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International Journal For Research in Applied Science and Engineering Technology



INTERNATIONAL JOURNAL FOR RESEARCH

IN APPLIED SCIENCE & ENGINEERING TECHNOLOGY

Volume: 6 **Issue:** I **Month of publication:** January 2018

DOI: http://doi.org/10.22214/ijraset.2018.1230

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International Journal for Research in Applied Science & Engineering Technology (IJRASET)
ISSN: 2321-9653; IC Value: 45.98; SJ Impact Factor :6.887
Volume 6 Issue I, January 2018- Available at www.ijraset.com

Hepatic-ALP Kinetics in Cichilid, Oreochromis Mossambicus (Peter)

Arun M. Chilke[1]
[1]*Division of Toxicology and Biomonitoring, Department of Zoology, Shree Shivaji Arts, Commerce and Science College, Rajura-442 905 (India)*

***Abstract:** As a metabolic center, liver is the key exocrine gland in Piscean body. The variety of cell types in liver performs multifaceted physiological functions. Alkaline phosphatase (ALP) is one of the lysosomal enzymes that participate in the mobilization of phosphate group. Virtually, this enzyme is versatile in its biochemical action in the animal tissues. In the present work, an attempt has been made to find out the kinetic properties of hepatic alkaline phosphatase in cichlid, Oreochromis mossambicus. The enzyme activity was found increased and the highest at pH-8.5 at temperature 37°C for 30 minutes incubation. However with extended period of time, the enzyme activity gradually increased at constant pH-10.5 and temperature 37°C. With the increase in enzyme and substrate concentration, the enzyme activity steadily increased. By using Lineweaver-Burk plot, the velocity maxima (Vmax) 106.5 µg/half hour (Hh) and Michaelis constant (Km) 0.216 mM were recorded for hepatic ALP of O. mossambicus.*
***Keywords** Alkaline phosphatase · Kinetics · Liver · Cichilid*

I. INTRODUCTION

Orthophosphoric monoester phosphohydrolase (EC: 3.1.3.1) also referred as alkaline phosphatase (ALP) in a generic term that describes a group of catalytic proteins sharing the capacity to hydrolyse phosphate ester in alkaline medium (30 & 34). Like a number of enzymes, ALP is a glycoprotein (6 & 23) but the carbohydrate content of ALP has been the subject of conflicting reports. The ALP is widely distributed in nature and is characterized by a high pH optima and broad substrate specificity (21). They are zinc metallo enzymes (20 & 27). This enzyme contains zinc atom near the active site and is viewed to be responsible for its catalytic activity (9 & 11).

Many authors have suggested the diversified functions of ALP, according to 5 & 16; it may be connected with protein synthesis in the cell. The enzyme is thought to be concerned with nucleoprotein and nucleotide metabolism (10). A functional relationship between ALP and RNA has been demonstrated (14) and ALP may play a role in controlling DNA synthesis and growth (29); the enzyme is capable of hydrolyzing both DNA (31) and RNA (26). ALP from many tissues possesses both pyrophosphatase (8 & 12) and phosphotransferase (4 & 22) activities, and, in the case of intestinal, kidney and bone phosphatases, the two catalytic activities are the result of the action of the same enzyme. In higher animals, an ALP activity is involved in bone formation and in membrane transport activities. In blue crab Callinectes sapidus, ALP modulates the osmoregulatory response (19).

As far as the ALP is concerned, some works have been carried out on fish with respect to biochemistry and histochemistry of ALP, but still no work is available pertaining to the kinetics of ALP in fish and hence an attempt was made to find out the Km and Vmax of hepatic ALP in O. mossambicus.

II. MATERIALS AND METHODS

Healthy, live Oreochromis mossambicus captured from River Wardha located near Rajura (M.S.), India were purchased from the fisherman. Liver from five fishes were removed after decapitation and brought to the laboratory in ice-cold 0.1 M PBS, pH-7.45. p-Nitrophenylphosphate (HiMedia, Mumbai) was used as substrate. Glycine, sodium hydroxide, p-nitrophenol were purchased from HiMedia, Pvt. Ltd. Mumbai.

Liver were soaked with clean blotting paper for removing the moisture. 0.1 gm of liver obtained from each fish was weighed and crushed in 0.1 M PBS, pH-7.45. The liver homogenate was raised to final concentration 1% with 0.1 M PBS, pH-7.45 Biochemical assay of alkaline phosphatase (ALP) was carried out according to the method described by 15. 1mM substrate prepared in alkaline glycine buffer (0.1 M, pH-10.5) was used. However, substrate of the same molar concentration prepared in 0.1 M alkaline-glycine buffer (six replicates with variation in pH ranging from 8 to 11.5) was used for studying the effect of pH on hydrolytic activity.

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For the determination of ALP activity, in the centrifuge tube was taken 0.8 ml alkaline-glycine buffer and 0.1 ml substrate (1 mM p-NPP) solution. Mixture was kept for 15 minutes in an incubation chamber. Later 0.1 ml liver extract of *O. mossambicus* was added to the mixture and incubation was carried out for 30 minutes. At the end of incubation, the reaction was terminated by adding 5.0 ml of 0.2 M NaOH. Later, the intensity of developed color was measured by spectrophotometer (Labtronics, India) at maximum wavelength of 405 nm.

Substrate concentration ranging from 2 to 10 mM was prepared in alkaline glycine buffer (0.1 M, pH-10.5), and by using the inverse value, Lineweaver-Burk Plot (double receiprocal plot) was plotted. For the effect of enzyme concentration, 1% stock liver extract was used with subsequent increased quantity. At the end of incubation, 5.0 ml of 0.2M NaOH was added to the assay mixture to terminate ALP activity. The mixture was transferred into a cuvette and absorbance was measured with a spectrophotometer (Labtronics, India) at maximum wavelength of 405 nm. The concentrations of p-NP produced in the various tubes were interpolated from standard curves (Fig.1).

III. RESULTS

Enzymes are very important as far as the biological functions are concerned but very little work has been carried out regarding the kinetic study of enzyme in fish. Hence in the present work, an attempt has been made to study the kinetic changes in an enzyme alkaline phosphatase (ALP) in the liver of *O.* mossambicus. The parameters studied were time, pH, and enzyme and substrate concentration. It was observed that all the said parameters variably affect the activity of hepatic ALP of O. mossambicus.

A. Effect Of Time

The time is an important factor requires accomplishing the enzymatic reaction. It was observed that in the beginning of incubation period the enzyme exhibited low rate of reaction. But with the extended time period the ALP activity gradually increased (Fig.2).

B. Effect Of Ph

ALP was found to work in alkaline pH. At low alkaline pH the enzyme activity was very low. At pH-7, no ALP activity was takes place. But at 7.5 quite negligible enzymes activity was noticed. The maximum enzyme activity was noticed at pH-8.5 (Fig.3). Later with the increase in pH from 9 to 11.5 the enzyme activity gradually declined. At pH-11.5, ALP activity got stopped.

C. Effect Of Enzyme Concentration

The enzyme concentration was found to affect the enzyme activity. At low enzyme concentration at pH-10.5 and temperature 37°C for half hour incubation low ALP activity was recorded. However at constant pH, temperature and time the ALP activity was increased with increased in the concentration of enzyme source (Fig.4).

D. Effect Of Substrate Concentration

Effect of substrate (p-Nitrophenylphosphate) concentration on enzyme activity was studied for the inhibitory action of substrate on the rate of reaction. The liver extract (source of enzyme ALP) of *O. mossambicus* was exposed to different substrate concentration i.e. 2mM to 10mM/Hh at 37°C temperature and pH 10.5. The reaction velocity starts to increase with increase in substrate concentration. The maximum velocity was noticed at 10mM and the minimum at 2mM substrate concentration.

E. Kinetcs Of Alkaline Phosphatase

Lineweaver-Burk double reciprocal (LBDR) plot was studied by considering the inverse values of substrate concentration against the reaction velocity. It was observed that the straight line intercepts the Y-axis and gave the value 0.00935ug/hh and the X-intercept -4.625 mM^{-3}. However, the corresponding Vmax and Km were calculated to be 106.95 µg/Hh and 0.216 mM/Hh.

IV. DISCUSSION

Some authors have studied the kinetics of alkaline phosphatase (ALP) in mammalian species (2, 3 & 25) and bacteria (13,28 & 33). But no data is available on fish. Hence in the present work an attempt was made to find out the kinetics of ALP in the extract of liver of *O.* mossambicus.

The time is an important factor require for the interaction of substrate and enzyme. In zero time no enzyme activity takes place, but with the increase in time of exposure the enzyme activity increases gradually at constant pH, substrate and enzyme concentration

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was recorded in the present investigation. The increased in enzyme activity with increase in exposure time could be due to increase in an interaction between the substrate and enzyme eventually to produce the product.

The pH of an enzyme often reflects the pH of its normal environment; the optimum value may not be precisely the same in every biological system. Noteworthy, as far back as five decades ago, 13, reported optimal pH for ALP activity of *Escherichia coli* to be about 8.0, while the bovine enzyme optimum pH was slightly higher (8.5) (17). Furthermore, study of ALP from human hydatidi form by 1 gave the optimum pH of 10.8. In the present investigation the optimum pH for hepatic ALP of O. mossambicus was recorded 8.5 at temperature 37°C for 30 minutes incubation. These findings are consistent with previous studies by 33, in which they observed that mammalian ALPs possess higher pH optima than prokaryotes (E.coli) enzyme and proposed that substitution of two amino acid residues with corresponding histidine molecules in the mammalian enzymes are responsible for this increase in optimum pH (18 &24). The pH of ALP in bovine and in the liver of studied fish was found same could be due to same amino acid sequence and structure of enzyme ALP.

The increase in enzyme activity due to increase in enzyme concentration at constant temperature, pH and substrate concentration was recorded. The gradual increase in its activity could be due to increase in binding competition of the enzymes with the substrate. The same increase in enzyme activity was noticed while increase in the concentration of substrate at constant parameters like pH, temperature and enzyme.

This study demonstrated the activity of ALP in liver extract of O. mossambicus. The occurrence and diversity of ALP in biological systems has been reported by several authors (7 & 34). For instance, 32 reported on sequence comparisons between different ALP indicated that about 25 to 30% homology exist between mammalian and E.coli alkaline phosphatase. Despite differences in amino acid sequences and three dimensional structures, all ALPs catalyse the hydrolysis of almost any phosphomonoester with release of inorganic phosphate and alcohol. In addition, although ALP exhibits broad specificity for wide range of phosphorylated substrates, the enzyme displays variable affinity for these substrates depending on the molecular nature of the substrate, isoform and source of the enzyme.

The Km and Vmax of ALP show variation in different organisms and also in the different tissues of same organism. These variations could be due to variation in amino acid sequence, number of amino acid residues, three dimensional structures and the binding site for the substrate. Beside this time of exposure, pH and temperature plays a very important role as far as Km and Vmax of ALP is concerned. In the present work, Lineweaver-Burk double reciprocal (LBDR) plot was studied by considering the inverse values of substrate concentration against the reaction velocity. It was observed that the straight line intercepts the Y-axis and gave the value 0.00935µg/Hh and the X-intercept -4.625 mM-3. However, the corresponding Vmax and Km were calculated to be 106.95 µg/Hh and 0.216 mM/Hh.

V. ACKNOWLEDGEMENT

Author is very thankful to Mr. Sanjay.N. Shende, Assistant Professor of English, Shree Shivaji Arts, Commerce and Science College, Rajura for editing the manuscript.

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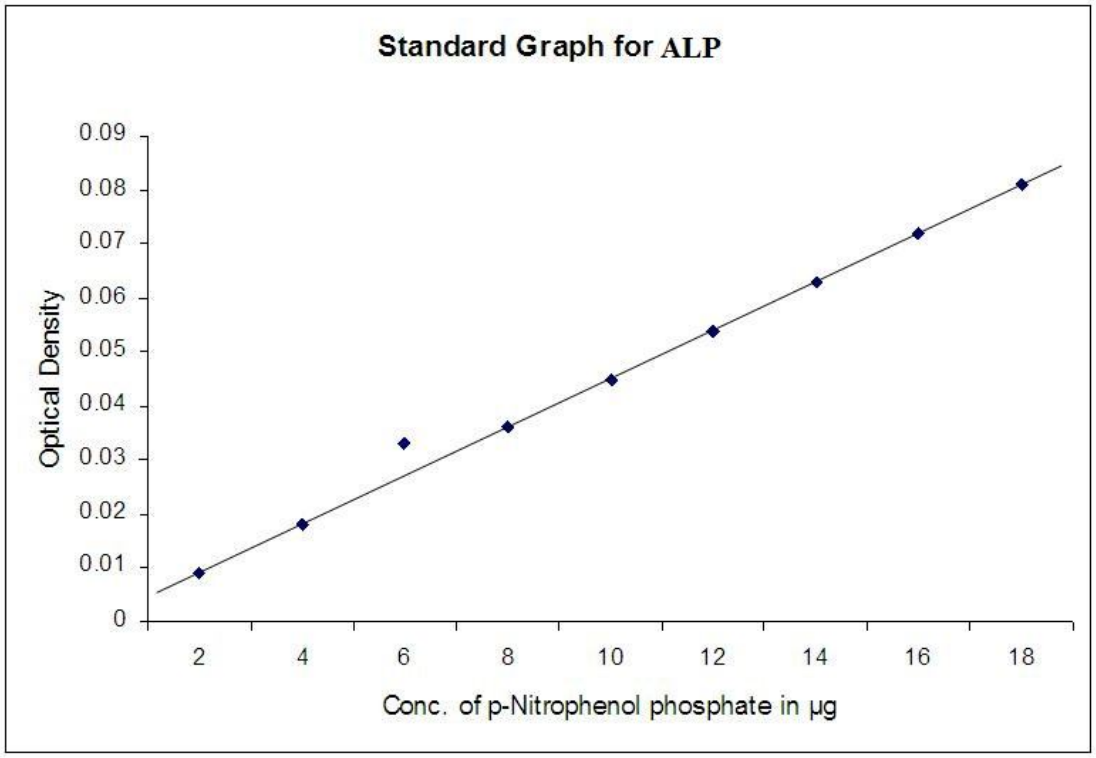


Fig.1 Shows the standard graph of p-Nitrophenol for determining the alkaline phosphatase (ALP) activity.

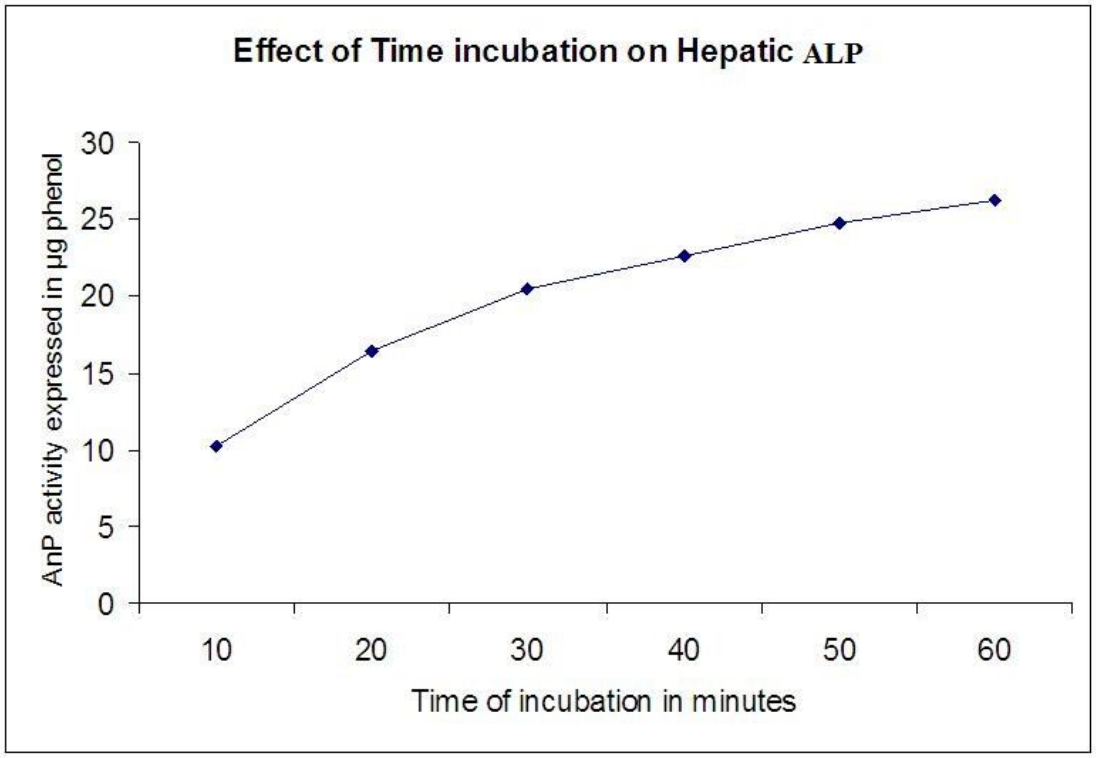


Fig.2 Shows gradual increase in enzyme activity with the extended period of incubation at constant pH 10.5 and temperature 37°C.

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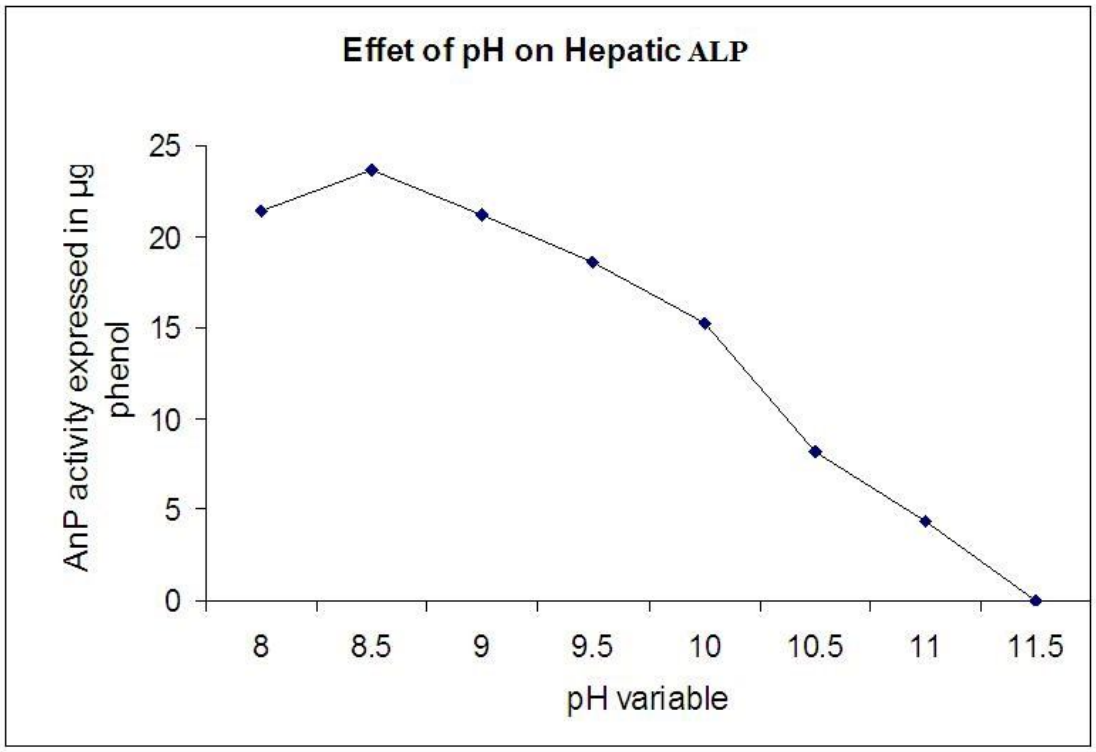


Fig.3 Shows increase in ALP activity at optimum pH 8.5 and temperature 37°C. Below and above this pH optima the enzyme activity declined.

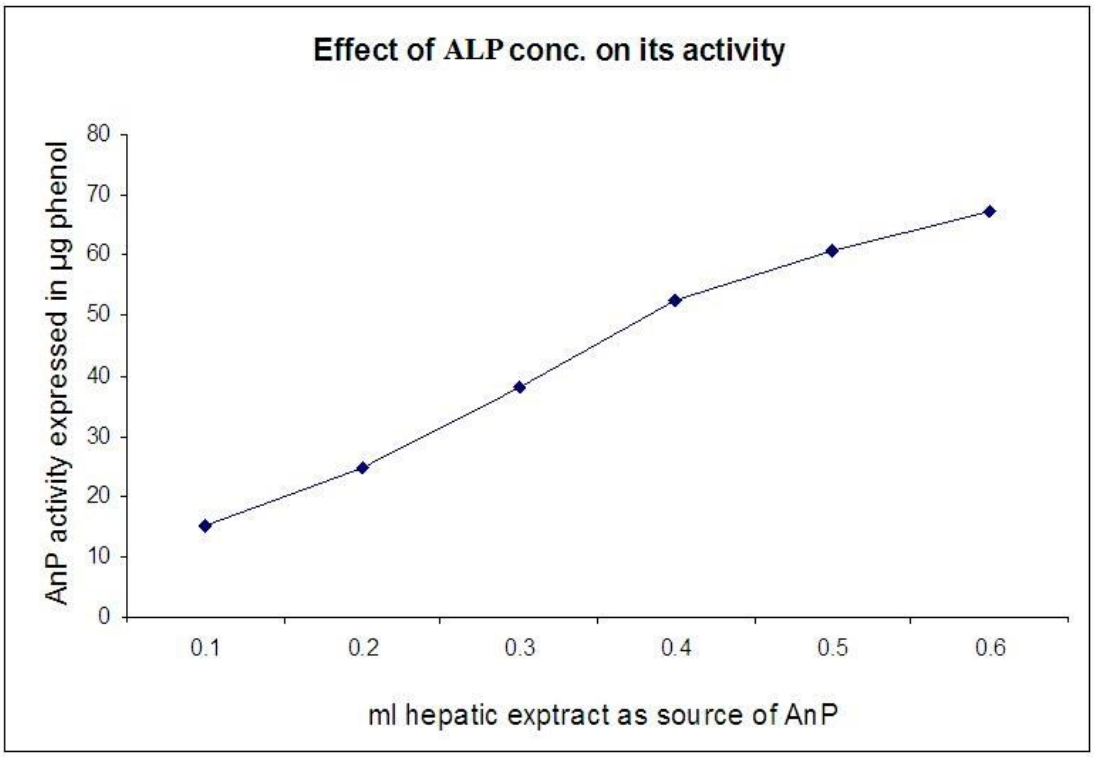


Fig.4 Shows consistent increased in enzyme activity corresponding to increase in concentrations of enzyme source at constant pH 10.5 and temperature 37°C.

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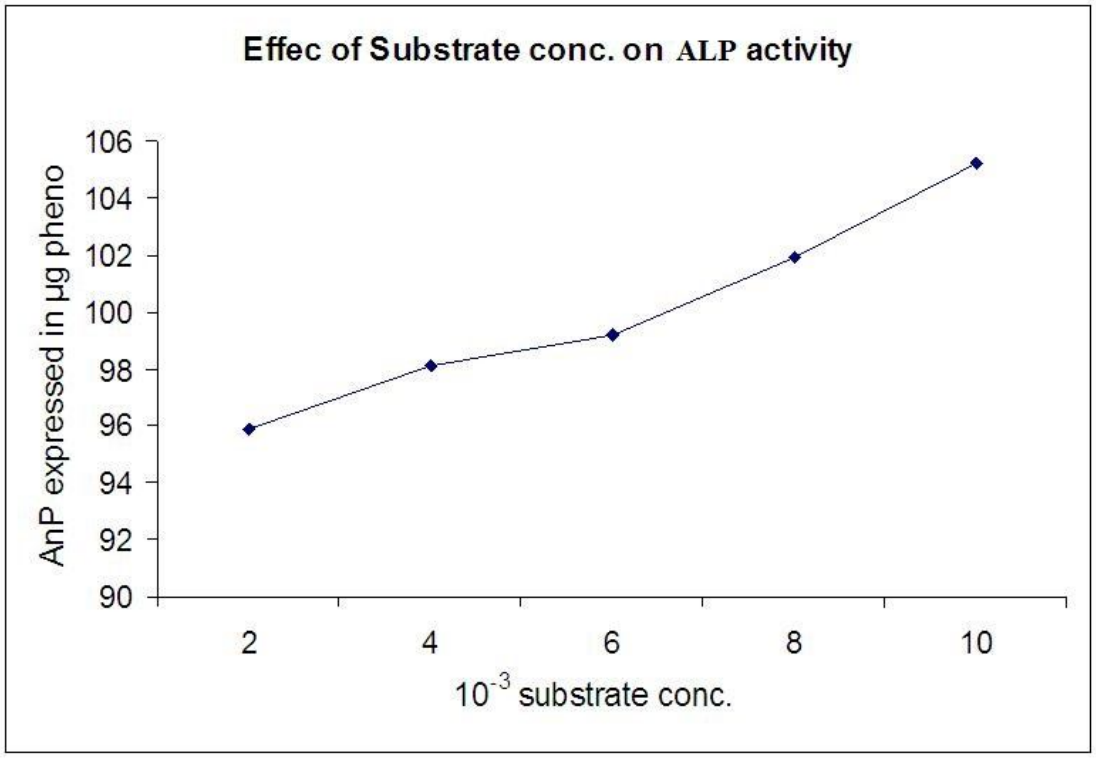


Fig.5 Shows consistent increased in enzyme activity corresponding to increase in concentration of substrate (2 mM to 10 mM) at constant pH 10.5 and temperature 37°C.

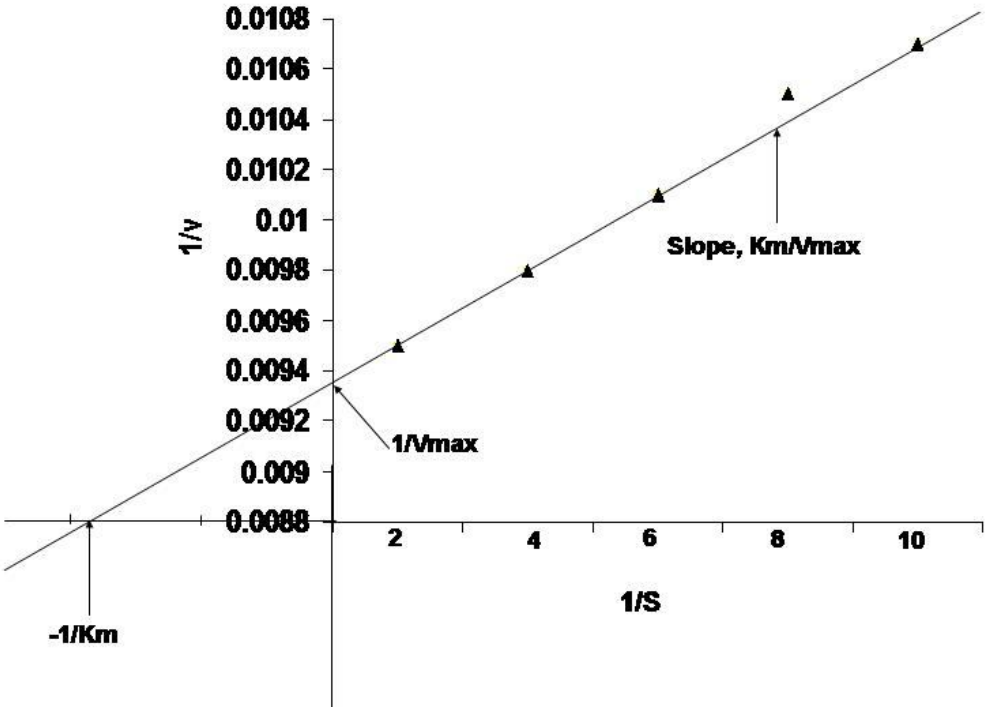
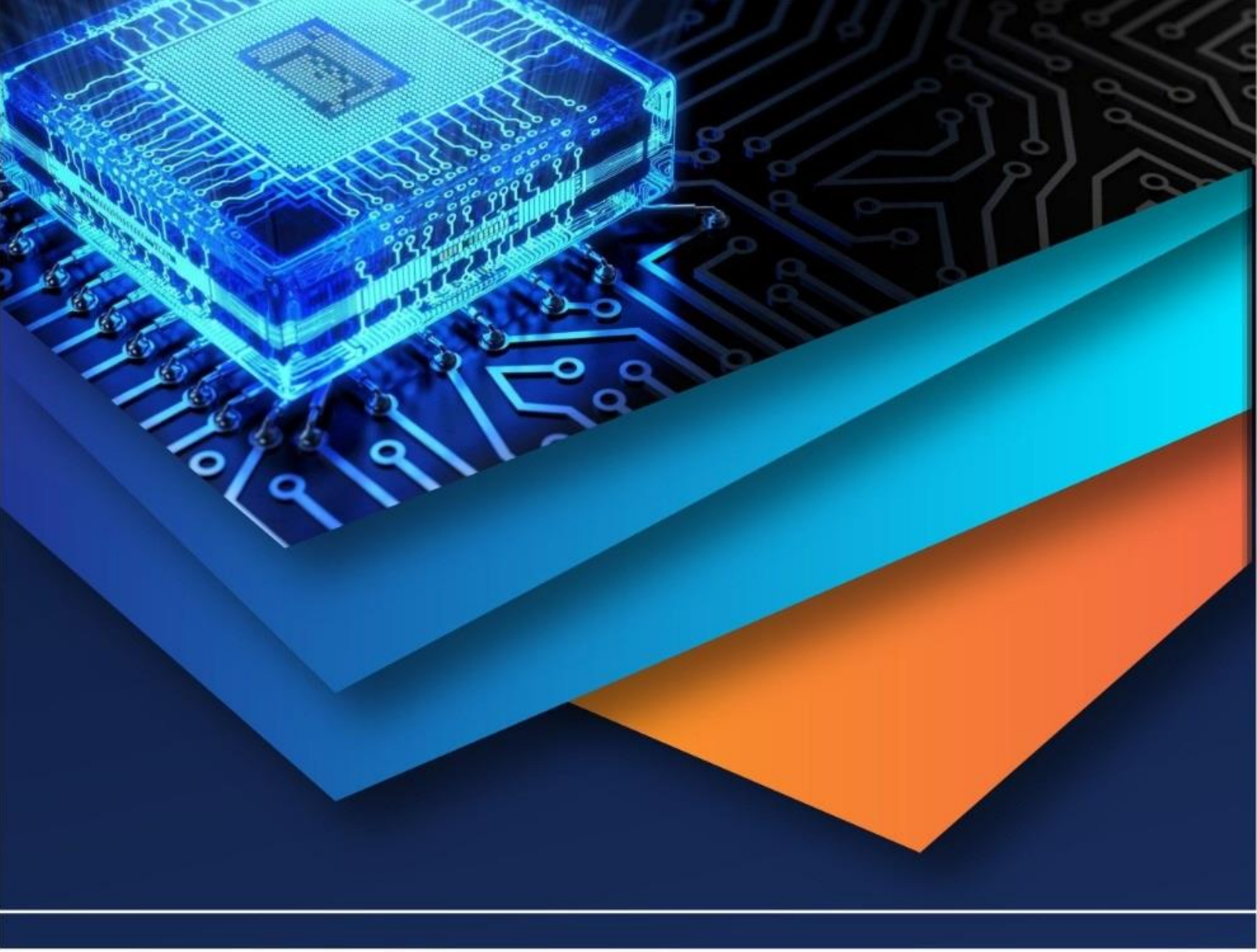


Fig.6 Lineweaver-Burk Plot against substrate concentration 2 mM to 10 mM showing straight line intercepting Y-axis giving the value 0.00935 µg/Hh and the X-intercept -4.625 mM^{-3} with Vmax 106.95 µg/Hh and Km, 0.216 mM/Hh at constant pH 10.5 and temperature 37°C.

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