Determination of enzyme activity of acid phosphatase through standard curve of Para-nitrophenol

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ABSTRACT: - The standard graph is prepared for determining the activity of acid phosphatases from it. The optical density of para-nitrophenol is plotted against its concentration on X-axis after diluting it with 1N nNaOH.Para-nitrophenol which is colourless in acidic medium gives yellowish colour in alkaline medium which has maximum absorption at 405nm.

KEYWORDS:-Standard Curve, Para-nitro phenol, acid phosphatase, spectrophotometry, monochromatic light, absorption

INTRODUCTION:-The standard curve is prepared with the help of spectrophotometer.Specttrophotometry is instrument which measures light absorbance as a function of wavelength in the ultraviolet as well as visible region. It is based on the law of absorption known as Beer Lambert Law

Beer Lambert Law: - It states that when a ray of monochromatic light of initial intensity (I) passes through a solution kept in a transparent vessel, some of the light is absorbed, so that the intensity of the transmitted light (I_O) is less than the initial intensity. There is some loss of light due to the scattering by particles in solution and reflection at the interface but mainly from the absorption of solution. The relationship between I and Io depends upon the path length of the absorbing medium and the concentration of the absorbing solution component

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Lambert law:-When a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the length of the medium increases.

Beer law:-When a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the concentration of the medium increases.

These two laws are combined together to give the Beer Lambert Law

$$I = I_O e^{-k3cl}$$

Where;

I_o= Intensity of incident light

I= Intensity of transmitted light
C= Concentration of an absorbing medium
L= Path length
K_3= Constant

Acid phosphates (orthoposphoric monoester phosphohydrolase, EC 3.1.3.2) are widely distributed in plants and animals. Acid phosphates have been purified and characterized from tubers **(Kameman,1984;Gellatly et al.,1994;Kusudo et al.,2003; Kouadio,2004),seeds(Ullah and Gibson,1988;Olczak et al.,1997;granjeiro et al.,1999),roots (Panara et al.,1990),**leaves **(Staswick et al.,1994),** bulbs **(Guo and Pesacreth,1997)** and **seedlings (Yenigum and Guvenilir,2003).**Acid phosphates catalytically breakdown a wide variety of phosphate esters and exhibit pH optima below 6.**0 (Vincent et al.,1992).**In plant roots ,acid phosphates seem to be involved in the solubilization of macromolecular organic phosphates in soils which can then

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be utilized by **plants (Panara et al.,1990).**F**rom tubers ,Kamenan (1984)** and **Kouadio (2004)** have reported an important role of acid phosphates in the transport of phosphate in the metabolic phenomena taking place during the preservation of yam (Dioscorea cayenensis rotundala) and cocoyam (Xanthosoma sp.) tubers.

From seeds and seedlings, the physiological function of the acid phosphatases is to provide germination, and many different phosphate esters of sugars and substrates stored in the seed and seedling need to be hydrolyzed during germination and growth (Gahan and McLean, 1969; **Schultz and Jensen, 1981; Akiyama and Suzuki, 1981; Hoehamer et al., 2005).**

MATERIALS AND METHODS:-

Conical Flask, Test Tubes, Pipettes of 5ml and 1ml,Stirrer,Buffer sodium acetate of 0.1M,Para-Nitrophentl phosphate,NaOH of 1 normal,Spectrophotometer,PH meter and water bath.

Acetate buffer was prepared (0.1M,PH=4.5).Ten test tubes were taken including one as a blank with no PNP.Different Volumes of Acetate Buffer were mixed with corresponding volumes of PNP to make the total volume to 3ml.The same set of 10 test tubes were then placed to incubate at 37^0C for 30 minutes(water bath).

Each sample was then treated with o.2 ml of NaoH to make the solution alkaline and the absorbance was taken at 405 nm (ʎ max).Then a graph was drawn where OD (Optical Density) was plotted on Y-axis against the concentration of PNP (µg), on X-axis.

Applications of Spectrophotometry:

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1. It is used to estimate the concentration of both coloured as well as colourles substances, which could absorb light.
2. It is also used to find out absorption maxima of component in a wide range of wavelengths.
3. Small volumes as small as o.1ml can be used for the estimation of precious sample.
4. It offers sensitivity in that each component in a solution or reaction can be singled out and estimated.
5. Because of its higher sensitivity, it is used to estimate extremely small quantities of substances in a matter of few minutes.

RESULT AND DISCUSSION: - The standard curve is linear, thus obeying Beer Lambert's Law. Thus Para-nitro phenol, which is colourless in acidic medium, gives yellowish colour in alkaline medium which has maximum absorption at 405nm.

Upon increasing the concentration of Para-nitro phenol by (0.50µgs) at constant temperature (370C) keeping the volume of sodium hydroxide same (0.2.ml), the optical density curve goes on increase linearly

Since the curve obtained is linear with a slight deviation from the linearity, it can be concluded that the experiment was performed almost accurately and is in accordance to the Beer-Lambert's law. This Standard curve can now be utilized to determine the concentration of PNP in a sample of unknown concentration.

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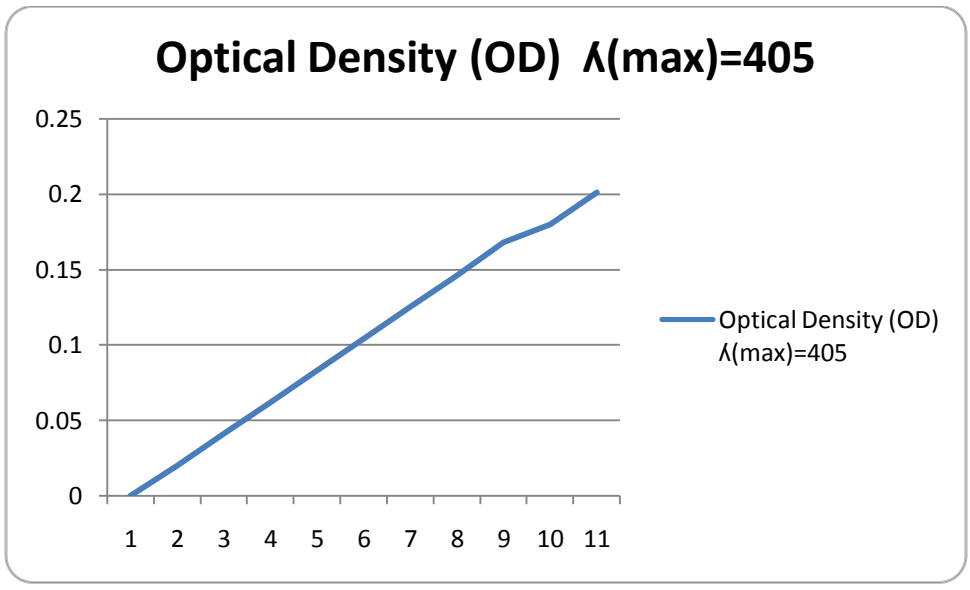
S.No	Volume of PNP(ml)	Concentration of PNP(µgs)	Volume of Acetate buffer (ml)	Incubation AT 37oC FOR 30 MINUTES	Volume of NaoH (ml)	Optical Density (OD) ʎ(max)=405
Blank	0.00	0.00	3.00		0.20	0.00
1	0.10	0.50	2.90		0.20	0.020
2	0.20	1.00	2.80		0.20	0.041
3	0.30	1.50	2.70		0.20	0.062
4	0.40	2.00	2.60		0.20	0.083
5	0.50	2.50	2.50		0.20	0.104
6	0.60	3.00	2.40		0.20	0.125
7	0.70	3.50	2.30		0.20	0.146
8	0.80	4.00	2.20		0.20	0.168
9	0.90	4.50	2.10		0.20	0.180
10	1.00	5.00	2.00		0.20	0.201



Standard Curve for Para-nitro phenol

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