The effects of enzyme concentration on the rate of an enzyme catalyzed reaction.

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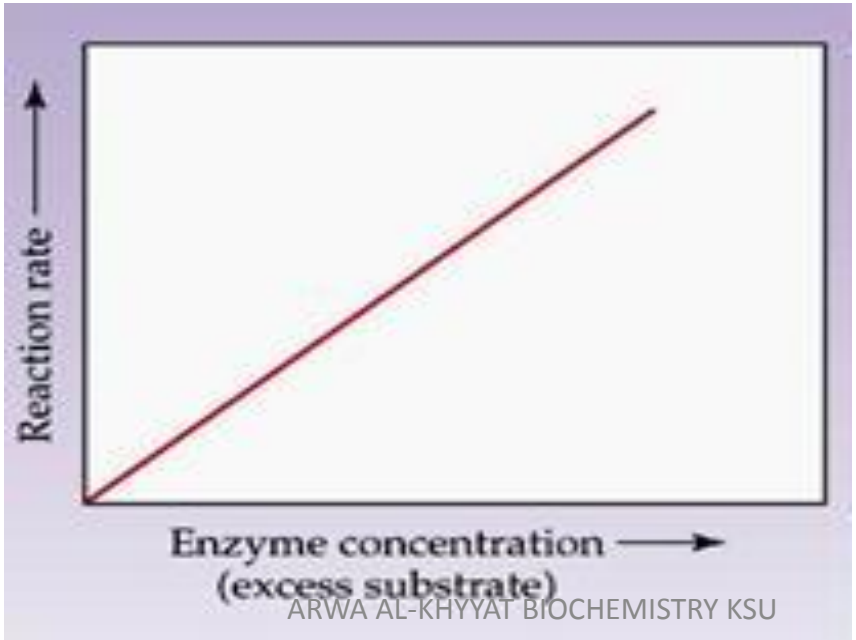
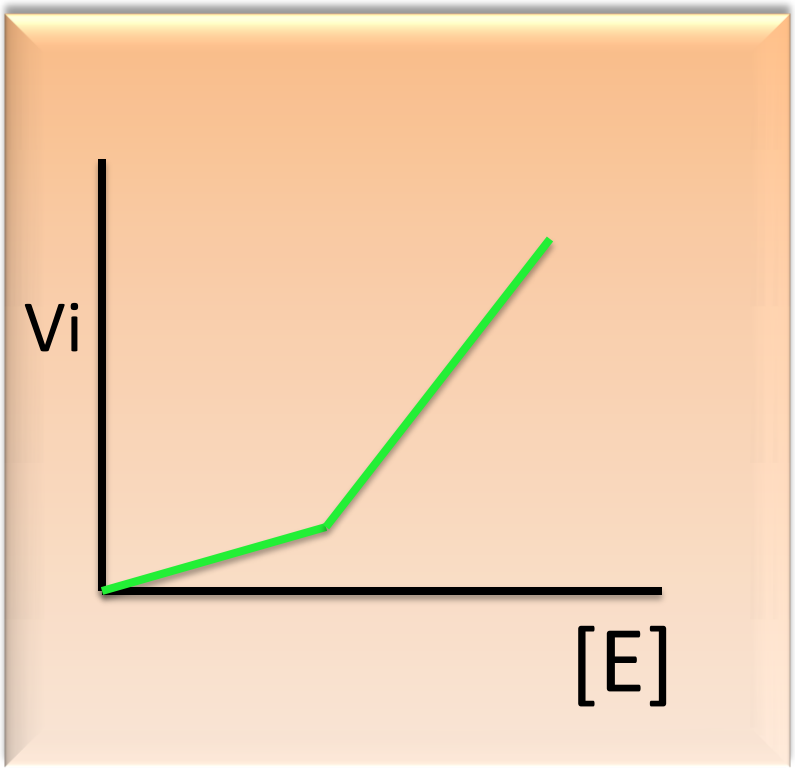
•The rate of enzyme catalyzed reaction is proportional to enzyme concentration

V α [E] ⟶ V = K [E]

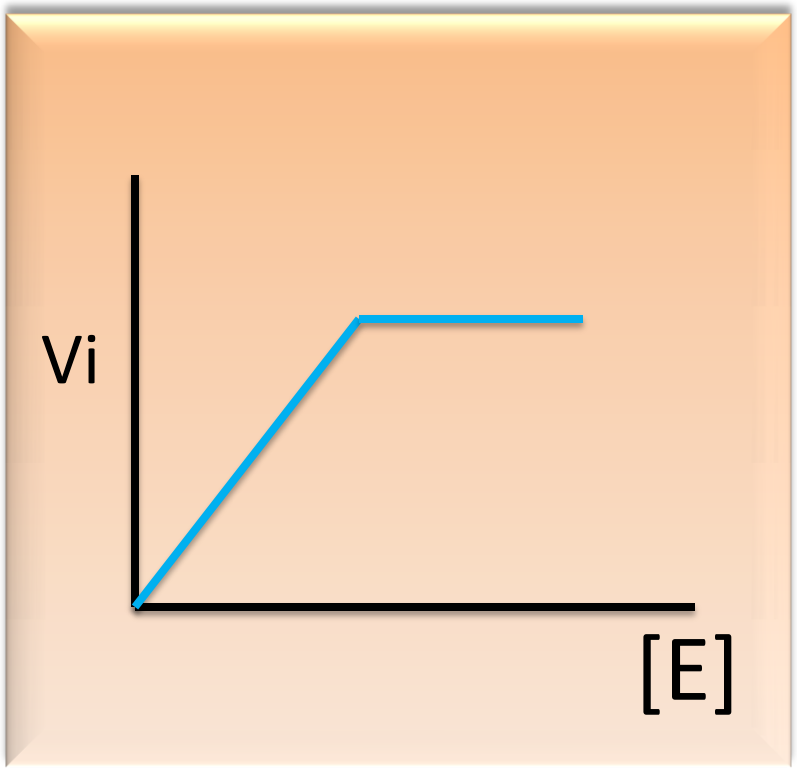
❖As the enzyme concentration increase the rate of enzymatic reaction increased which give a **liner curve** but deviation can occur



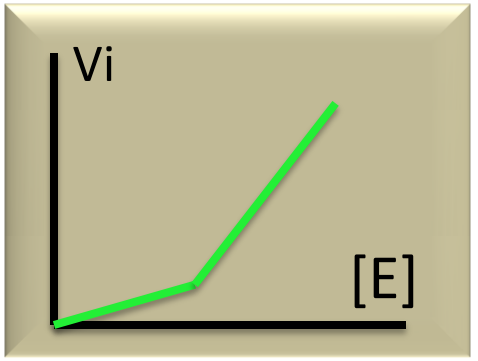
❖ Upward curve



❖ Downward curve



❖ Upward curve



In initial reaction the rate of the reaction is low but as E concentration increase the rate of reaction increase.
Because:

1. Presence of small amount of some highly toxic impurity in one of the component of the reaction mixture other than enzyme it self.

2. The percent of dissociable activator or co-enzyme in the initial reaction is low compeer with enzyme concentration.

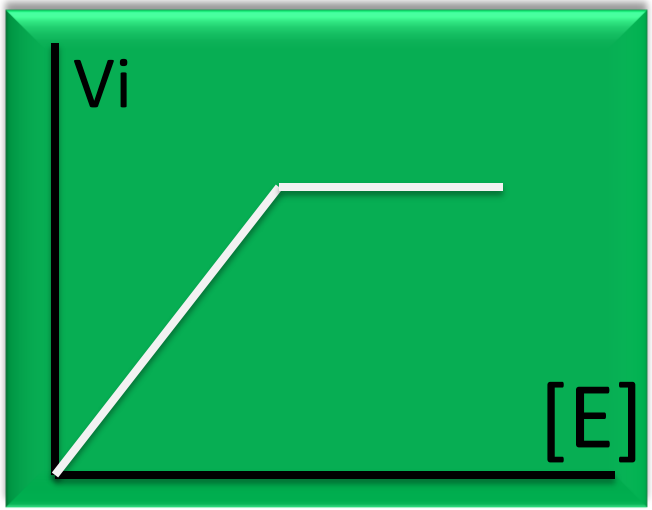
3. Some enzyme consist of complex subunit which are become inactivation when singly and when E subunit aggregate together ⟶ activation.

❖Downward curve

In initial reaction the rate of the reaction is increase as E concentration increase, then the rate of reaction become down and constant from liner curve

Because:

1. Limitation co-enzyme or activator
2. The substrate consume
3. Change PH
4. Presence of reversible inhibitor
   (Feed back inhibition)



Method:

- Label 7 test tubes (A, B, C, D, E, F, and G) and blank.
- Pipette the following solutions as indicated in the following table:

Tube no.	Buffer pH 5.7ml	$MgCl_2$ (ml)	Substrate (ml)	Dis. Water (ml)
A	0.5	0.5	0.5	5.3
B	0.5	0.5	0.5	5.2
C	0.5	0.5	0.5	5.1
D	0.5	0.5	0.5	5.0
E	0.5	0.5	0.5	4.9
F	0.5	0.5	0.5	4.7
G	0.5	0.5	0.5	4.5
Blank	0.5	0.5	0.5	5.5

•Place the tubes in the water bath at 37 ºC for 5 minutes.
•Start the reaction by adding the enzyme at 2 minutes intervals as in the following table:
•Stop the reaction by adding 0.5 ml KOH. After 5 min. as indicated in the previous table.
•Read the absorbance at 405 nm against the blank.

Tube no.	Enzyme conc. (ml)	Start the reaction (min.)	Stop the reaction (min.) o.5ml KOH
Blank	0	0	0
A	0.2	0	5
B	0.3	2	7
C	0.4	4	9
D	0.5	6	11
E	0.6	8	13
F	0.8	10	15
G	1.0	12	17

Read absorbance at 405nm

calculations:

velocity (v) =((A*10) /(E* 5min))

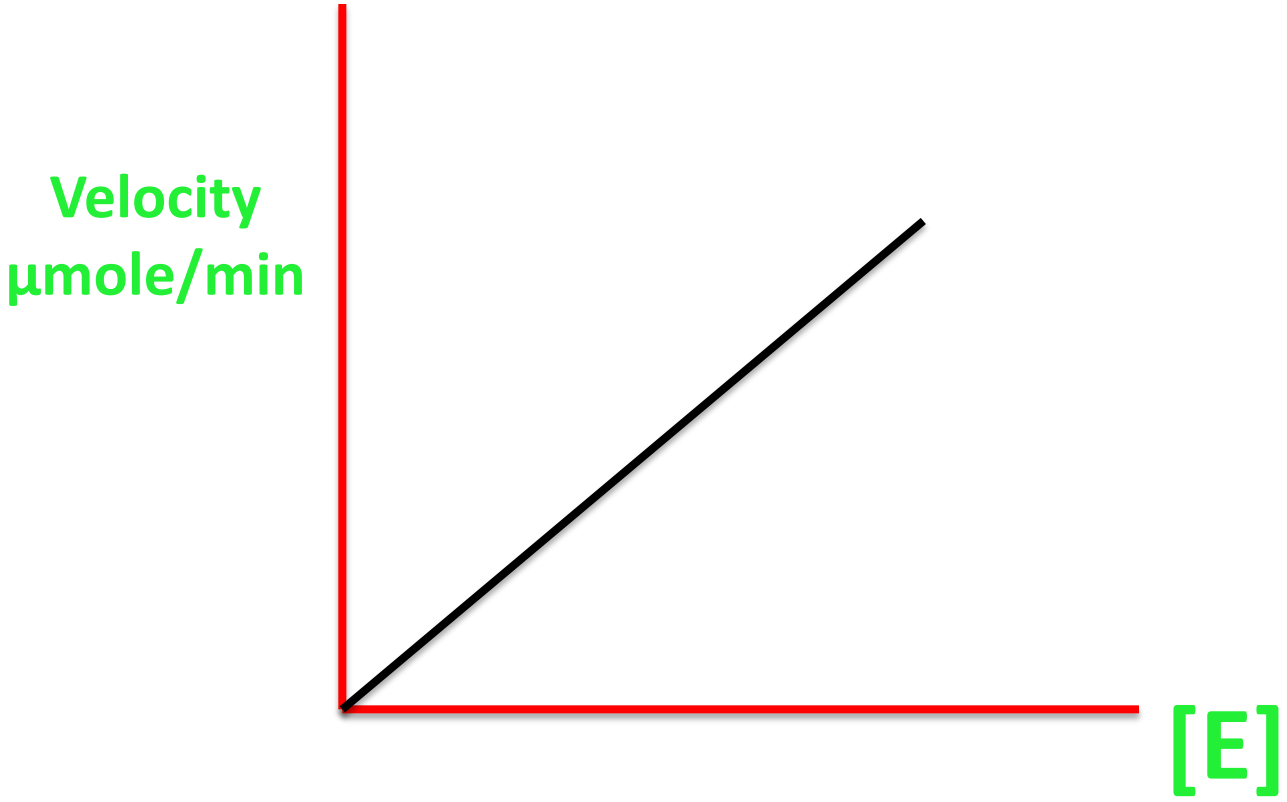
µmole/min

E= extension coefficient=18.8*10

A= absorbance

Results :

Tube	Enzyme concentration (ml)	Absorbance 405nm	Velocity µmole/min


Velocity
µmole/min
[E]