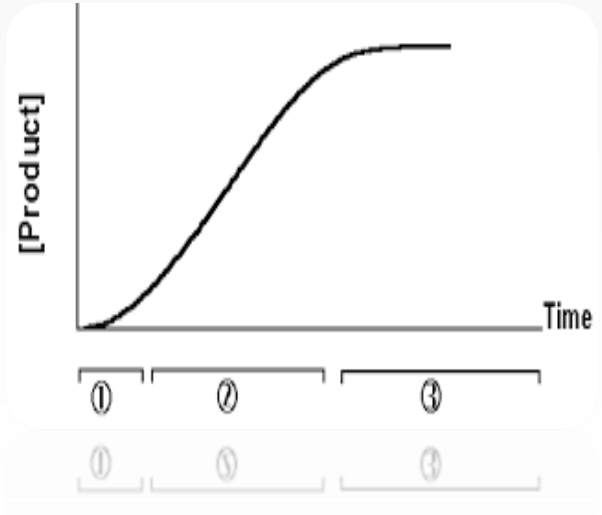
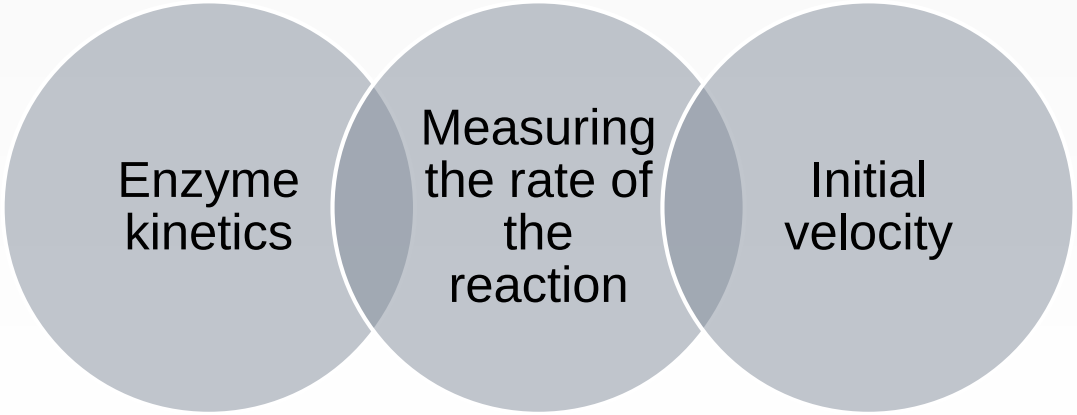
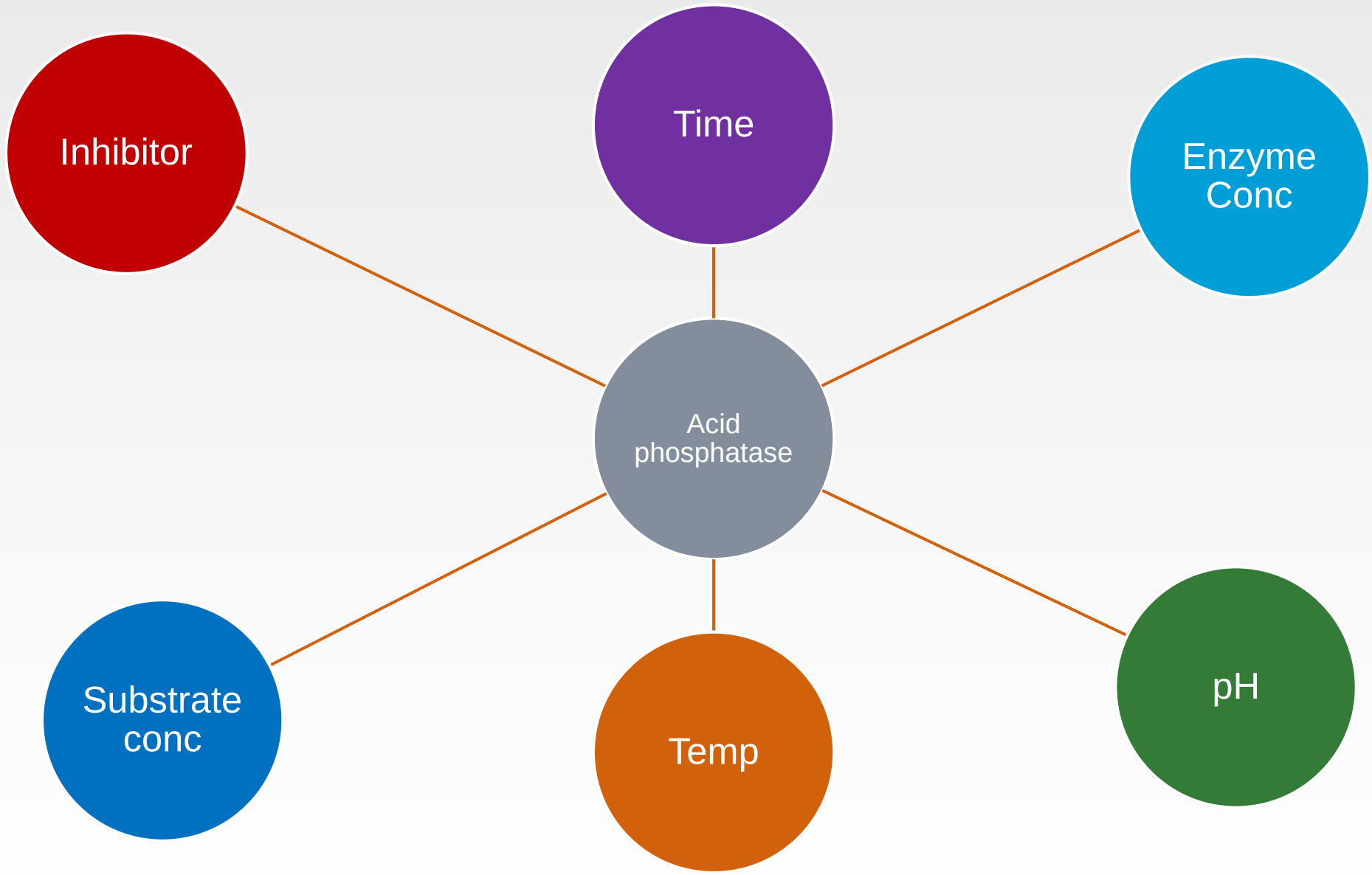
The effect of incubation time on the rate of an enzyme catalyzed reaction



Objectives

- To monitor the progress of an enzyme catalyzed reaction (Acid phosphatase).
- To determine the initial rate of the reaction ($\mathbf{V_i}$).
- **Important terms and points:**




Inhibitor
Time
Enzyme Conc
Acid phosphatase
Substrate conc
Temp
pH

Enzyme kinetics

- The central approach for studying the mechanism of an enzyme-catalyzed reaction is to study enzyme kinetics.

- 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**.

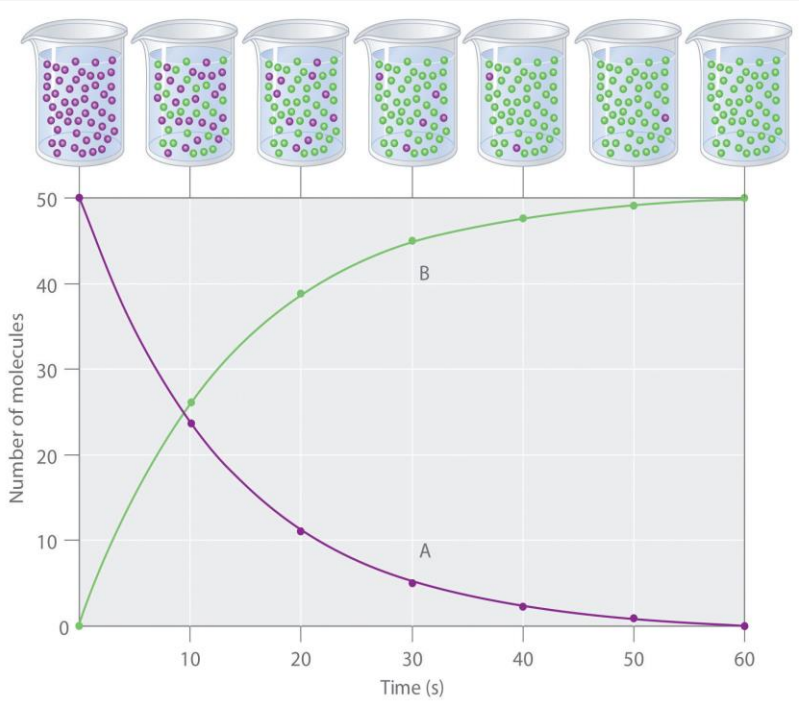
Measuring the rate of reaction (velocity)

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**

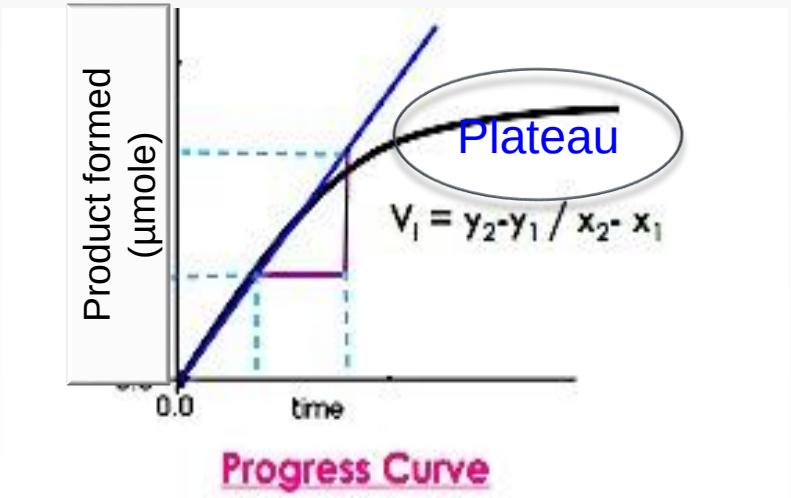
Velocity (V) = **rate of reaction**= change in [P] or [S] per unit time
Unit : **µmoles/minute**



Initial velocity

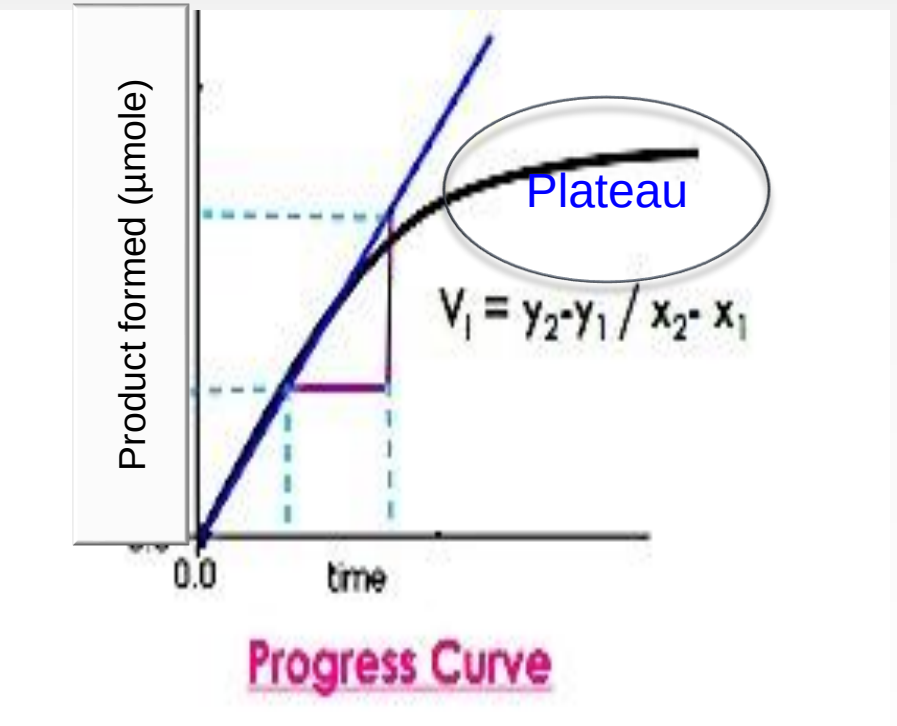
- **The initial rate of reaction is used in the study of enzyme kinetics**

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



Why we measure initial velocity ?

- **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.
- **The Solution is** to Measure V at very early times in reaction, before [S] decreases significantly
- The initial rate of reaction, vi, measured as the **slope** to the above curve at the origin (time= 0)



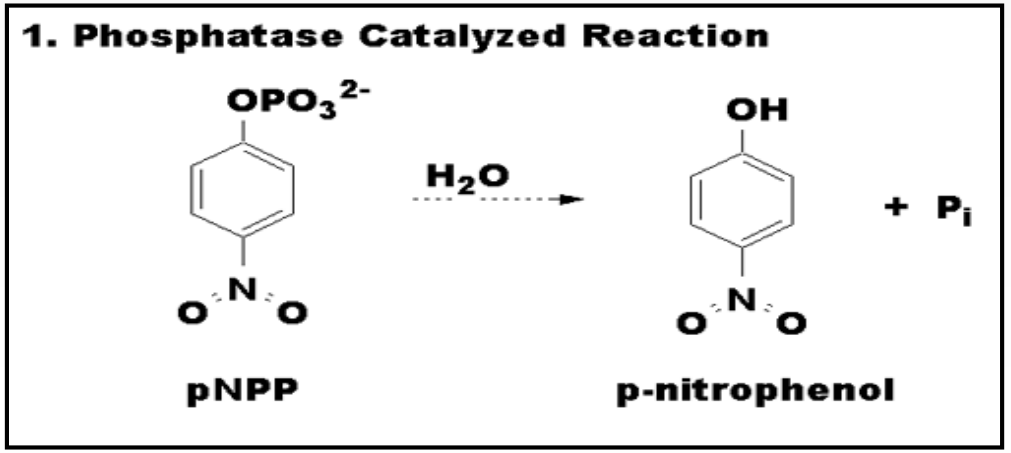
Acid phosphatase

- 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.
- Acid phosphatase is an important enzyme that plants use to obtain their energy from phosphate. Acid phosphatase's function in plants is to hydrolyze phosphate esters during energy metabolism.

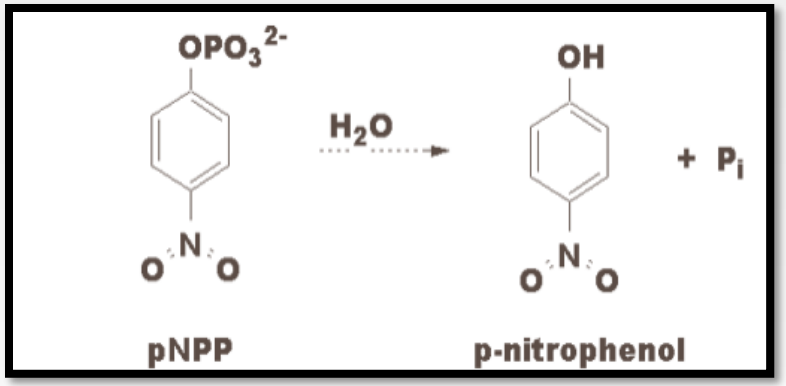
Acid phosphatase from wheat germ

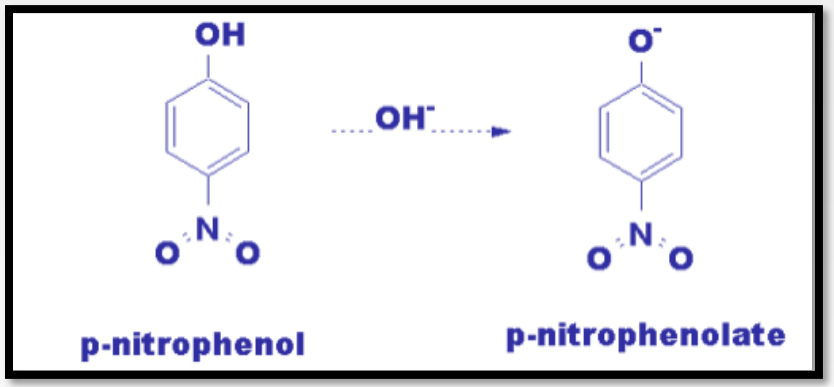
1. The enzyme catalyzes the hydrolysis of **p-nitrophenyl phosphate** to **inorganic phosphate** and **p-nitrophenol**, under **acidic conditions (pH=5.7)**, with **optimum temperature 37◦C**

- Mg^{++} ion act as an activator for the enzyme



Principal of the enzyme assay in vitro





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

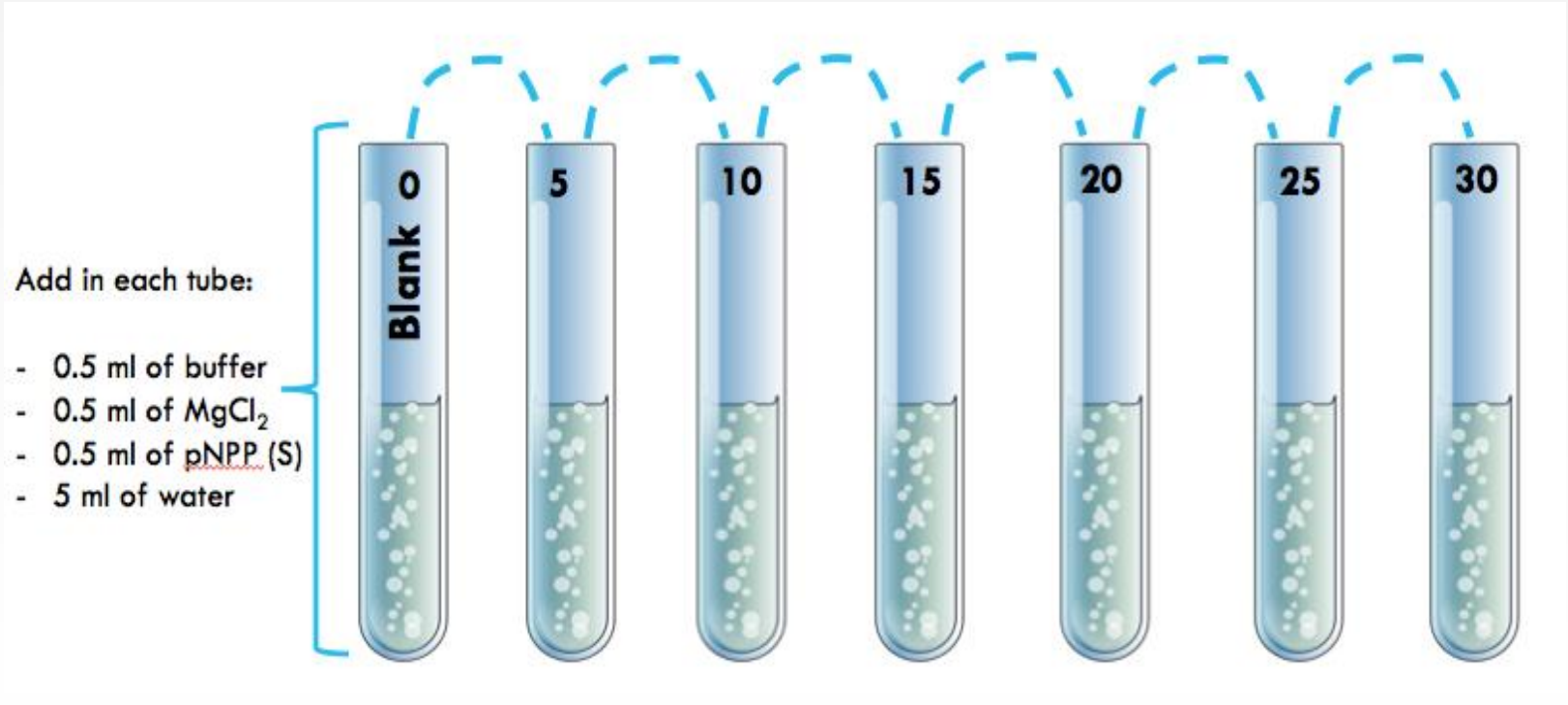
Note: Since ACP works under acidic condition, adding alkaline will cause the **enzymatic reaction to stop**.

Method: The effect of incubation Time

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

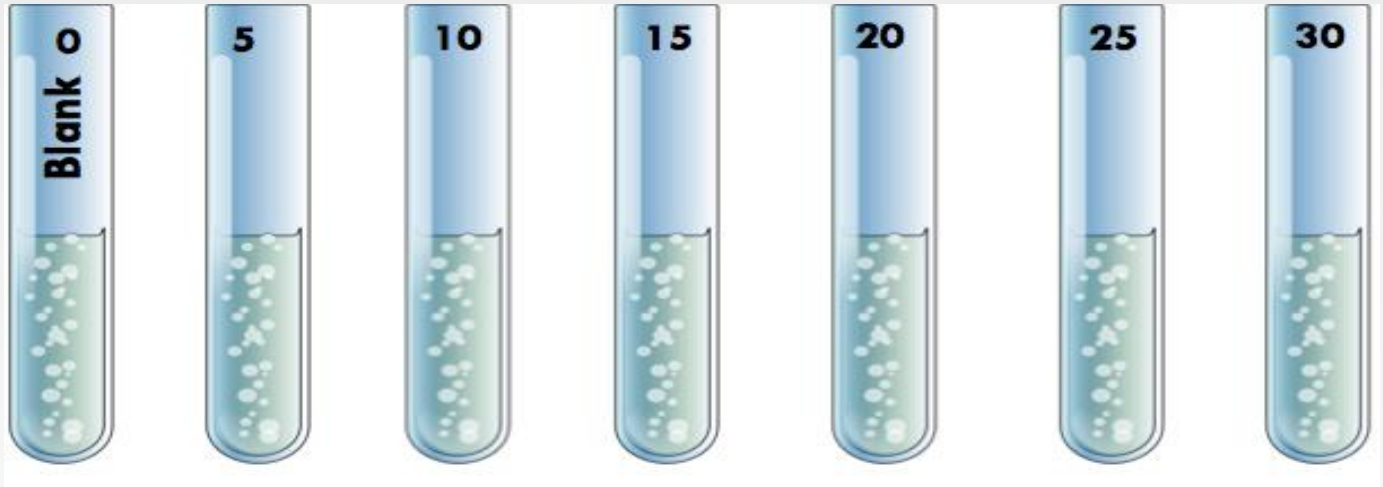
Method:

- Prepare a series of **seven reaction tubes** labeled 0 through 30 minutes at 5 minute intervals (Blank, 5, 10 … minutes).
- Follow the following addition protocol for **all the tubes:**



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

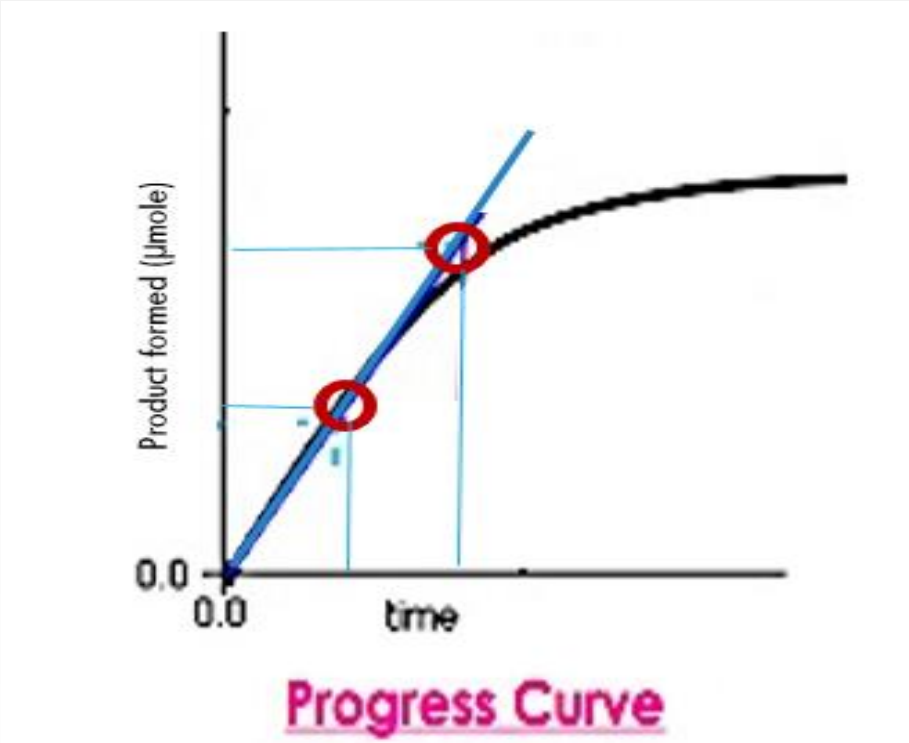
Time (min)	Absorbance at 405nm	[P] (µmoles)
0		
5		
10		
15		
20		
25		
30		

[P] = (A x 10^6) / E X L= µmole

E= extension coefficient=18.8×10^3

A= absorbance

L= path length (1 cm)



Progress Curve

$$V_1 = y_2 - y_1 / x_2 - x_1$$

Discussion

- Draw the graph [do not forget the title and the units]
  - X axis = Time
  - Y axis = [P]
  - 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?