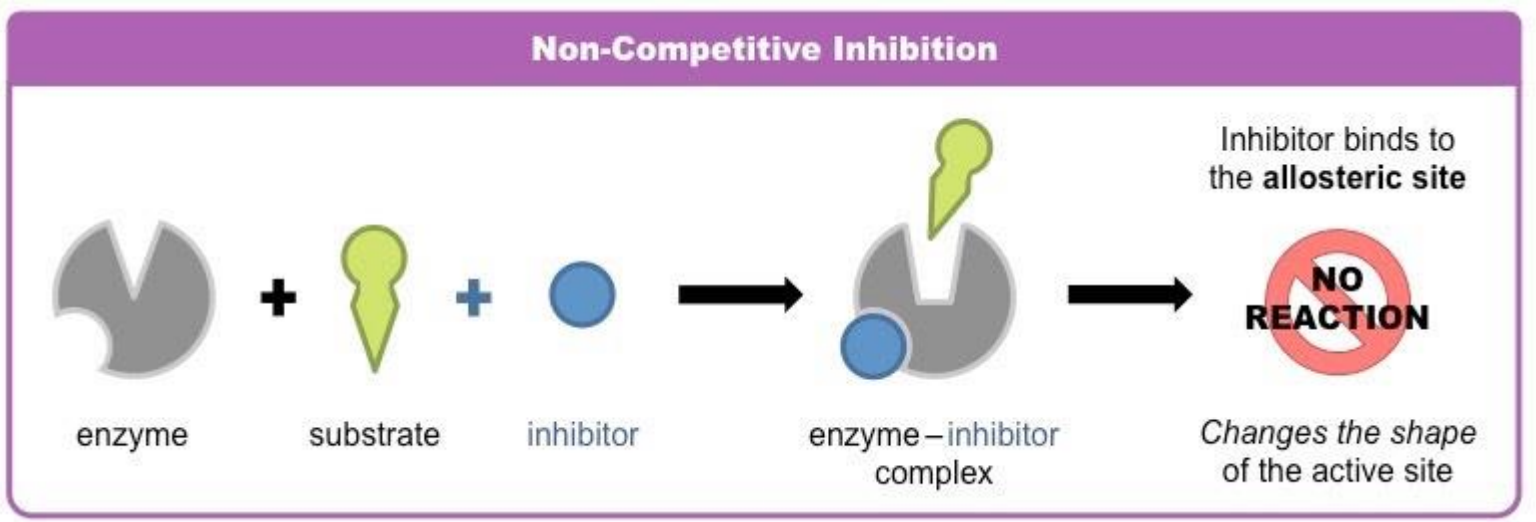
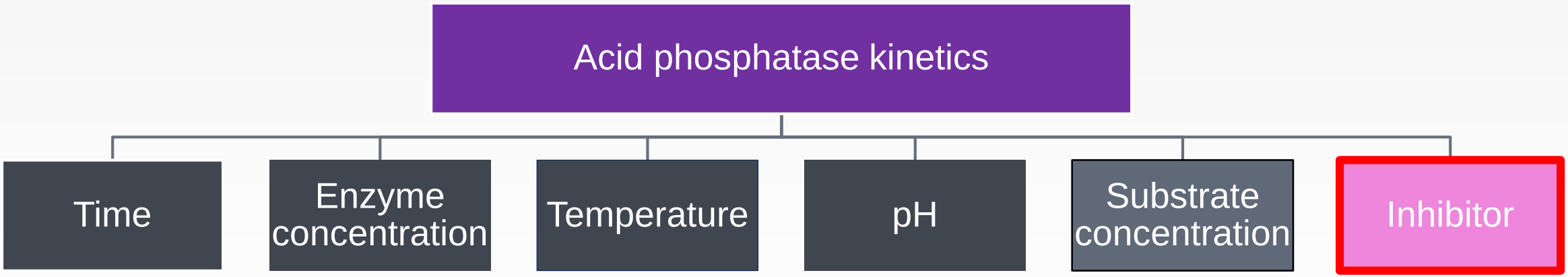
The effect of inhibitors (Inorganic phosphate & Sodium fluoride) on the rate of an enzyme catalyzed reaction



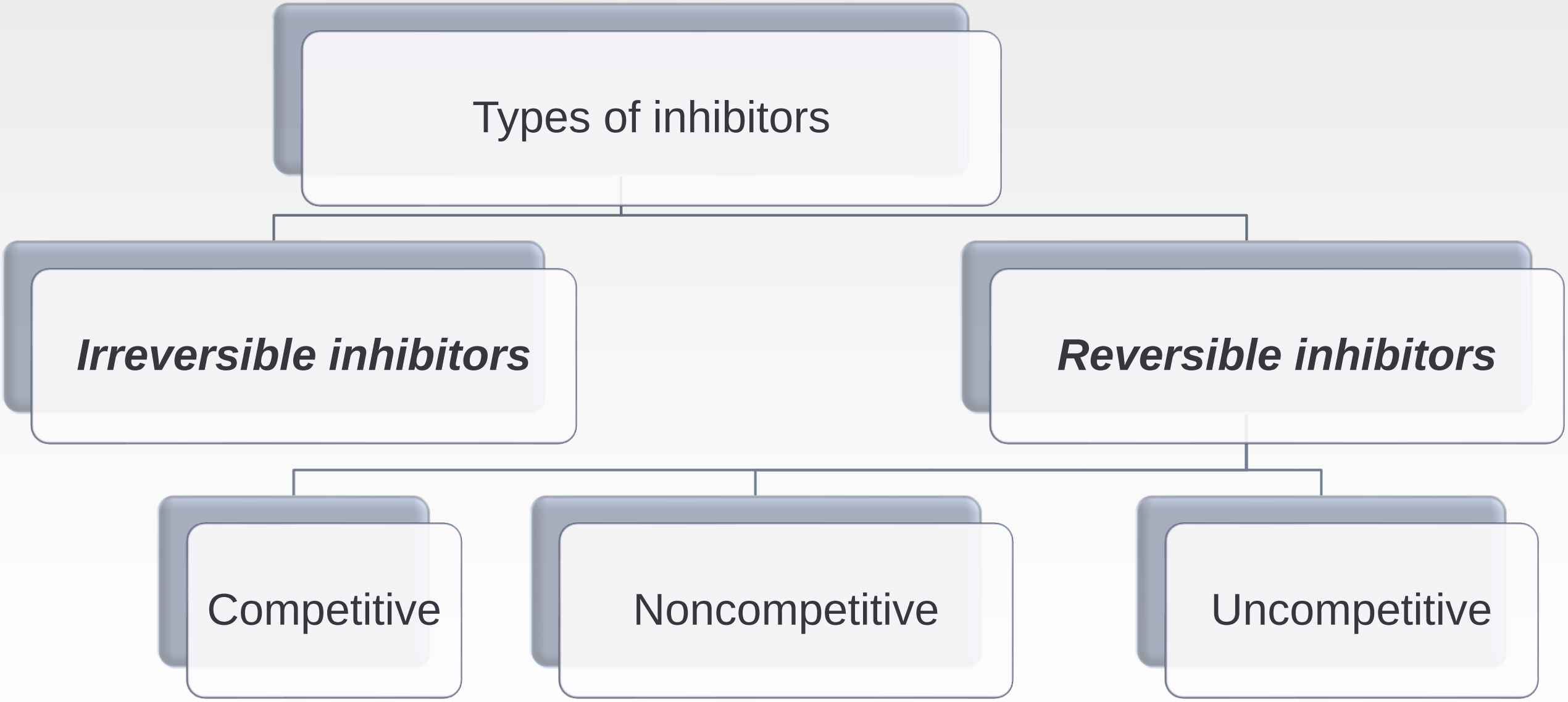
- **In this experiment, we will continue to study acid phosphatase kinetics.**



Objectives

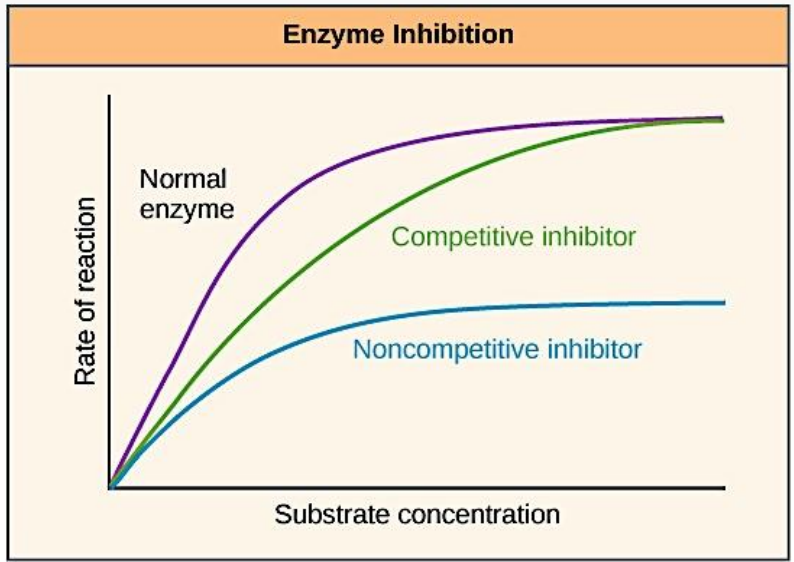
- To study the effect of inhibitors on the rate of an enzymatic reaction.
- To determine the type of inhibition of acid phosphatase by inorganic phosphate and sodium fluoride.

- **Inhibitors** are chemicals that reduce the rate of enzymatic reactions.
- They are usually specific and they work at low concentrations.
- They block the enzyme but they do not usually destroy it.
- Since blocking an enzyme's activity can kill a pathogen or correct a metabolic imbalance, *many drugs are enzyme inhibitors*.


Types of inhibitors
Irreversible inhibitors
Reversible inhibitors
Competitive
Noncompetitive
Uncompetitive

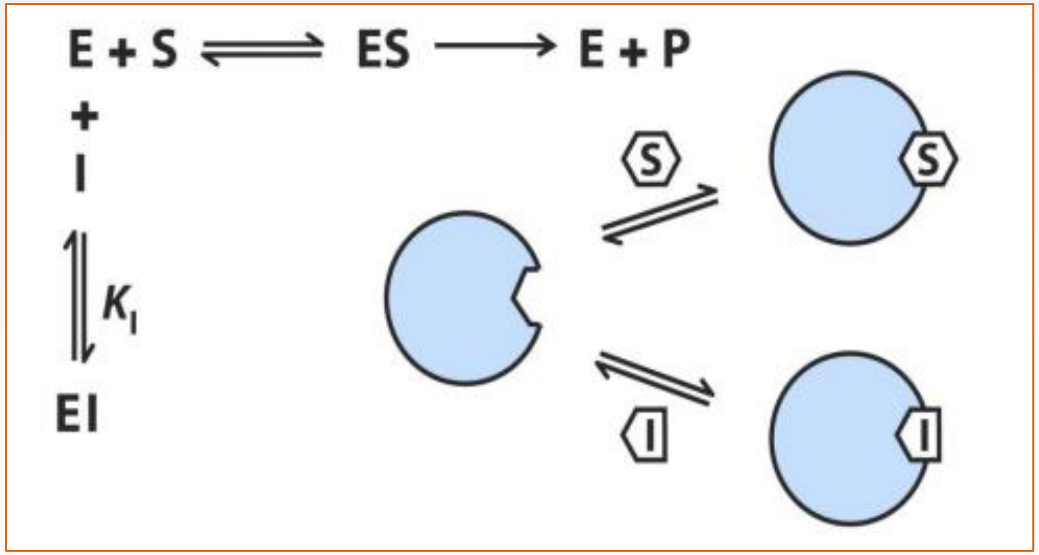
	Irreversible inhibitors	Reversible inhibitors
Type of bonds with E	Inhibitors bind **covalently** with enzyme	Inhibitors bind **non-covalently** with enzyme
Removal	Cannot be removed by dialysis or other way	Can be removed by dialysis
Activity Restoration	Permanently modify the active site residues (functional group) which the enzyme become inactive.	Removal of the inhibitor restores enzyme activity

It is relatively simple to **distinguish the three types of reversible inhibition** by comparing the Michaelis-Menten and Lineweaver-Burke kinetics (Vmax and Km) in the presence and absence of the inhibitor.

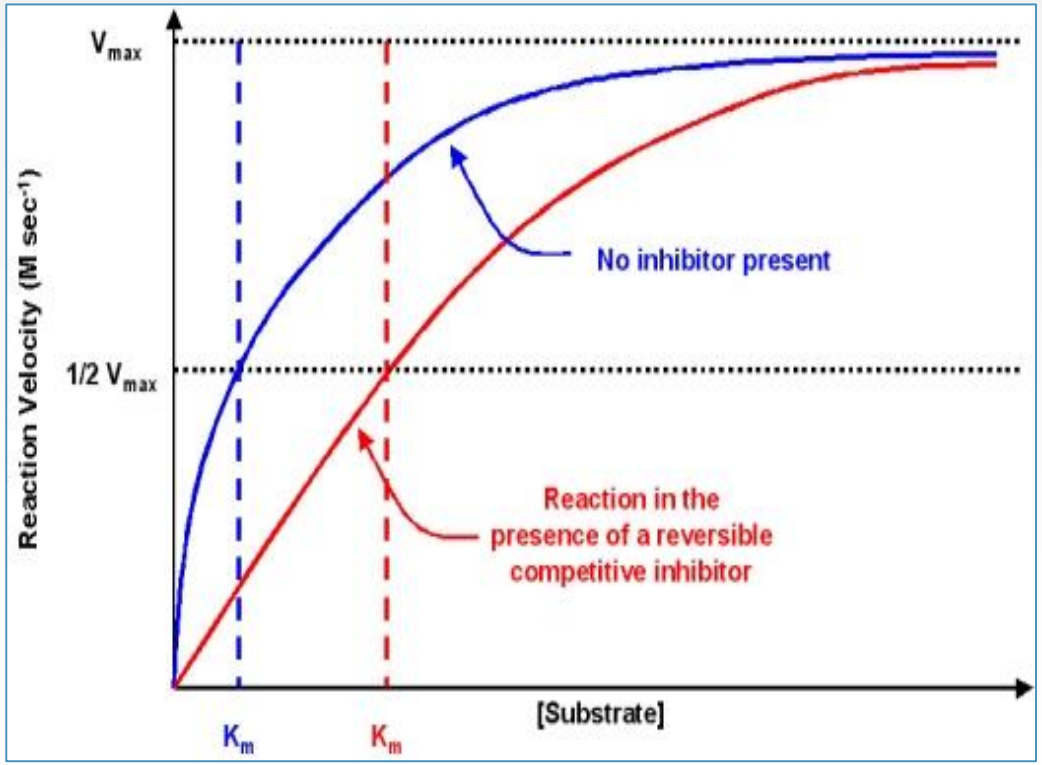


Competitive inhibitors

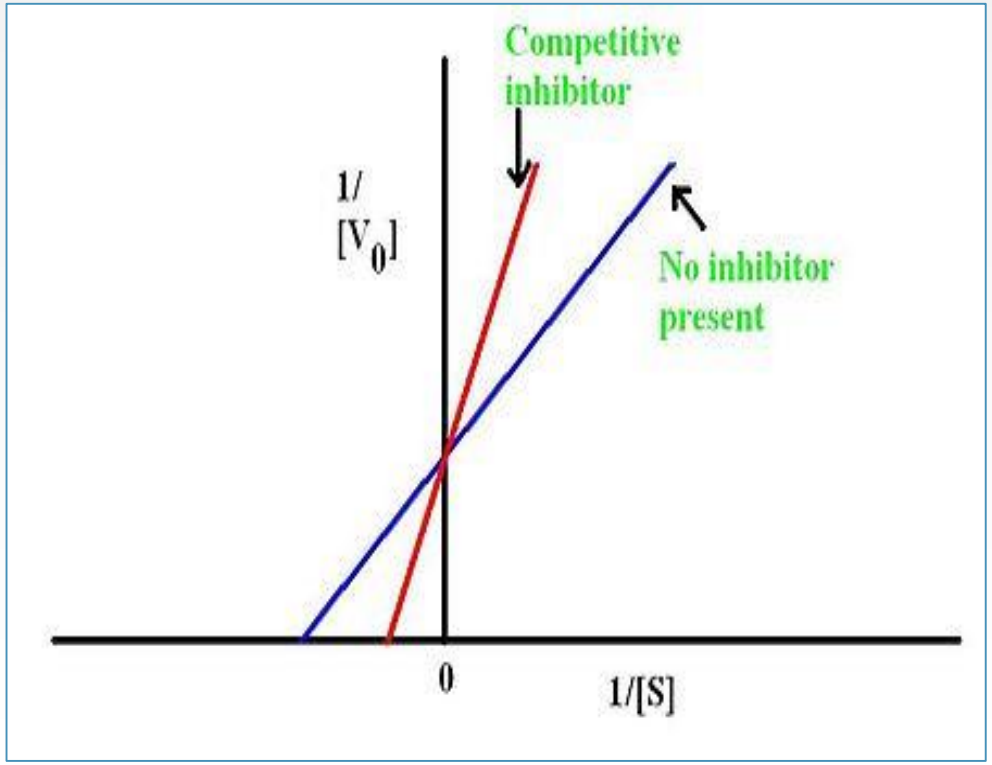
- As the name implies, the inhibitor compete with the substrate for active site of the enzyme.
- **The structure of substrate** and inhibitors are similar.
- Competitive inhibitor will **not affect** the Vmax
- **Increase the Km** ---→decrease the affinity
- This type of inhibition can be overcome by sufficiently high concentrations of substrate.



- Michaelis-Menten

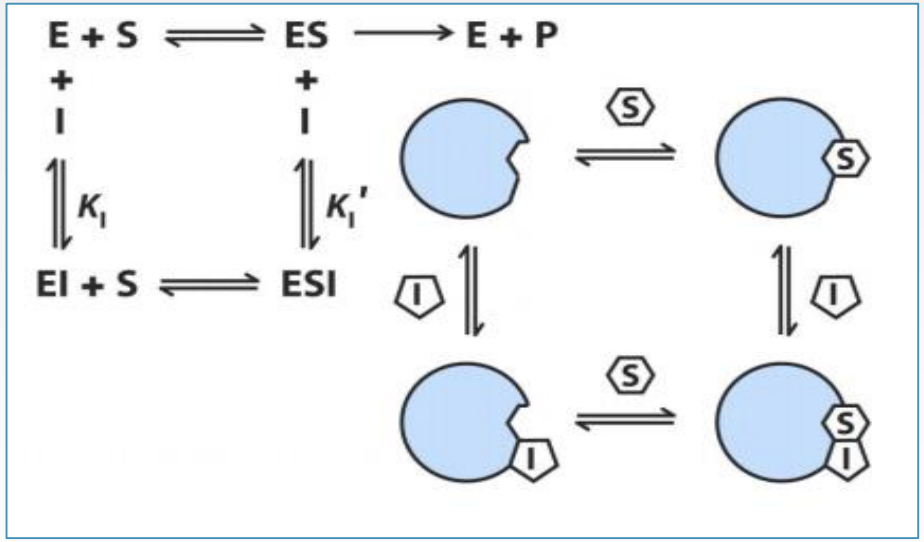


- Lineweaver-Burke

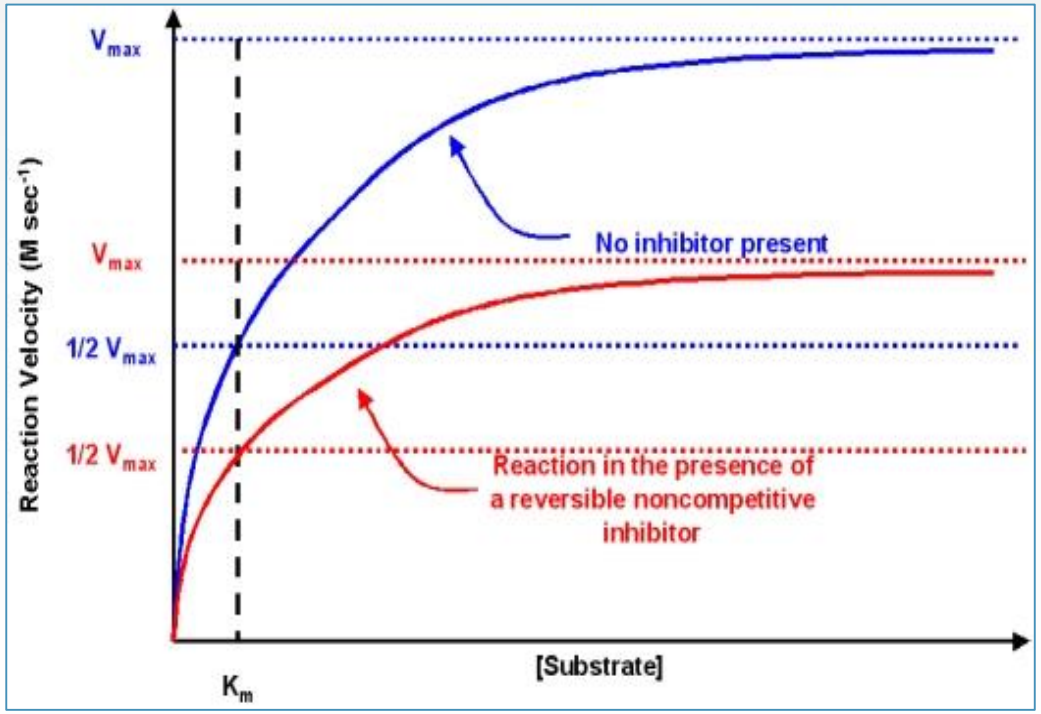


Noncompetitive inhibitors

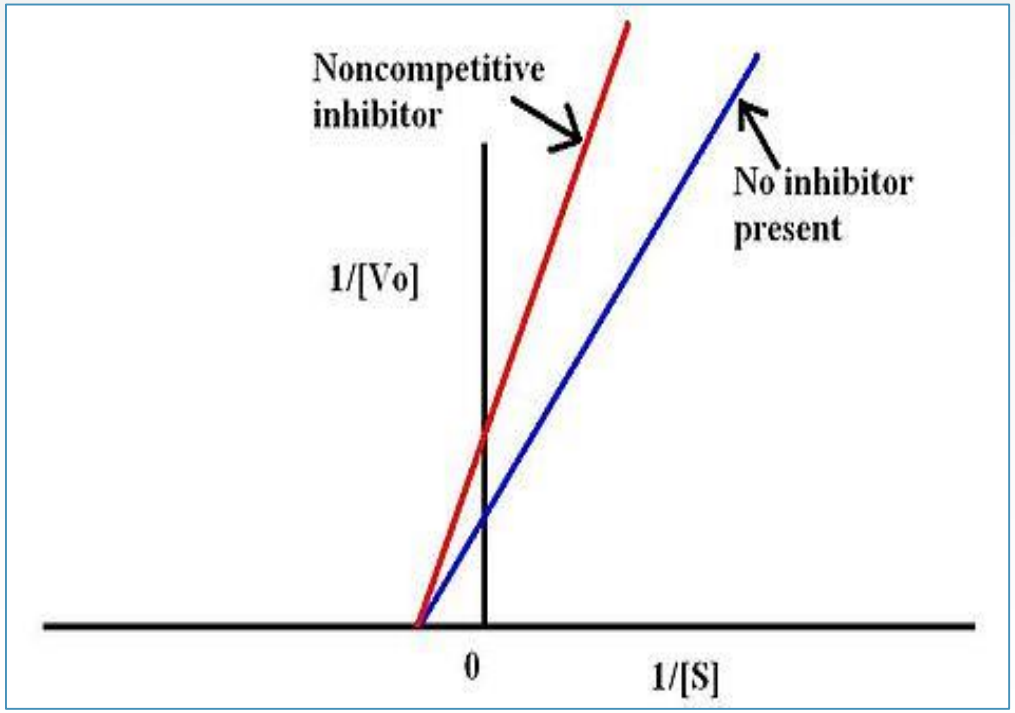
- A noncompetitive inhibitor is one that binds reversibly to the enzyme, but not at the active site itself, so that the substrate can still bind at the active site, but there's no catalyzed transformation.
- They can bind with E or ES complex.
- Have the **same Km** (with I OR without I).
- **low Vmax** (with I).
- This type of inhibition cannot be overcome by a large amount of substrate, thus noncompetitive inhibition.
- Km will not change with the inhibitor and the Vmax will decrease.



- Michaelis-Menten

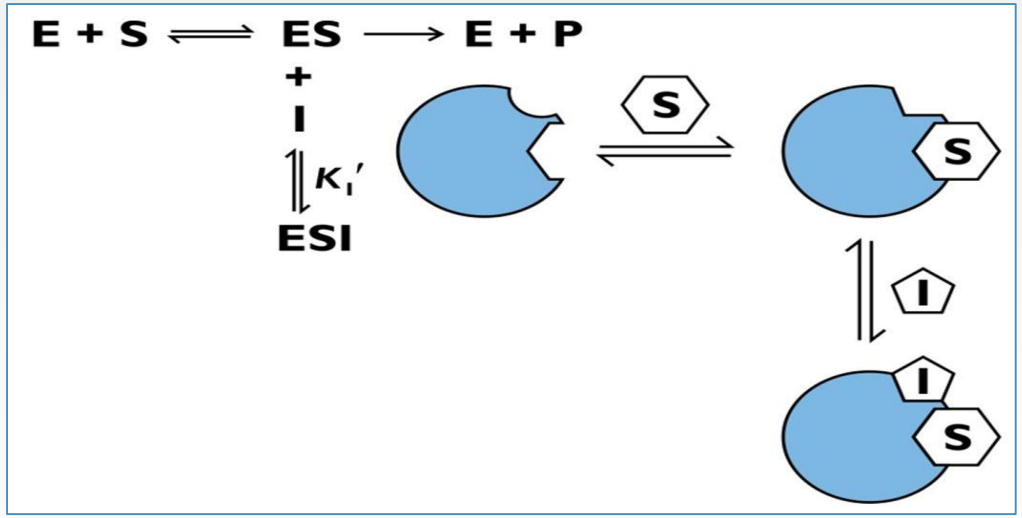


- Lineweaver-Burke

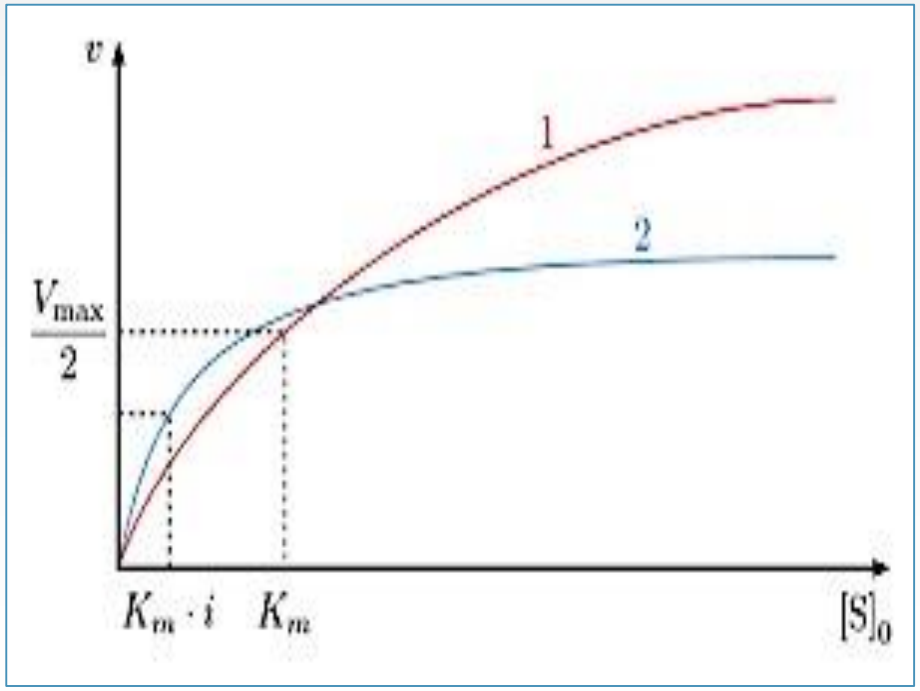


Uncompetitive inhibitors

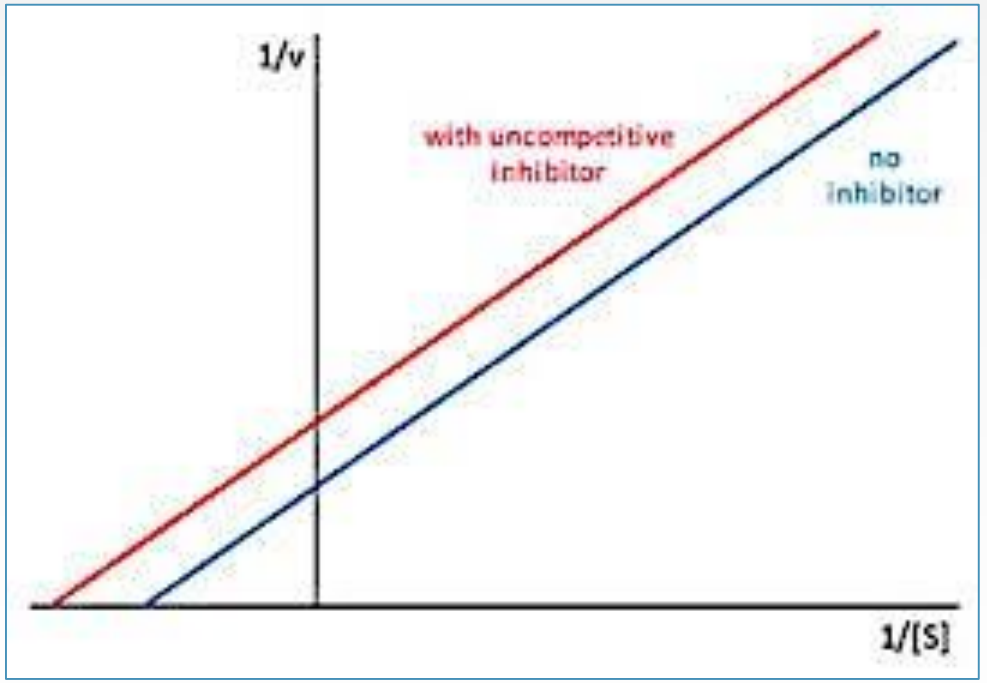
- The inhibitor binds only to the substrate-enzyme complex
- Both Vmax and Km are low (with I)



- Michaelis-Menten



- Lineweaver-Burke



Method:

- **Inorganic phosphate (Pi) and sodium fluoride** are inhibitors of acid phosphatase and it is your task to determine whether they are competitive, noncompetitive, or uncompetitive inhibitor.
- The setup is basically the same as in the experiment for the effect of substrate concentration on reaction velocity, except that a constant amount of inhibitor is added.
- The kinetics for the uninhibited reactions must be **compared** with those of reactions run in the presence of the inhibitor.
- Determinations of V_{max} and K_m will help you to determine the specific mode of inhibition

Method:

Without I

- Prepare 8 tubes labeled as follows

Tube	A	B	C	D	E	F	G	H
[S] mM	0	0.5	1	2.5	5	10	25	50

- To each of these tubes add

Chemical	Volume (ml)
pH sodium acetate buffer	0.5
0.1M $MgCl_2$	0.5
Corresponding p-nitrophenyl phosphate (pNPP)	0.5
Water	5

With I

- Prepare 8 tubes labeled as follows

Tube	A	B	C	D	E	F	G	H
[S] mM	0	0.5	1	2.5	5	10	25	50

- To each of these tubes add

Chemical	Volume (ml)
pH sodium acetate buffer	0.5
0.1M $MgCl_2$	0.5
Corresponding p-nitrophenyl phosphate (pNPP)	0.5
Water	4
K_2HPO_4 or Sodium fluoride (NaF)	1

- Place the tubes in a test tube rack situated in 37°C water bath and let stand

- Start the reaction by adding 0.5 ml enzyme and stop it by adding 0.5 ml KOH as in the following table:

Tube	Start the reaction	Stop the reaction
A	0 min	0 min
B	0 min	5 min
C	2 min	7 min
D	4 min	9min
E	6 min	11 min
F	8 min	13 min
G	10 min	15 min
H	12 min	17 min

- Determine the absorbance at 405 nm for each sample, using the first tube (0 mM of S) as the blank.

Results

Tube	[S] (mM)	1/[S] (1/mM)	Abs at 405 nm		V=(A x 10^6) /(18.8 x 10^3x time) (µmole of PNP/min)	1/V (1/ µmole of PNP/min)
			Without I	With I		
A	0					
B	0.5					
C	1					
D	2.5					
E	5					
F	10					
G	25					
H	50					

Results

- Draw the curve using Michaelis-Menten, determine Vmax and Km for acid phosphatase of both inhibited and not inhibited reaction
- Prepare the double –reciprocal plot of Lineweaver-Burk and determine the Km and V max from the x and y intercepts of both inhibited and not inhibited reaction

Discussion

- An introductory statement
- Principle
- **Compare** the Vmax and Km obtained Michaelis-Menten and Lineweaver-Burk graphs of both inhibited and uninhibited reactions with each other to **determine the type of inhibition**
- Determine if inorganic phosphate and sodium fluoride are a competitive, noncompetitive, or uncompetitive inhibitor? Justify your answer and discuss the difference you find
- Investigate the literature to determine how your results compare with those of previous workers.