Immobilized Enzyme Systems

Enzyme Immobilization:
To restrict enzyme mobility in a fixed space.
UNIT-III

R.KAVITHA,M.PHARM
LECTURER,
DEPARTMENT OF PHARMACUTICS
SRM COLLEGE OF PHARMACY
SRMUNIVERITY
Immobilized Enzyme Systems

Enzyme Immobilization:

- Easy separation from reaction mixture, providing the ability to control reaction times and minimize the enzymes lost in the product.

- Re-use of enzymes for many reaction cycles, lowering the total production cost of enzyme mediated reactions.

- Ability of enzymes to provide pure products.

- Possible provision of a better environment for enzyme activity

- Diffusional limitation
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- Methods of Enzyme Immobilization:
  - Entrapment
  - Surface Immobilization
  - Cross-linking
Entrapment Immobilization is based on the localization of an enzyme within the lattice of a polymer matrix or membrane.

- retain enzyme
- allow the penetration of substrate.

It can be classified into matrix and micro capsule types.
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Entrapment
- Matrix Entrapment
- Membrane Entrapment (microencapsulation)

entrapped in a matrix
entrapped in droplets
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Matrix Materials:

Organics: polysaccharides, proteins, carbon, vinyl and allyl polymers, and polyamides. e.g. Ca-alginate, agar, K-carrageenin, collagen

Immobilization procedures:

Enzyme + polymer solution $\rightarrow$ polymerization
$\rightarrow$ extrusion/shape the particles

Inorganics: activated carbon, porous ceramic.

Shapes: particle, membrane, fiber
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**Entrapment**

challenges:
- enzyme leakage into solution
- diffusional limitation
- reduced enzyme activity and stability
- lack of control micro-environmental conditions.

It could be improved by modifying matrix or membrane.
Surface immobilization

According to the binding mode of the enzyme, this method can be further sub-classified into:

- **Physical Adsorption**: Van der Waals
  Carriers: silica, carbon nanotube, cellulose, etc.
  Easily desorbed, simple and cheap, enzyme activity unaffected.

- **Ionic Binding**: ionic bonds
  Similar to physical adsorption.
  Carriers: polysaccharides and synthetic polymers having ion-exchange centers.
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Surface immobilization

- **Covalent Binding**: covalent bonds

Carriers: polymers contain amino, carboxyl, sulfhydryl, hydroxyl, or phenolic groups.

- Loss of enzyme activity
- Strong binding of enzymes
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Cross-linking is to cross link enzyme molecules with each other using agents such as glutaraldehyde.

Features: similar to covalent binding.

Several methods are combined.
Summary of Immobilization Methods

Methods of Enzyme immobilization:

- Entrapment
  - matrix
  - membrane (microencapsulation)
- Surface immobilization
  - physical adsorption
  - ionic binding
  - covalent binding
- Cross-linking
Recycle packed column reactor:
- allow the reactor to operate at high fluid velocities.
Fluidized Bed Reactor:
- a high viscosity substrate solution
- a gaseous substrate or product in a continuous reaction system
- care must be taken to avoid the destruction and decomposition of immobilized enzymes
- An immobilized enzyme tends to decompose upon physical stirring.

- The batch system is generally suitable for the production of rather small amounts of chemicals.
Factors Affecting Enzyme Kinetics

- pH effects
  - on enzymes
    - enzymes have ionic groups on their active sites.
    - Variation of pH changes the ionic form of the active sites.
    - pH changes the three-Dimensional structure of enzymes.
  - on substrate
    - some substrates contain ionic groups
    - pH affects the ionic form of substrate
  ➔ affects the affinity of the substrate to the enzyme.
Factors Affecting Enzyme Kinetics

- Temperature
  - on the rate of enzyme catalyzed reaction

\[ v = \frac{d[P]}{dt} = k_2 [ES] \]

\[ k_2 = A \exp(-E_a/R \times T) \]

\[ T \uparrow \quad k_2 \uparrow \quad v \uparrow \]

- enzyme denaturation

\[ \frac{d[E]}{dt} = k_d [E] \]

\[ k_d = A_d \exp(-E_a/R \times T) \]

\( k_d \): enzyme denaturation rate constant;
\( E_a \): deactivation energy