Ca^{2+} , Mn^{2+} and Co^{2+} on *Agama agama* liver ALP to compare their activatory or inhibitory influence relative to Mg^{2+} ions.
5. In vivo studies are recommended to determine the physiological significance of Mg^{2+} modulation of ALP activity in reptiles and its implications for metabolic regulation.

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APPENDIX

Table 4: Alkaline phosphatase activity in the absence and presence of varying Mg^{2+} concentrations.

p-NPP	Activity($\mu\text{Mol/min}$)			
Concentrations(mMol/L)				
	0.000mg/L	0.7800mg/L	0.1560mg/L	0.0312mg/L
0.000	0.0250	0.0313	0.0501	0.0834
0.500	0.0131	0.0151	0.0184	0.0239
0.250	0.0089	0.0108	0.0118	0.0133
0.125	0.0058	0.0062	0.0066	0.0069

Table 5: Reciprocal of alkaline phosphatase activity in the absence and presence of varying Mg^{2+} concentrations.

1/p-NPP	1/Activity			
Concentration	$(\mu\text{Mol/min})^{-1}$			
$(\text{mMol/L})^{-1}$				
	0.000mg/L	0.7800mg/L	0.1560mg/L	0.0312mg/L
0.0000	39.8760	31.9450	19.9451	11.9836
2.0000	75.7576	65.7895	54.1026	41.7284
4.0000	111.9670	92.5926	84.7458	74.6269
8.0000	172.4140	161.2900	151.515	143.636

Table 6 : Kinetic parameters of ALP extracted from *Agama agama* liver in the absence and presence of Mg^{2+} ions.

Concentration of $MgCl_2$ (mg/L)	K_m (mM)	V_{max} (μ Mol/min)
0.0000	0.3923	0.0237
0.0312	1.6416	0.0992
0.1560	0.8086	0.0494
0.7800	0.5055	0.0315

Table 7: Determination of activation constant (K_a) of ALP extracted from *Agama agama* liver in the absence and presence of Mg^{2+} ions.

Magnesium concentrations(mg/L)	$1/V_{max}(\mu$ Mol/min) $^{-1}$
0.0000	10.0800
0.0312	20.2540
0.1560	31.7430
0.7800	42.1390