# Immobilization of Enzymes

An immobilized enzyme is an enzyme attached to inert, insoluble material such as Calcium alginate (produced by reacting a mixture of sodium alginate solution and enzyme solution with calcium chloride).

This can provide increased resistance to changes in conditions such as pH or temperature.

It also lets enzymes be held in place throughout the reaction, following which they are easily separated from the products and may be used again - a far more efficient process and so is

widely used in industry for enzyme catalysed reactions.

**Immobilisation** is the imprisonment of an enzyme in a distinct phase that allows exchange with, but is separated from the bulk phase in which the substrate, effector or inhibitor molecules are dispersed and monitored.

# Advantages of Immobilized enzymes

- Reuse, continuous use, less labour intensive.
- Saving in capital cost, minimum reaction time.
- Less chance of contamination in products, more stability.
- Improved process control, high enzyme: substrate ratio.
- Increased functional efficiency of enzyme.

# Methods of Enzyme immobilization

- Adsorption
- Covalent binding
- Entrapment
- Microencapsulation

# Adsorption

- It is oldest and simplest method.
- Nelson and Griffin used charcoal to adsorb invertase for the first time in 1966.
- Enzyme is adsorbed to the external surface of support.
- The surface or carrier used may be of different type-
- I. Mineral support( eg. aluminium oxide, clay)
- II. Organic support ( eg. Starch)
- III. Modified sapharose and ion exchange resins.

- There is no permanent bond formation between enzyme and carrier. Only weak bonds stabilize enzymes to support or carrier.
- The weak bonds involved are Ionic interaction, Hydrogen bond and Van der Waal forces.

# Methods of Adsorption

- Static process- Enzyme is immobilized on carrier simply by allowing the solution containing enzyme to contact the carrier without stirring.
- Dynamic batch process- Carrier is placed into the enzyme solution and mixed by stirring or agitated continuously in a shaker.
- The reactor loading process – Carrier is placed in reactor and then Enzyme solution is transferred to reactor with continuous agitation.
- The electrodeposition process – Carrier is placed near to electrode in an enzyme and then current is put on, under the electric field an enzyme migrate to carrier and deposited on its surface.
- **Advantage** – Easy to carry out, no reagents are required, comparatively cheap method of immobilization, minimum activation steps are involved.
- **Disadvantages** – Efficiency is less, Desorption of enzyme from carrier.

# Covalent bonding

- Covalent bond is formed between the chemical groups of enzyme and chemical groups on surface of carrier.
- One of the widely used method.
- Hydroxyl groups and amino groups of support or enzyme form covalent bond more easily.
- Important functional groups of enzymes that provide chemical groups to form covalent bonds with support are amino groups, imino groups, hydroxyl groups, carboxyl groups, thiol groups, methylthiol groups, imidazole groups and phenol ring.
- Carriers or supports used for covalent bonding are – Carbohydrates, synthetic agents, protein carriers, inorganic carriers, amino group bearing carriers.
- Cynogen bromide (CNBr) agarose and cynogen bromide sepharose.

# Methods of covalent bonding

- Diazoation- bonding between amino group of support and a tyrosyl or histidyl group of enzyme.
- Peptide bond- bonding between amino or carboxyl group of the support and amino or carboxyl group of enzyme.
- Polyfunctional reagents – Use of bifunctional or multifunctional reagents which forms bonding between amino group of support and amino group of enzyme.
- **Advantages** – Comparatively simple method, no leakage or desorption problem, wide applicability , a variety of support with different functional groups available.
- **Disadvantages –** Chemical modification of enzyme leads to loss of functional conformation of enzyme.

# Entrapment

- Enzymes are physically entrapped inside a matrix (support) of water soluble polymer such as polyacrylamide type gels, and naturally derived gels. Eg., cellulose triacetate, agar, gelatin, carrageenan, alginate etc.
- Pore size of matrix should be adjusted to prevent loss of enzyme from matrix due to excessive diffusion.
- There is possibility of leakage of low molecular weight enzymes from gel.
- Agar and carrageenan have large pore size.
- **Methods of Entrapment** -

1. Inclusion in gels- enzyme is entrapped in gel.
2. Inclusion in fibres – enzyme is entrapped in fibre format.
3. Inclusion in microcapsules – enzyme entrapped in microcapsule formed by monomer mixtures such as polyamine and calcium alginate.

**Advantages** – fast method, cheap, mild conditions are required, less chances of conformational change in enzyme.

**Disadvantages** – Leakage of enzyme, chances of contamination, not much success in industrial process.

# Microencapsulation

- It is done by enclosing enzymes in membrane capsule.
- The capsule will be made up of semipermeable membrane like nitrocellulose or nylon.
- **Advantages –** Cheap and simple method. Large quantity of enzymes can be encapsulated.
- **Disadvantages** – Pore size limitation, only small substrate molecule is able to cross the membrane.

# Applications of Enzyme immobilization

- Immobilized enzymes have become an important tool in pharma industry. Among other things, they are used to manufacture penicillin and other antibiotics.
- They also form the basis of various processes in food industry, for example in the production of fructose enriched syrup.
- Production of steroids - Prednisolone is produced from cortisone using immobilized enzyme obtained from curcuria lanata.
- To produce amino acid - Immobilized "*L-aspartase-4-decarboxylase"* enzyme helps in conversion of L-Aspartic acid to L-Alanine an amino acid.
- In production of DOPA - L-DOPA is a drug used in treatment of Parkinsonism. This can be produced by using immobilized enzyme "β-tyrosinase."
- **In production of:** Co-enzyme-A (Vit-B5), Pro-insulin, interleukin-2, prostaglandins, monoclonal anti-bodies.
- **To produce** medicinal plant derivatives like anthraquinone (a glycoside to relieve constipation).
- Immobilized enzymes are used to study the type of disease present in the patient and also to study the pathological conditions.
- Immobilized enzyme "*Aldolase"* is used in diagnosis of **muscle disorders**.
- *"Glutamate oxalo acetate transfrerase"* to diagnose **myocardial infarction**.
- *"Iso-citrate dehydrogenase"* is used in diagnosis of **acute hepatitis**.
- **Prostate cancer** is detected using immobilized enzymes "*Acid phosphatase*."
- Immobilized enzymes are also used as **bio-sensors** to measure the concentration of biologicals in the body fluids.