Type of Analytic Method

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1.END POINT METHOD

- In this method, the reaction between substrate (analyte) and a specific reagent react and form a colored complex.
- It require time period until reaction end.
- In end point , OD is taken at the end of reaction (completion of reaction)
- So , At the end of the reaction,the amount of product (colour complex) formed is in equil propotional to the substrate (analyte).

- Glucose + o2+ H2O $\xrightarrow{GOD}$ gluconic acid + H_2O_2

H_2O_2+phenol + 4amino-antipyrine $\xrightarrow{POD}$ Quinoneimine + 2H20

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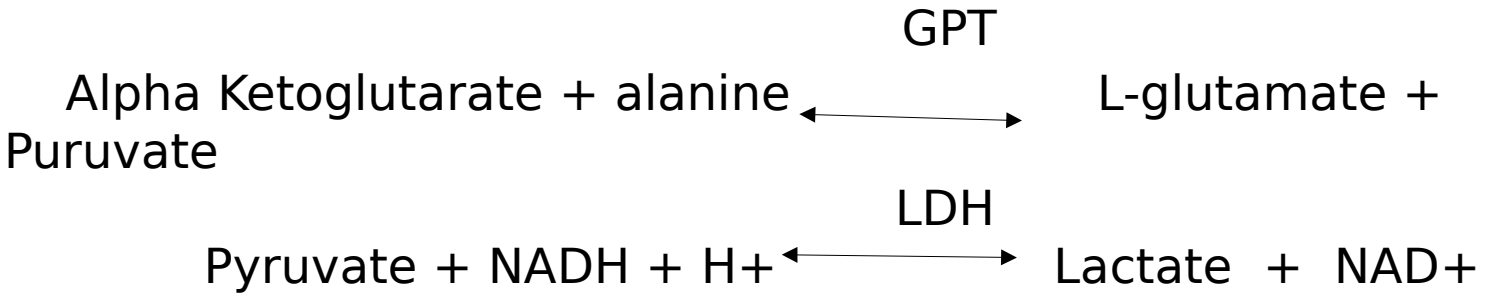
2.KINETIC METHOD

- In this method,if the concentration of the substrate is sufficiently high in comparison to enzyme then rate (velocity) of reaction is proportional to the concentration of enzyme.
- Thus the amount of product formed in a given period of time is proportional to the amount to concentration of enzyme present.
- OD is taken in time period of 1 min (delta OD)
- Delta OD = $OD_{60second}$ - $OD_{0\ second}$
- In this method,the rate of reaction is proportional to the concentration of enzyme(analyte).

2.KINETIC METHOD

- Principle of ALT:



More GPT
More NADH converted to NAD+
Decrease NADH propotional to concentration of GPT
More difference of OD of NADH in One Minute
More Delta OD
More GPT concentration

FIXED TIME KINETIC METHOD

- PRINCIPLE OF CRETININE :

Picrate + NAOH $\xleftrightarrow{\text{Non-Enzymatic}}$ Alkaline Picrate Complex

Alkaline Picrate complex + Creatinine $\xleftrightarrow{\text{Non-Enzymatic}}$ Janovaski Complex (Red-Orange Colour)

3. FIXED TIME KINETIC METHOD

- Velocity of reaction is measured in fix interval
- But Here in reaction , analyte is as substrate in reation.
- While in kinetic method, analyte is as enzyme.
- So, Analyte (substrate) is used up in reaction
- So , As Reaction goes on
  - Decrease substrate concentration
  - Decrease velocity of reaction
- So Delta OD is taken in very intial time period of reaction
- It is Called “Fix Time Kinetiv” Because
  - Analyte is as substrate in reaction, like End point method
  - Measuring velocity of reaction as Delta OD, Like Kinetic method