

THE EFFECT OF INCUBATION TIME ON THE RATE OF AN ENZYME CATALYZED REACTION

OBJECTIVES

- To monitor the progress of an enzyme catalyzed reaction.
- To determine the initial rate of the reaction (V_i).

ENZYME KINETICS

- Enzyme kinetics is the study of the chemical reactions that are catalyzed by enzymes.
- Kinetics analysis of enzymes are used for characterization of enzyme catalyzed reaction
- It determines the rate of the enzymatic reaction (**velocity**) and its changes in response with the changes in parameters such as **substrate concentration**, **enzyme concentration**, **pH**, **temperature**.

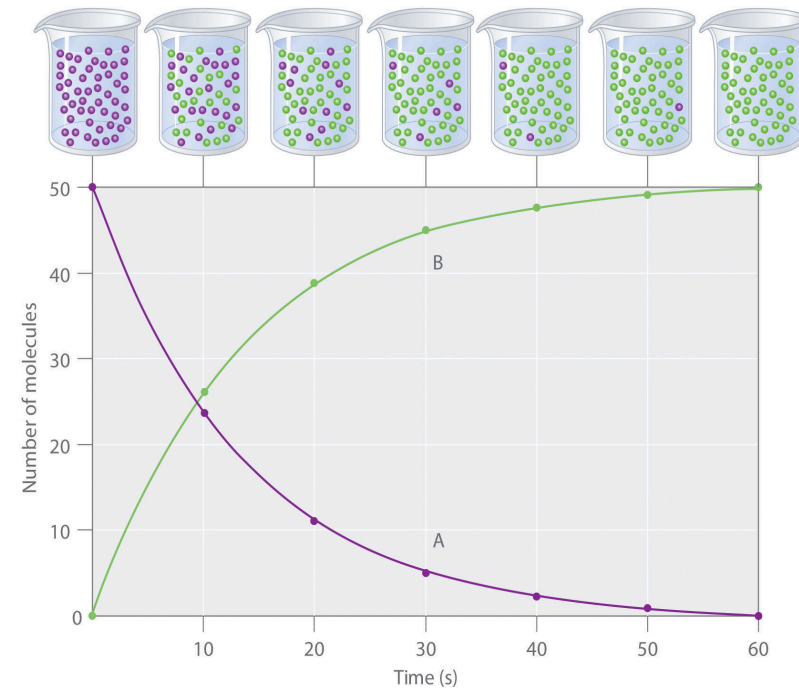
MEASURING THE RATE OF REACTION (VELOCITY)

The progress of an enzyme catalyzed reaction may be followed by measuring either the

- **amount of substrate consumed**, or
 - **Amount of product formed**
- } per unit time.

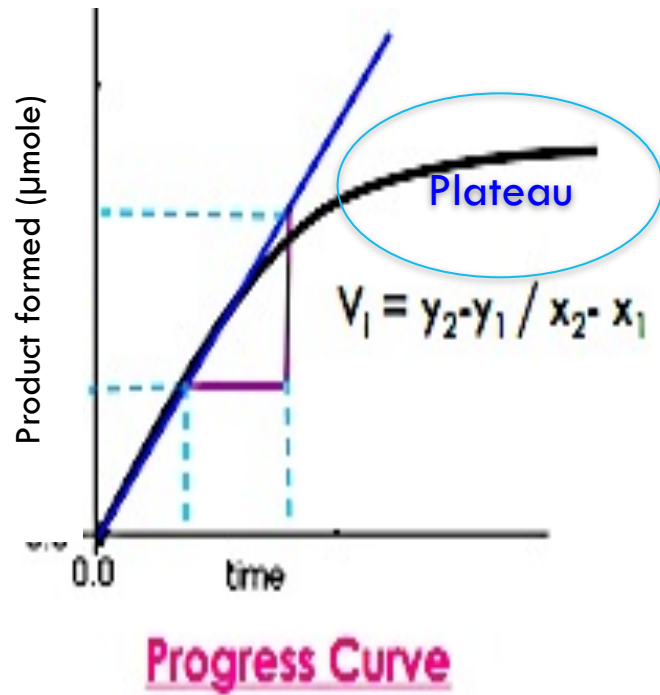
Velocity (V) = **rate of reaction** = change in [P] or [S] per unit time

Unit : $\mu\text{moles/minute}$



INITIAL VELOCITY

- **The initial rate of reaction (V_i)** is measured as the slope at the origin (time= 0).



The effect of time on the enzyme catalyzed reaction:

- The rate of the reaction is highest at time zero and decreases with increasing time, eventually falling to zero itself, reaching a plateau.
- This usually occurs either when all the substrate is used up or when equilibrium is reached.

ACID PHOSPHATASE

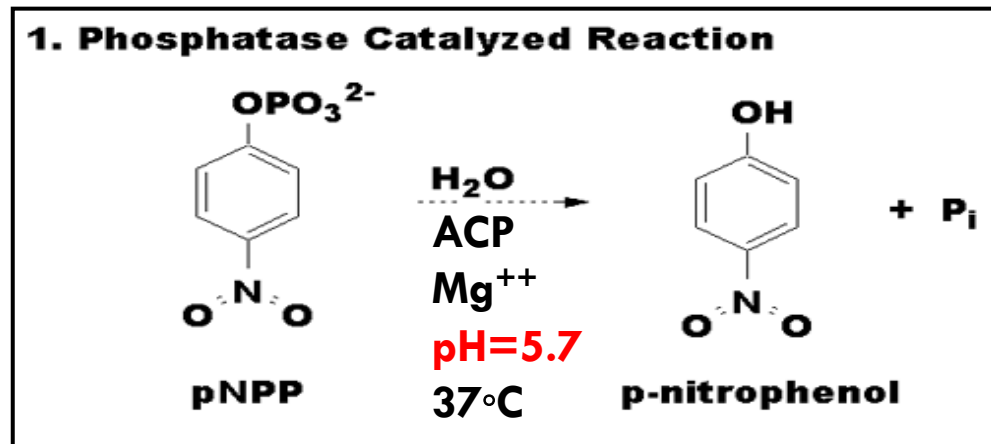


- In this experiment, you will measure the velocity of the reaction catalyzed by purified acid phosphatase (**ACP**) from wheat germ.
- Acid phosphatase is a **phosphatase** that acts on **monoesters** of orthophosphoric acid. It does **not act** on phosphoric diesters or triesters.
- The hydrolysis of phosphate esters is an important process in energy metabolism, metabolic regulation and a wide variety of cellular signal transduction pathways

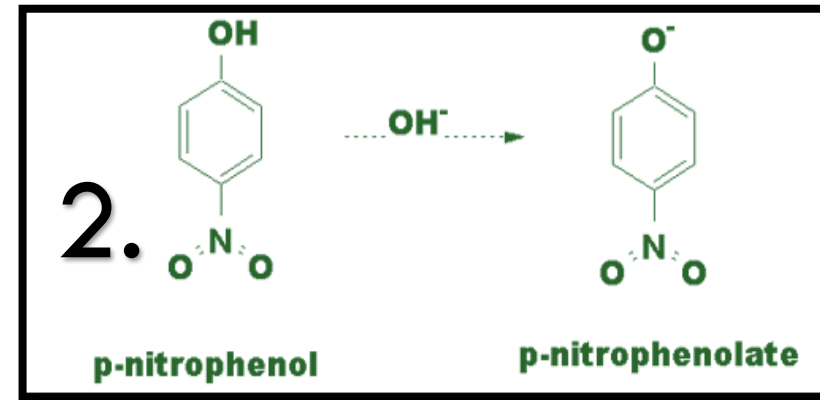
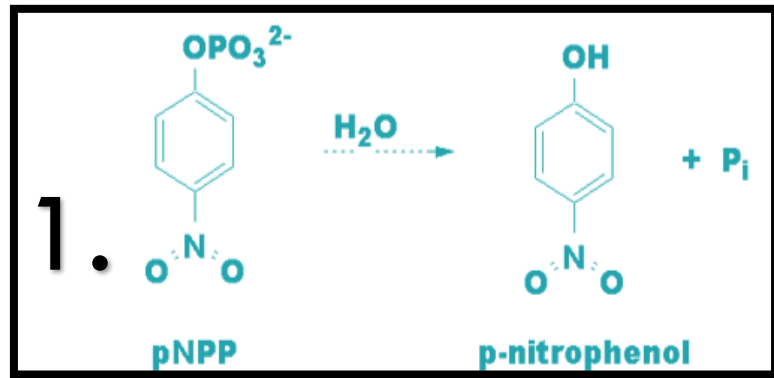
ACID PHOSPHATASE FROM WHEAT GERM



- The enzyme catalyzes the hydrolysis of p-nitrophenyl phosphate to inorganic phosphate and p-nitrophenol, **under acidic conditions (pH=5.7), with optimum temperature 37°C**
- Mg^{++} ion act as an activator for the enzyme



PRINCIPAL OF THE ENZYME ASSAY IN VITRO



1. Both p-nitrophenyl phosphate and p-nitrophenol are colorless at acidic pH values.
2. Under **alkaline conditions**, p-nitrophenol is converted to a **p-nitrophenolate** (yellow color) and concentration can be measured at 405 nm.

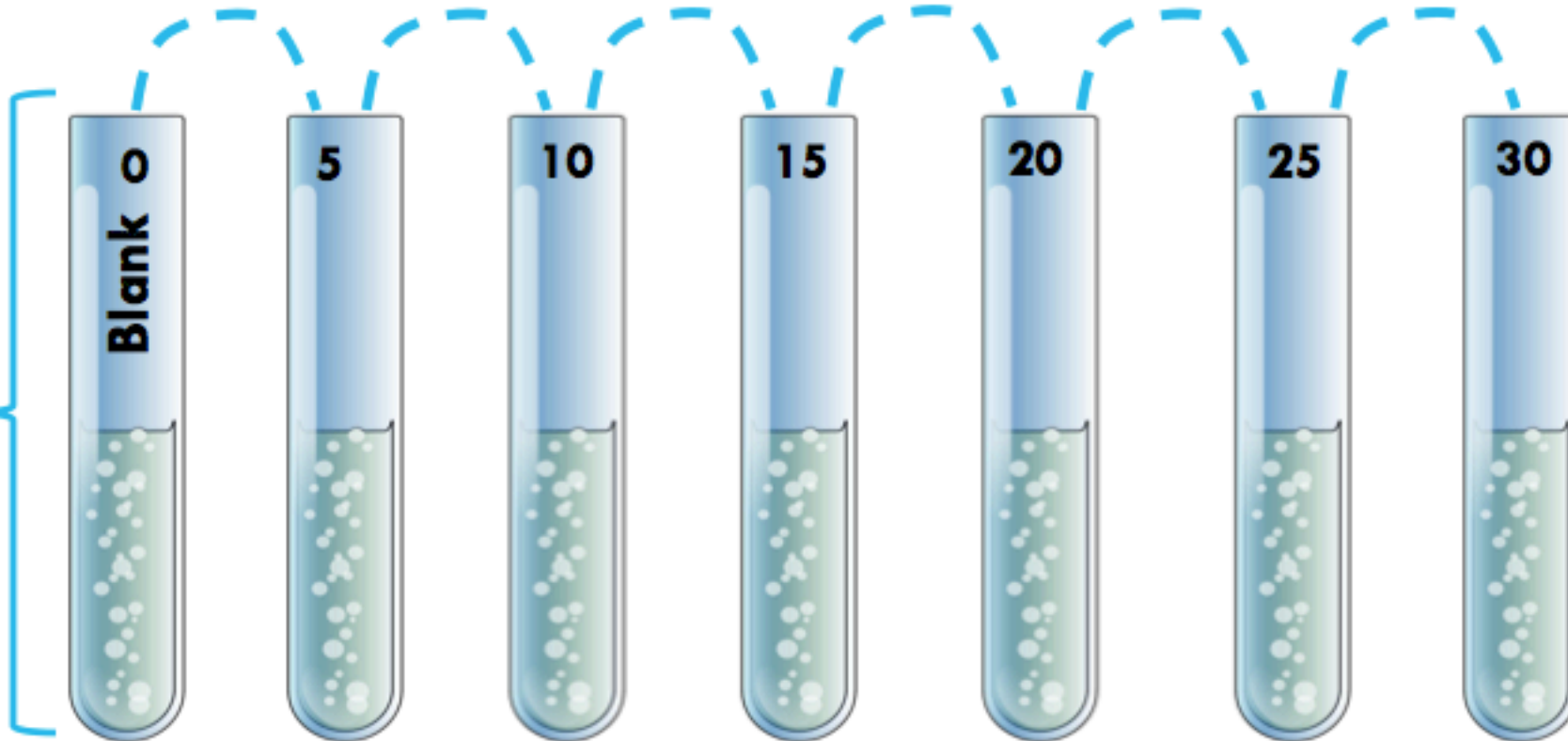
Note: Since ACP works under acidic condition, adding alkaline will cause the enzymatic reaction to stop.

METHOD

The reaction is *stopped after 5 minutes intervals* (by Addition of KOH), hence called **Fixed-time assay**

Add in each tube:

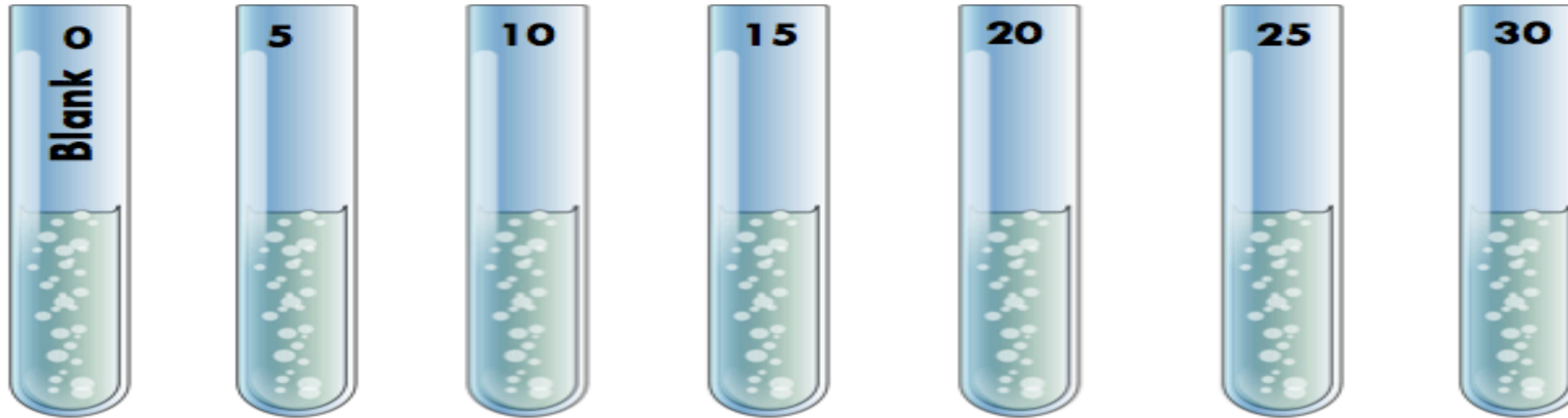
- 0.5 ml of buffer
- 0.5 ml of MgCl_2
- 0.5 ml of pNPP (S)
- 5 ml of water



Place in a water bath maintained at 37 °C for 5 minutes.

To start the reaction → add 0.5ml of E

To stop the reaction → add 0.5ml of KOH



Start at (min)	0	0	2	4	6	8	10
Stop at (min)	0	5	12	19	26	33	40



Add KOH to blank ((FIRST)), to prevent the reaction from happening.

After all the reactions have been terminated, determine the absorbance at 405 nm for each sample against blank.

Tube	5	10	15	20	25	30
Start at (min)	0	2	4	6	8	10
Stop at (min)	5	12	19	26	33	40

Time (min)	Tube	Addition 0.5 ml of
0	5	Enzyme
2	10	Enzyme
4	15	Enzyme
5	5	KOH
6	20	Enzyme
8	25	Enzyme
10	30	Enzyme
12	10	KOH
19	15	KOH
26	20	KOH
33	25	KOH
40	30	KOH

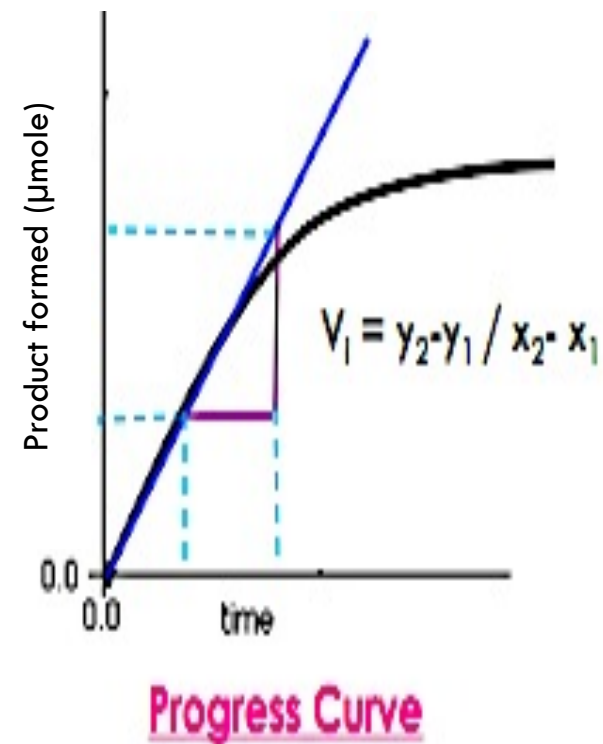
To convert the time table to an easier way try the following



RESULTS

Time (min)	Absorbance at 405nm	[P] (μmoles)
0		
5		
10		
15		
20		
25		
30		

[P] = (A x 10⁶) / E X L = μmole
 E= extension coefficient=18.8x10³
 A= absorbance
 L= path length (1 cm)



DISCUSSION

- Draw the graph [do not forget the title and the units]
 - X axis = Time (**min**)
 - Y axis = [P] (**μmoles**)
- Calculate from the graph the value initial velocity
- Principle
- Comment on the graph that you get in details

QUESTIONS

- How is the initial velocity of an enzymatic reaction determined?
- What are the factors that influence the rate of enzyme catalyzed reactions?
- What is the pH which the acid phosphatase activity is measured?