Biology and Biochemistry Department
BIO C331
biochemistry Lab

Experiment #10

Alkaline Phosphatase: Progress Curve, Effect of pH, Enzyme Concentration, Activators, and Inhibitors

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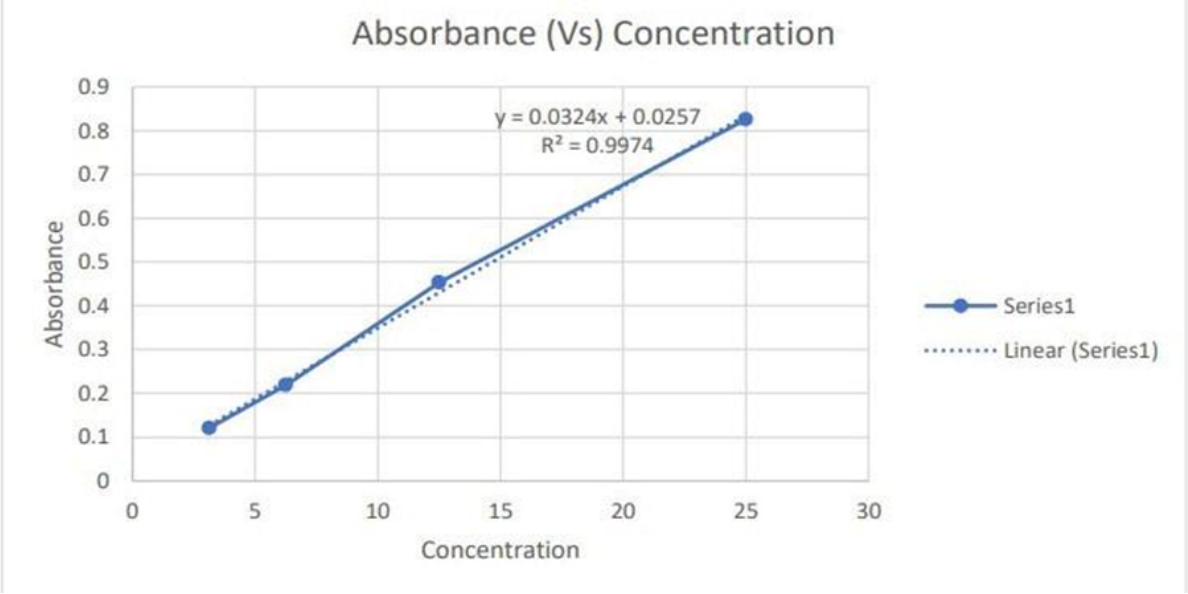
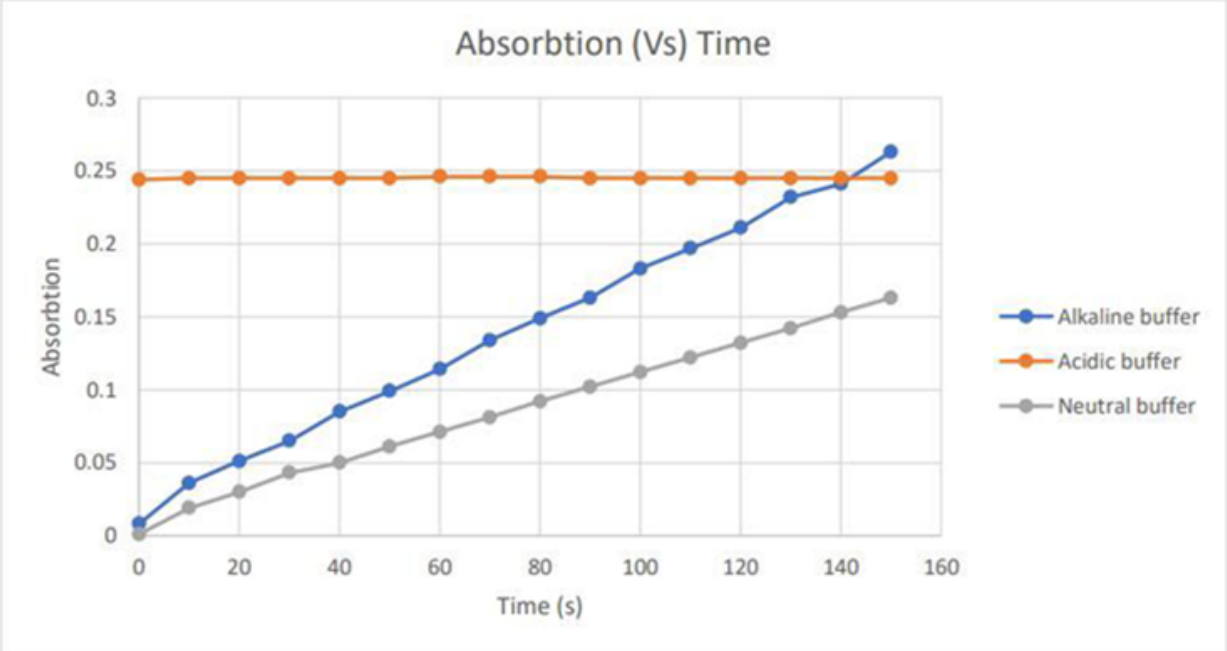
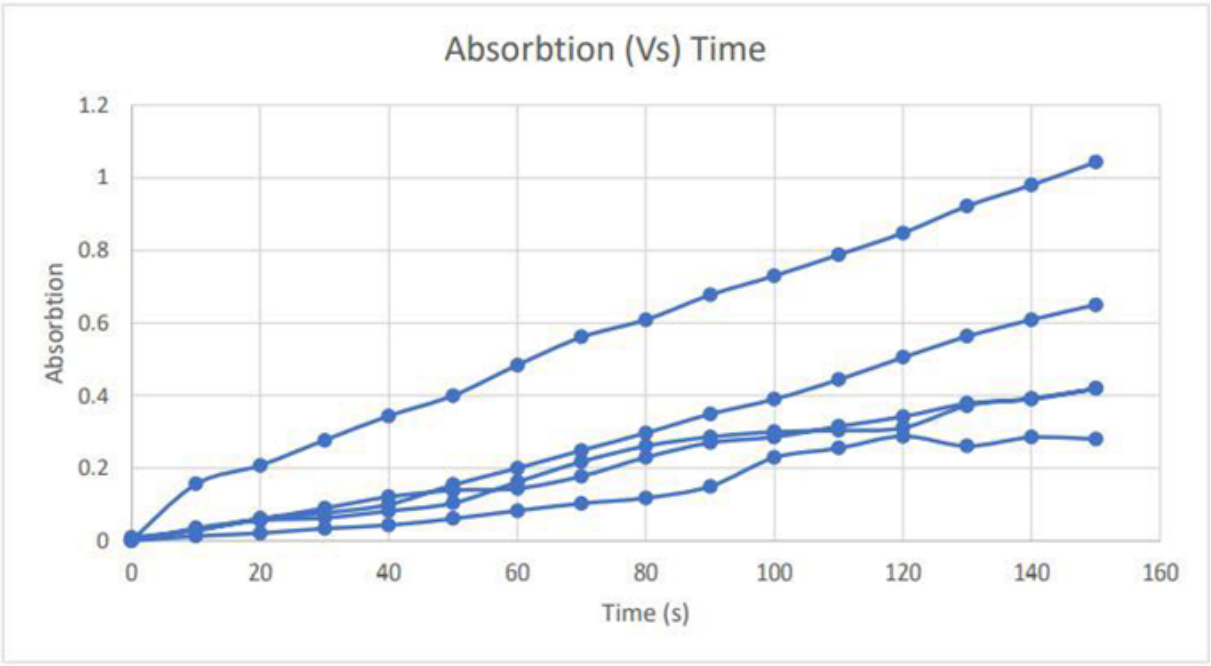
Data and results:



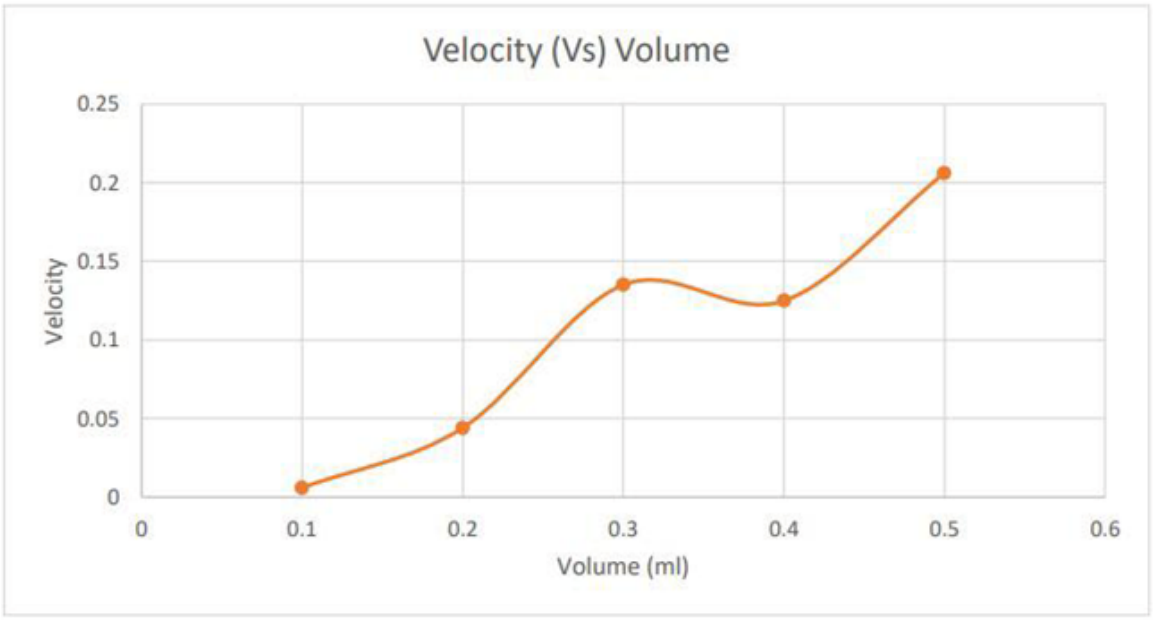
Graph 1: the standard curve of p-nitrophenol based on the absorbance 405nm and concentration.



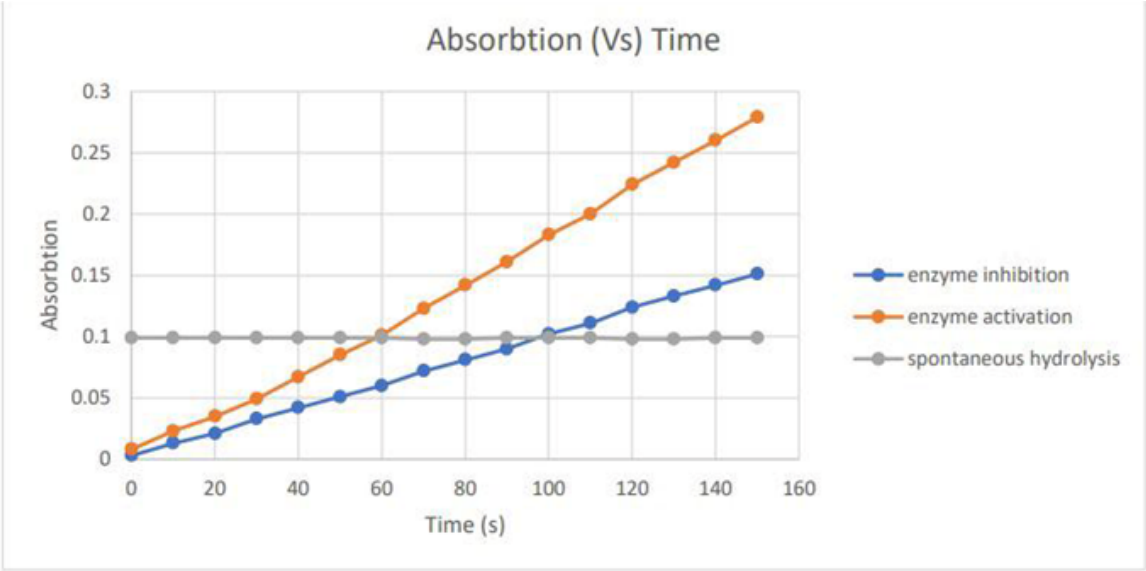
Graph 2: Plots of p-nitrophenol concentration (M) vs. time (seconds) in neutral, acidic, and alkaline buffers



Graph 3: Plots of p-nitrophenol(M) concentration vs. time (seconds) for various enzyme concentrations.



Graph 4:Plot of Alkaline Phosphatase Activity (µM/sec) Vs. Its Volume (ml)



Graph 5:absorption vs time of different inhibitors and activators.

Discussion:

Alkaline phosphatase has been extensively studied analytically, and it has been proven to work ideally under alkaline conditions with the aid of magnesium and zinc metals at its active site, displaying Michaelis-Menten kinetics, as its name indicates.

The goal of this experiment was to look at the properties of AP. According to the findings of the first section of the experiment (figure 1), the concentration of p-nitrophenol reduced as the pH declined from alkalinity through neutrality to acidity. This is accompanied by a decrease in enzyme activity, indicating an alkaline optimal pH, and an increase in enzyme inactivation when pH decreases. (1)

Enzymatic inactivation, also known as denaturation, occurs when the tertiary structure of an enzyme is lost owing to changes in the ionization states of key amino acid residues in the active site, making the enzyme non-functional. The optimal pH of AP, on the other hand, might vary depending on the concentration and kind of substrate utilized, as well as the type of buffer used.

In the second phase, the effect of raising enzymic concentration was studied. The obtained data revealed that as the enzyme's concentration grew, so did its activity. This finding is consistent with the predicted rise in reaction velocity as the number of catalytic active sites grows, resulting in an increase in the concentration of the enzyme-substrate complex, and therefore the concentration of the product, p-nitrophenol, generated per unit time. (2)

The final component of the experiment looked at how HPO42- and Mg2+ affected AP activity. The activity of AP in the presence of HPO42- or Mg2+ was lower than that of the enzyme in the solution with the lowest AP concentration observed in the preceding section, indicating possible inhibitory mechanisms. In theory, HPO42 competes with the substrate, p-nitrophenyl phosphate, for the same active site, causing the enzyme to be inhibited and its activity to be reduced. Magnesium, on the other hand, controls the occupancy of the catalytic active sites and so stabilizes and modifies the enzyme's activity. As a consequence, it's clear that the MgCl2 solution employed in this experiment had a low concentration, which might have resulted in this incorrect conclusion. (3)

Conclusion:

Alkaline phosphatase is a ubiquitous dimeric metalloenzyme that performs best in alkaline environments. When combined with magnesium ion, this enzyme exhibits enhanced activity, but when mixed with hydrogen monophosphate ion, it exhibits decreased competitively-inhibited activity.

References:

1. Hung, H. C., & Chang, G. G. (2001). Differentiation of the slow-binding mechanism for magnesium ion activation and zinc ion inhibition of human placental alkaline phosphatase. *Protein science : a publication of the Protein Society*, *10*(1), 34–45. https://doi.org/10.1110/ps.35201

2. https://www.journalofdairyscience.org/article/S0022-0302(57)94519-8/pdf

3. **Studies on Single Alkaline Phosphatase Molecules: Reaction Rate and Activation Energy of a Reaction Catalyzed by a Single Molecule and the Effect of Thermal DenaturationThe Death of an Enzyme**
   Douglas B. Craig, Edgar A. Arriaga, Jerome C. Y. Wong, Hui Lu, and Norman J. Dovichi
   Journal of the American Chemical Society 1996 *118* (22), 5245-5253
   DOI: 10.1021/ja9540839