Immobilized Enzyme Systems

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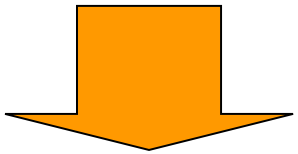
THE ECONOMIC ARGUMENT FOR IMMOBILISATION

- Enzymes are expensive, they should be utilized in an efficient manner
- As catalytic molecules, enzymes are not directly used up. After the reaction the enzymes cannot be economically recovered for re-use and are generally wasted
- This enzyme residue remains to contaminate the product and its removal may involve extra purification costs



Simple and economic methods must be used to separate the enzyme from the reaction product

Separation of enzyme and product using a two-phase system;

* One phase containing the enzyme
* The other phase containing the product

This is known as **IMMOBILISATION**

What is an Immobilized Enzyme?

- An immobilized enzyme is one whose movement in space has been restricted either completely or to a small limited region.

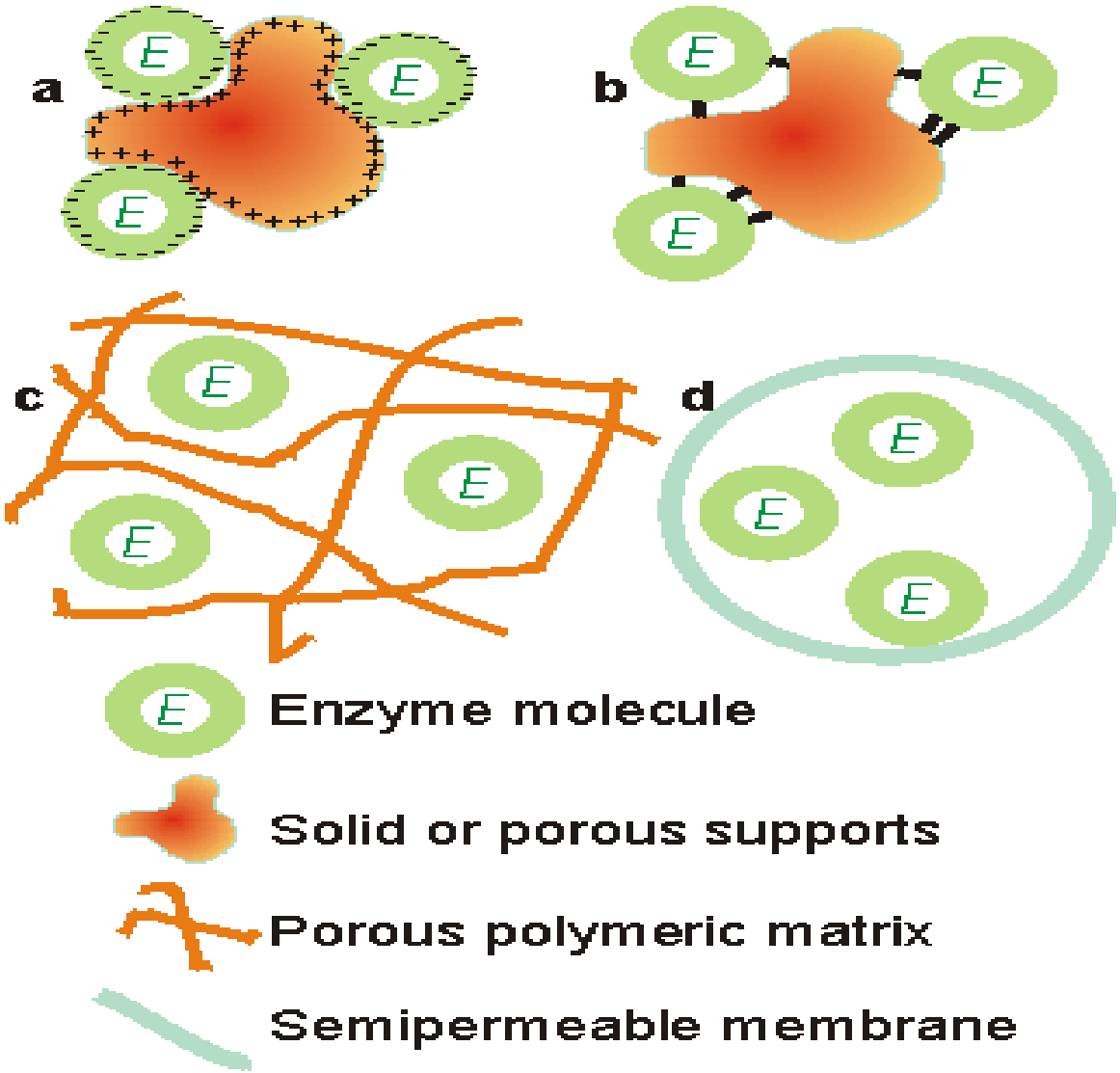
- Attachment to solid structure, incorporation in gels etc for use.

Benefits of Immobilizing an Enzyme

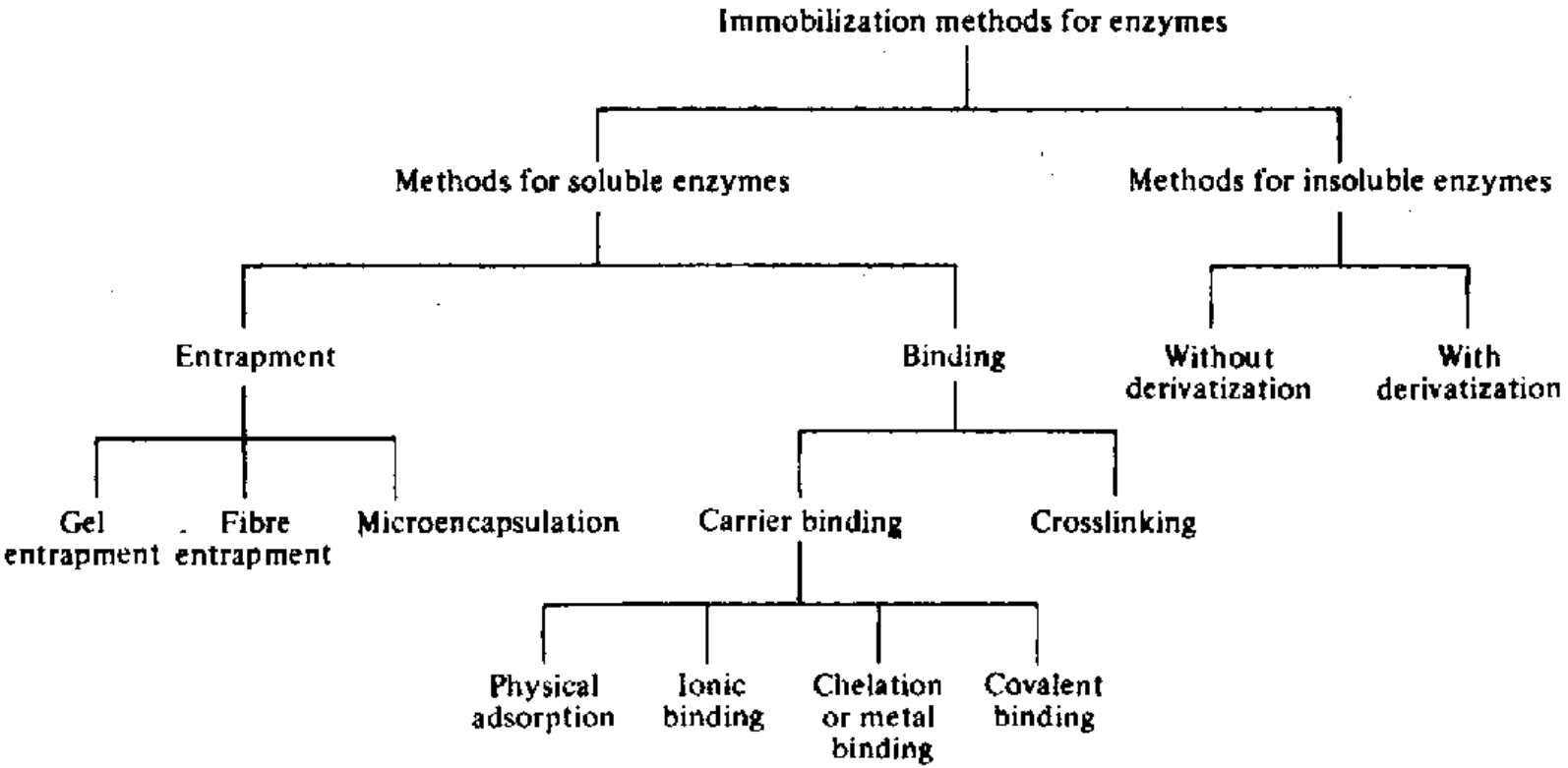
There are a number of advantages to attaching enzymes to a solid support and several major reasons are listed below:

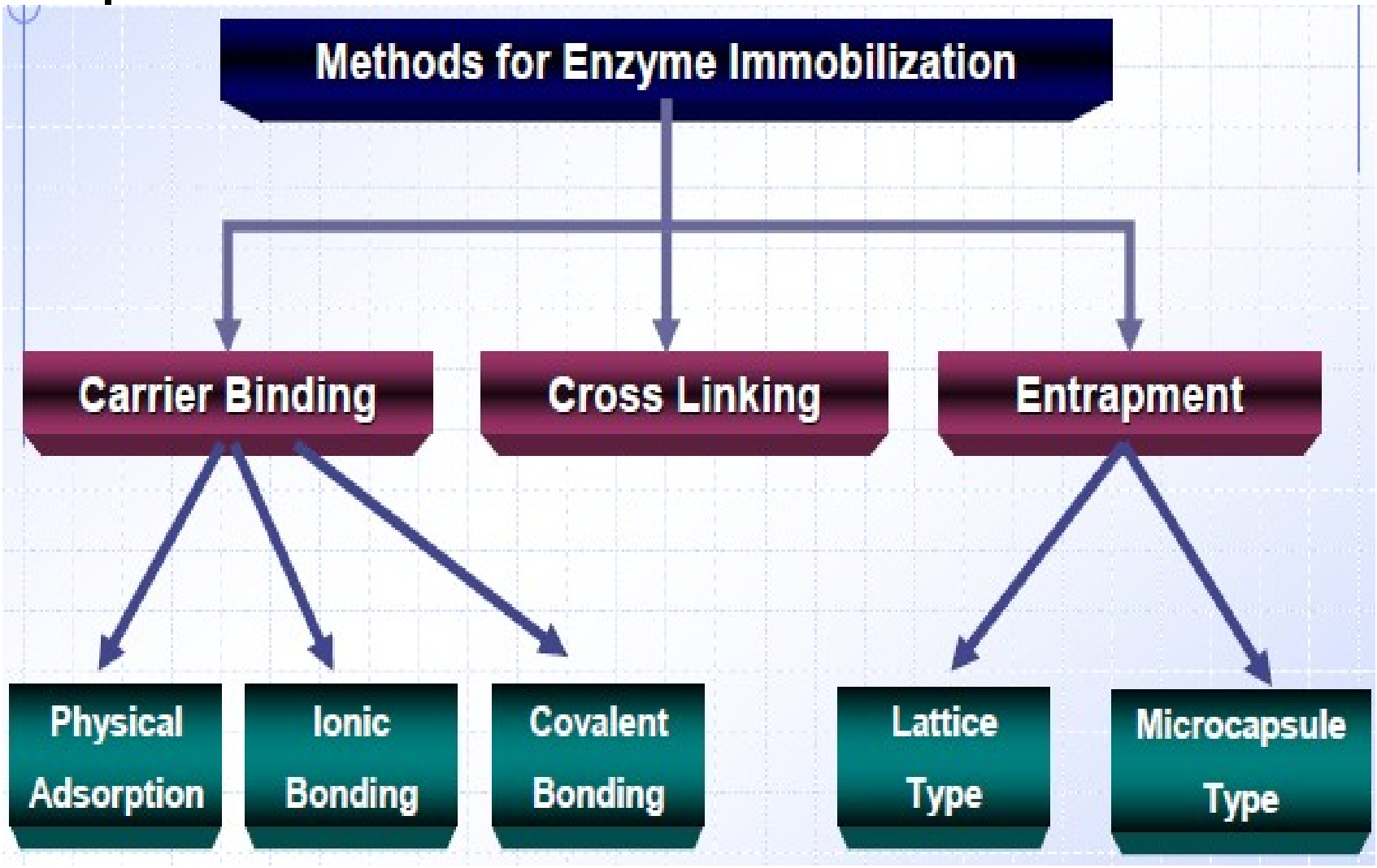
- Multiple or repetitive use of a single batch of enzymes
- The ability to stop the reaction rapidly by removing the enzyme from the reaction solution (or vice versa)
- Enzymes are usually stabilized by binding
- Product is not contaminated with the enzyme (especially useful in the food and pharmaceutical industries)
- Easy seperation of enzyme from the product
- Allows development of a multienzyme reaction system
- Reduces effluent disposal problems

METHODS OF IMMOBILISATION



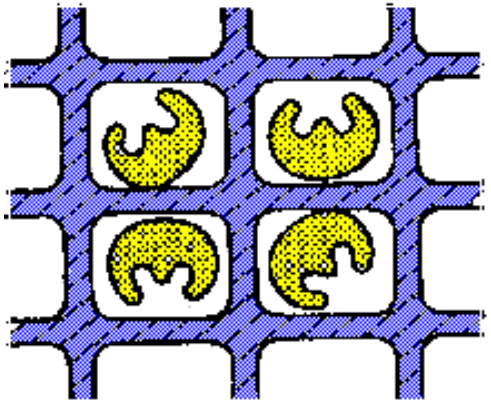
Classification of Immobilization Methods for Enzymes



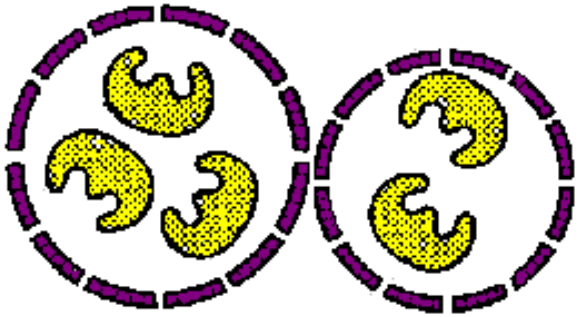

Methods for Enzyme Immobilization
Carrier Binding
Cross Linking
Entrapment
Physical Adsorption
Ionic Bonding
Covalent Bonding
Lattice Type
Microcapsule Type

1. Entrapment

The entrapment method of immobilization is based on the localization of an enzyme within the lattice of a polymer matrix or membrane. It is done in such a way as to retain protein while allowing penetration of substrate. It can be classified into **lattice** and **micro capsule** types.



entrapped in a matrix



entrapped in droplets

Lattice-Type entrapment involves entrapping enzymes within the interstitial spaces of a cross-linked water-insoluble polymer. Some synthetic polymers such as *polyarylamide, polyvinylalcohol, etc...* and natural polymer (starch) have been used to immobilize enzymes using this technique.

Microcapsule-Type entrapping involves enclosing the enzymes within semi permeable polymer membranes.

2. Membrane confinement

Membrane confinement of enzymes may be achieved by a number of quite different methods, all of which depend for their utility on the semipermeable nature of the membrane.

This must confine the enzyme whilst allowing free passage for the reaction products and, in most configurations, the substrates.

- Hollow fiber type of membrane can be used
- Although expensive, easy to use and convenient for any kinds of enzymes
- Liposome can be used for this purpose

3. Adsorption

- Simple method and high enzyme loading
- Incubating supporter with enzymes in an appropriate pH and ionic strength
- Driving force is hydrophobic intxn and salt bridge

% bound at	DEAE-Sephadex anion exchanger	CM-Sephadex cation exchanger
pH 2.5	0	100
pH 4.7	100	75
pH 7.0	100	34

Preparation of immobilised invertase by adsorption

4. Covalent Binding Mode

The covalent binding method is based on the binding of enzymes and water-insoluble carriers by covalent bonds.

- Maximum 0.2 g enzyme/g matrix
- Very little leakage
- The most common methods in laboratory scale

Advantages and Disadvantages of Covalent Binding Method

Advantages

- Covalent binding is usually thought to be *stable method* by the enzyme carrier bond, which *prevents elution of protein* into the production stream.

- *The wide range of choices* is possible by selecting carrier materials and binding method. This allows a great deal of flexibility in designing an immobilized enzyme with specific physical and chemical properties, such as charge distribution, hydrophobe/hydrophile ration, spacer arm separation, partitioning capabilities, etc.

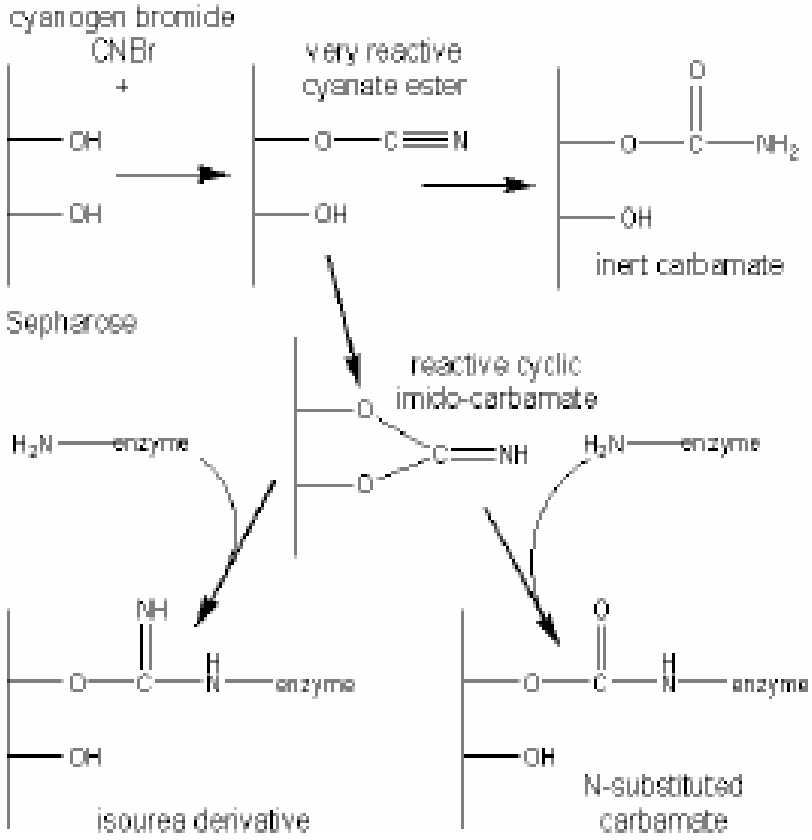
Disadvantages

- Covalent methods are *the relatively expensive and complicated in procedures* which are involved. Also, activity yields may be low due to exposure of the enzyme to harsh environments or toxic reagent.
- *Active site may be modified* through the chemical reactions used to create covalent bonding.

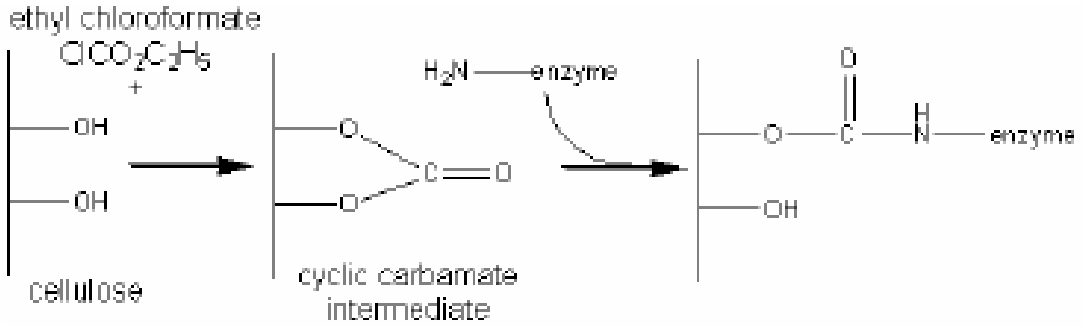
Methods of Covalent Binding of Enzymes to Supports

1. CNBr method

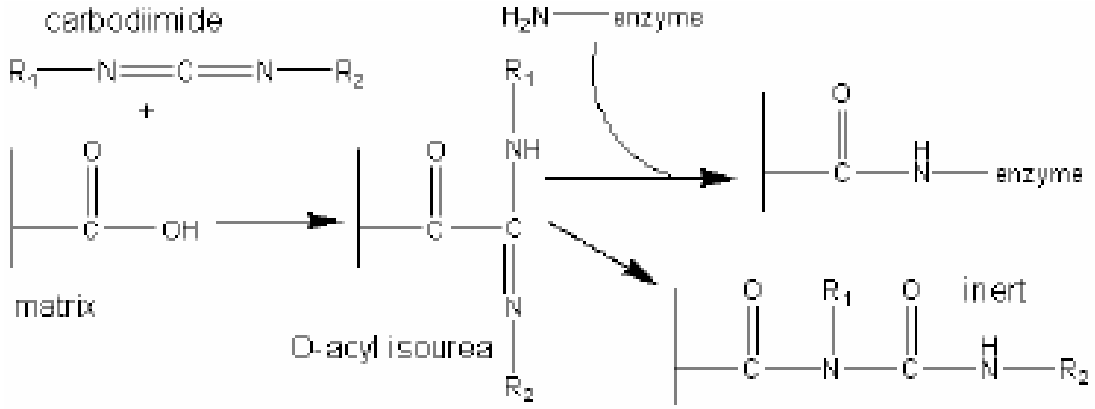
   CNBr– activated Sepharose



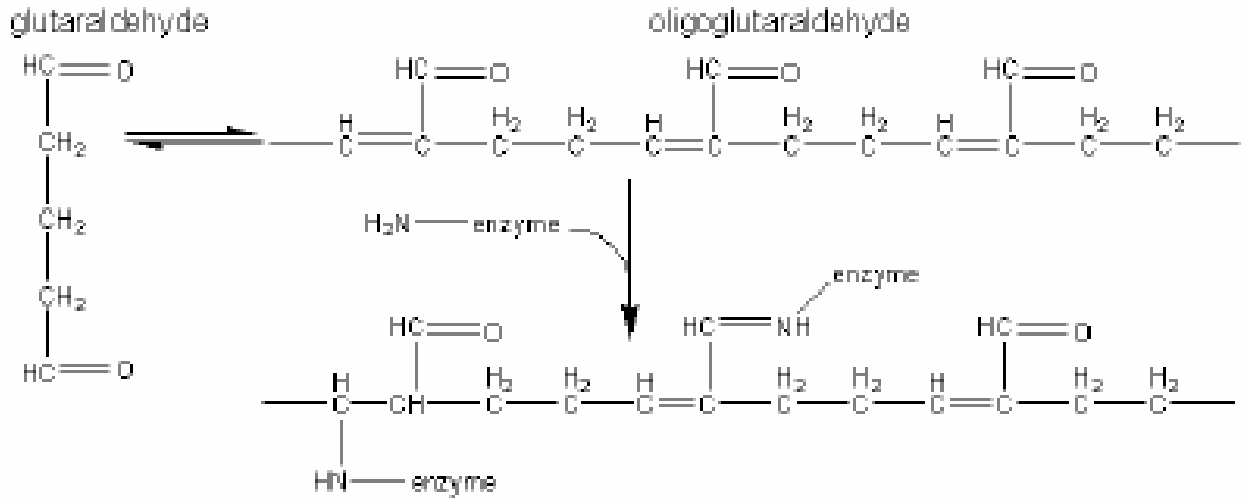
2. ethyl chloroformate : less toxic than CNBr



3. carbodiimide

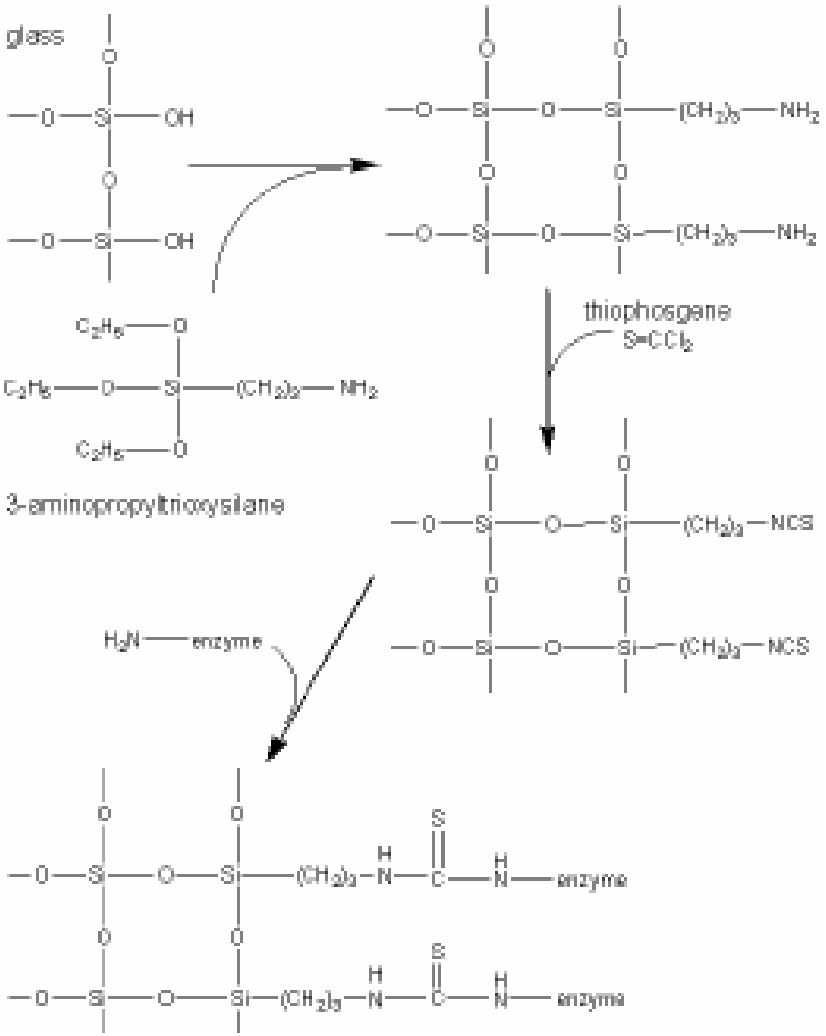


4. glutaraldehyde



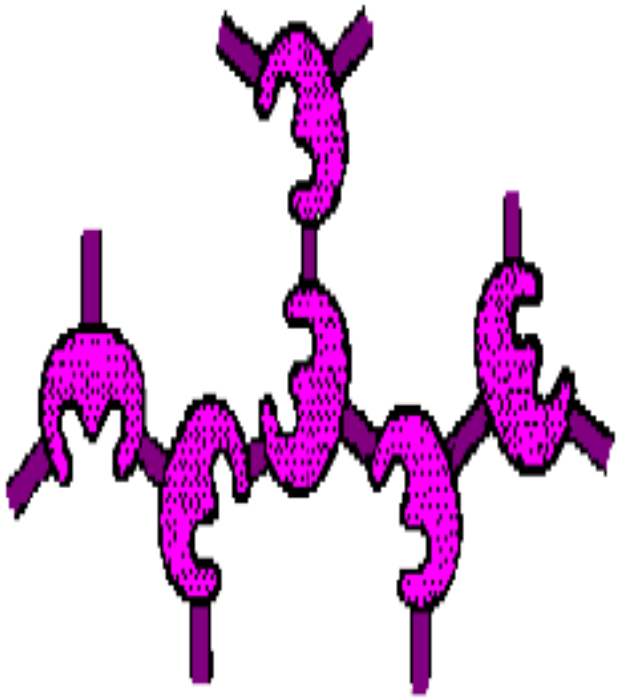
Covalent Coupling

5. 3-aminopropyltriethoxysilane



Cross-Linking

This method is based on the formation of *covalent bonds between enzyme molecules*, by means of bi- or multi-functional reagent, leading to three-dimensional crosslinked aggregates.



Advantages and Disadvantages

Advantages

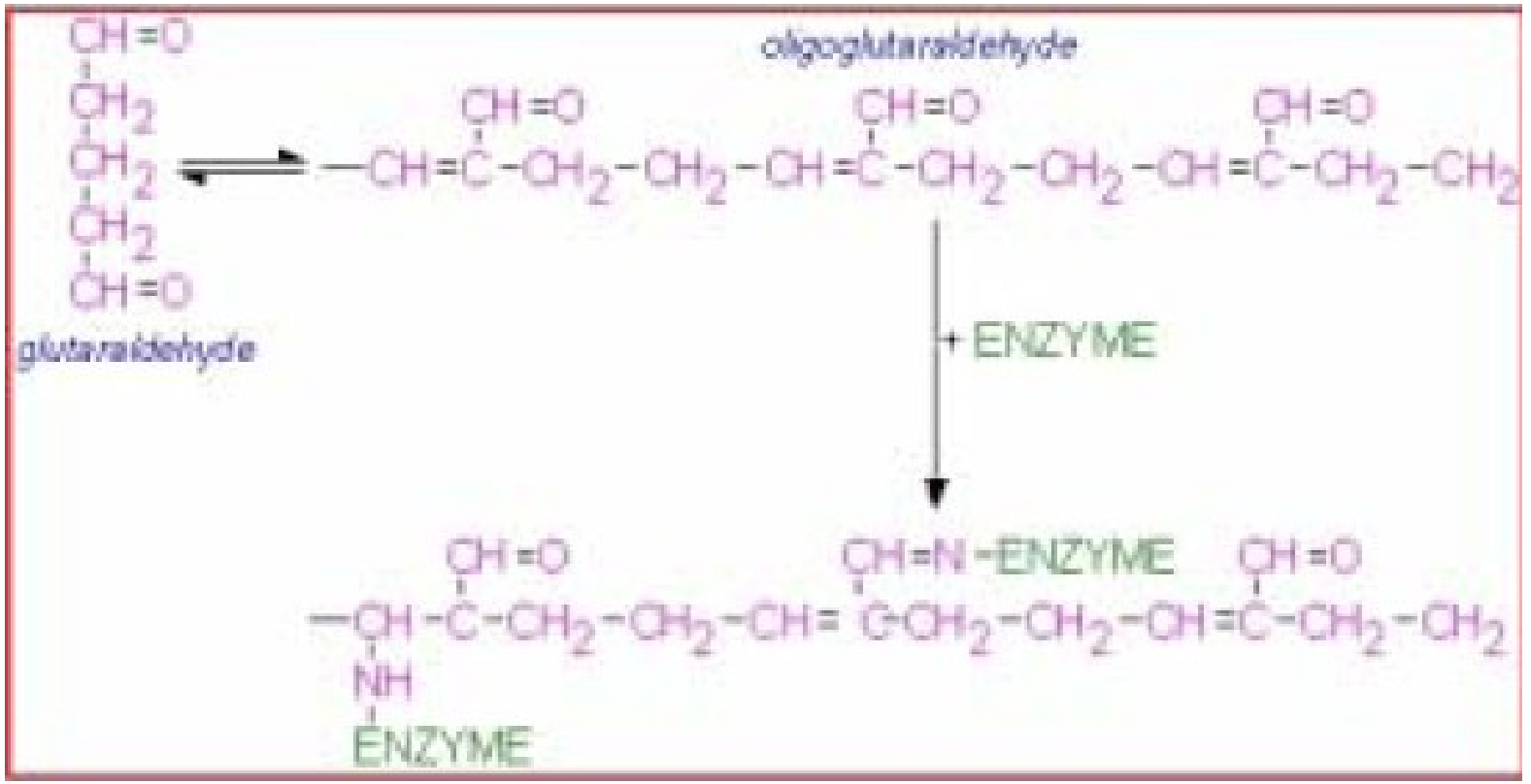
- Very *little desorption* (Enzyme strongly bound)
- Cross-linking is best used in *conjunction* with one of the other methods. It is used mostly as a means of *stabilizing adsorbed enzymes* and also for *preventing leakage.*

Disadvantages

- Cross-linking may cause *significant changes in the active site* of enzymes, and also severe *diffusion limitation* may lead to significant loss of activity.
- *Loss of enzyme activity* during preparation.

The most common reagent used for cross-linking is

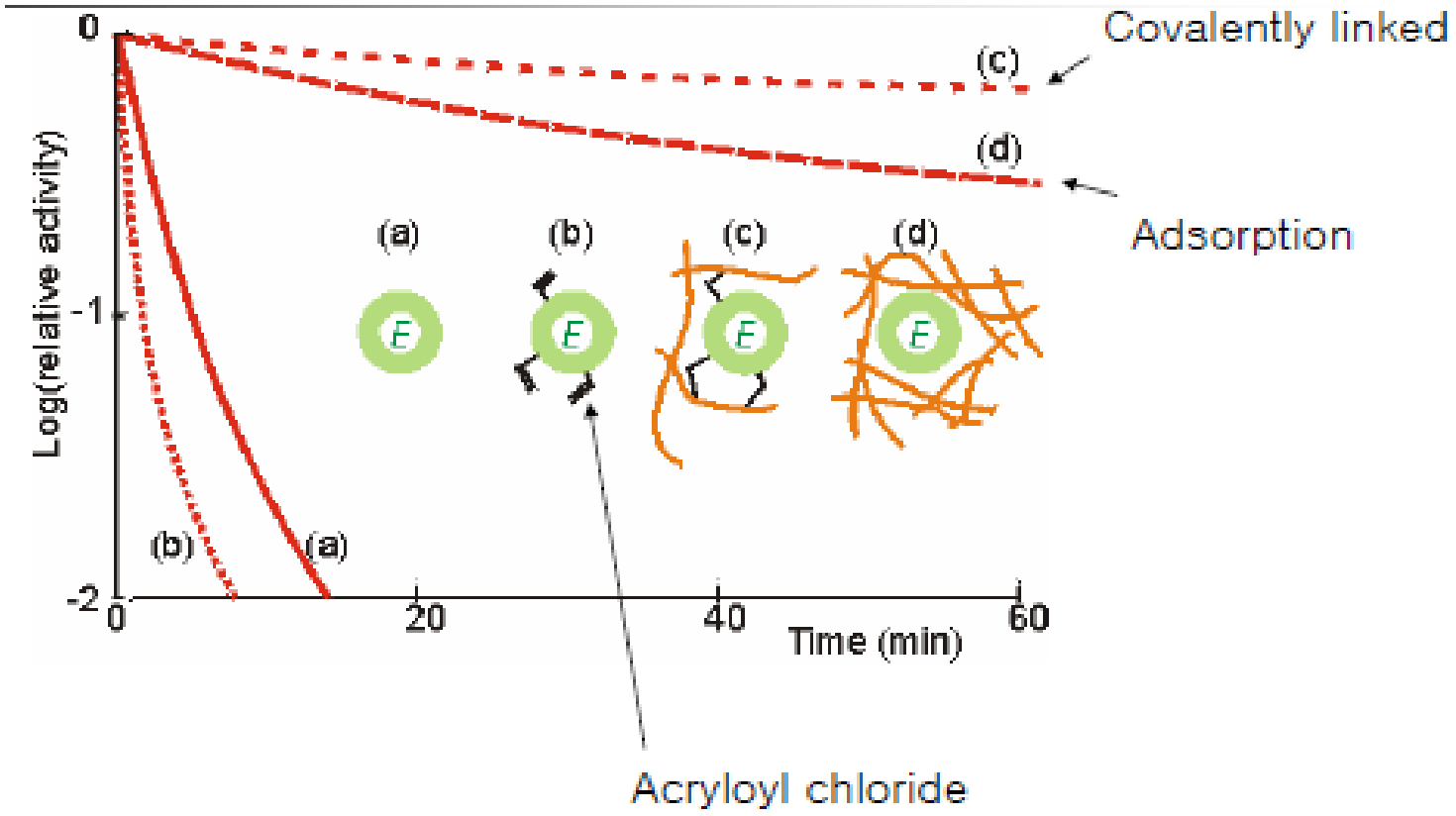
glutaraldehyde



Comparison between the methods

Characteristics	Adsorption	Covalent binding	Entrapment	Membrane confinement
Preparation	Simple	Difficult	Difficult	Simple
Cost	Low	High	Moderate	High
Binding force	Variable	Strong	Weak	Strong
Enzyme leakage	Yes	No	Yes	No
Applicability	Wide	Selective	Wide	Very wide
Running Problems	High	Low	High	High
Matrix effects	Yes	Yes	Yes	No
Large diffusional barriers	No	No	Yes	Yes
Microbial protection	No	No	Yes	Yes

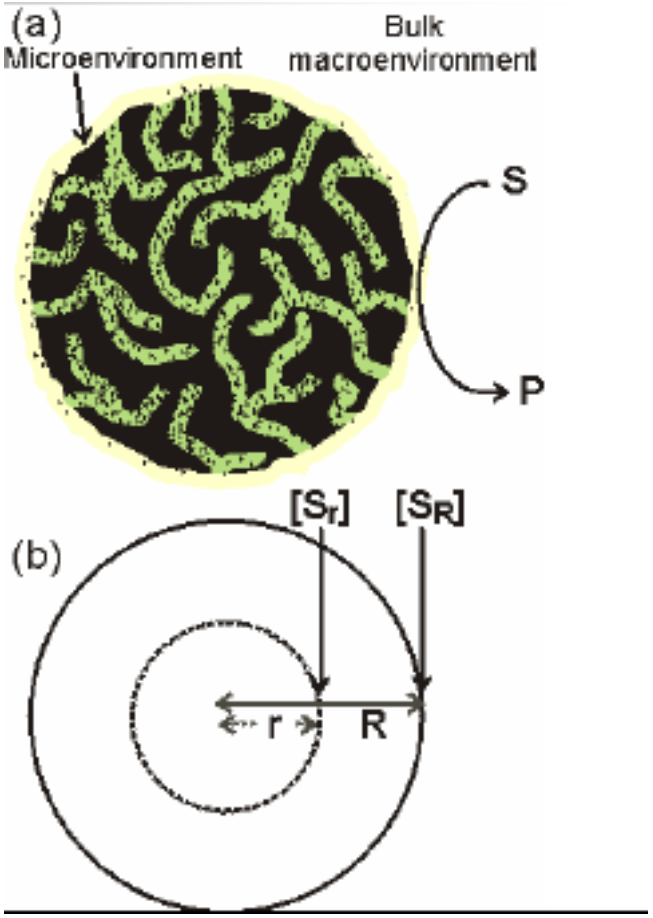
Immobilization enhances the stability of the enzyme



Activities of the chymotrypsin at 60 °C

Kinetics of immobilized enzyme

- Km and Vmax are changed
- Specificity can be changed (trypsin hydrolyze pepsinogen into 15 fragments in solution, but into 10 as immobilized form)



Kinetics of immobilized enzyme in nonporous solid support

- Assuming steady state,

$$J_s = k_L([S_0]-[S]) = \frac{V_{max}[S]}{K_m + [S]}$$

Michaelis-Menten eqn is defined in moles per unit time per unit area

- Defining dimensionless Damköhler number

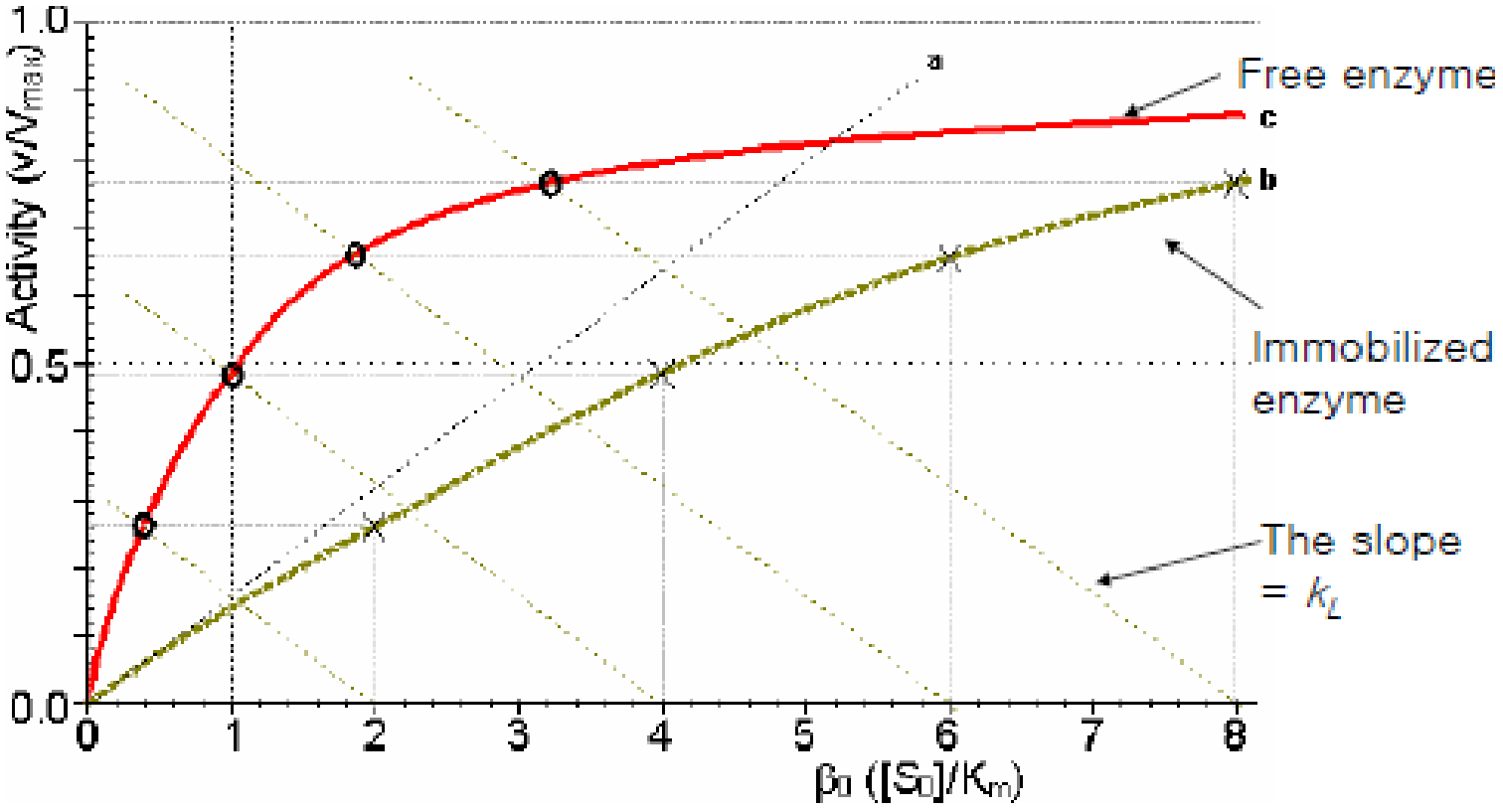
$$Da = \frac{V_{max}}{k_L[S_0]}$$

Maximum reaction rate/maximum diffusion rate

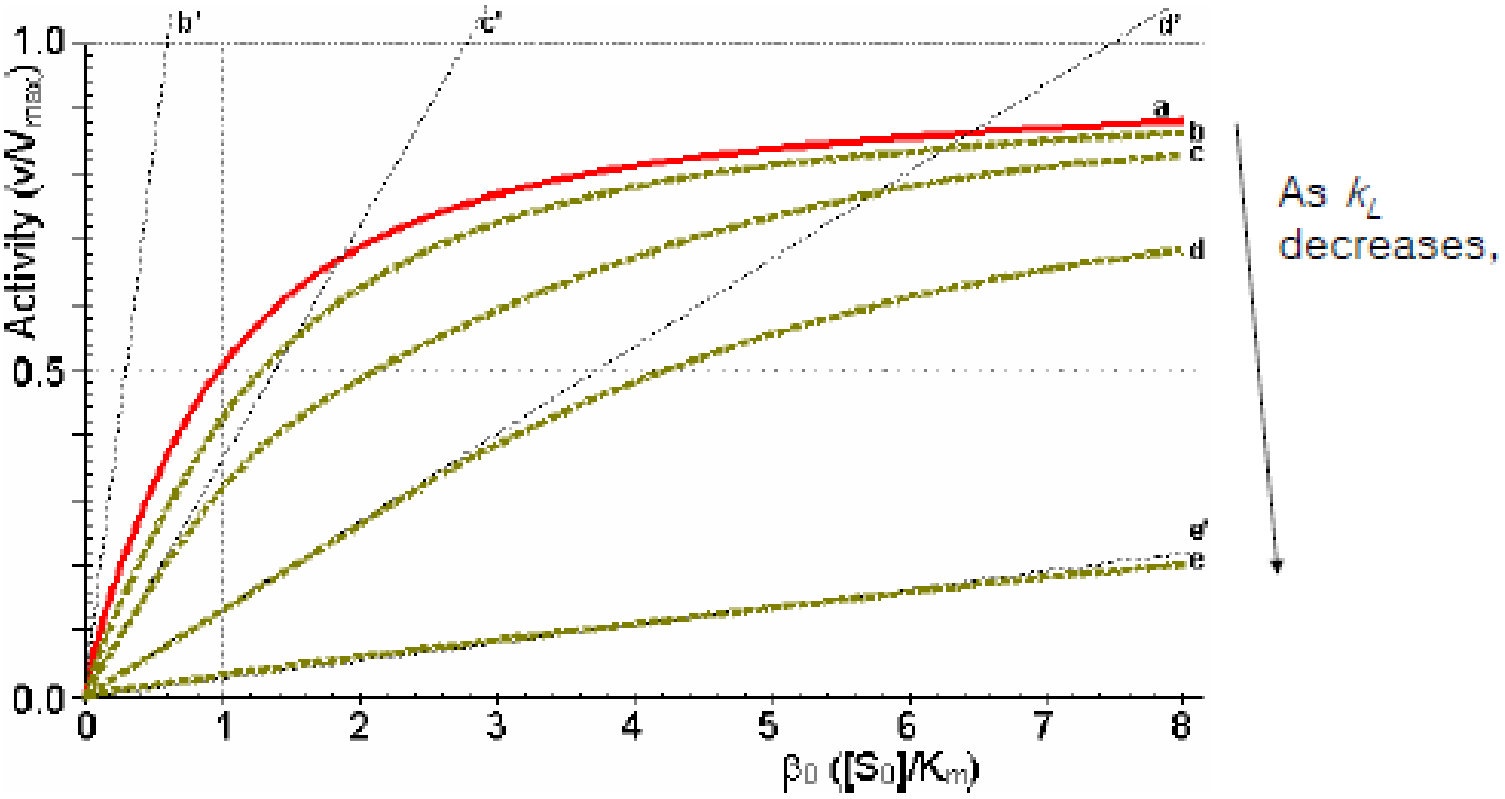
If Da>>1, diffusion rare is limiting

If Da<<1, reaction rate is limiting

Kinetics of immobilized enzyme in nonporous solid support



Kinetics of immobilized enzyme in nonporous solid support



Kinetics of immobilized enzyme in porous matrix

- Assuming steady state,

$$J_s = D_e\left(\frac{d^2[S]}{dr^2} + \frac{2}{r}\frac{d[S]}{dr}\right) = \frac{V_{\max}[S]}{K_m + [S]}$$

Assuming that no external diffusion limitation

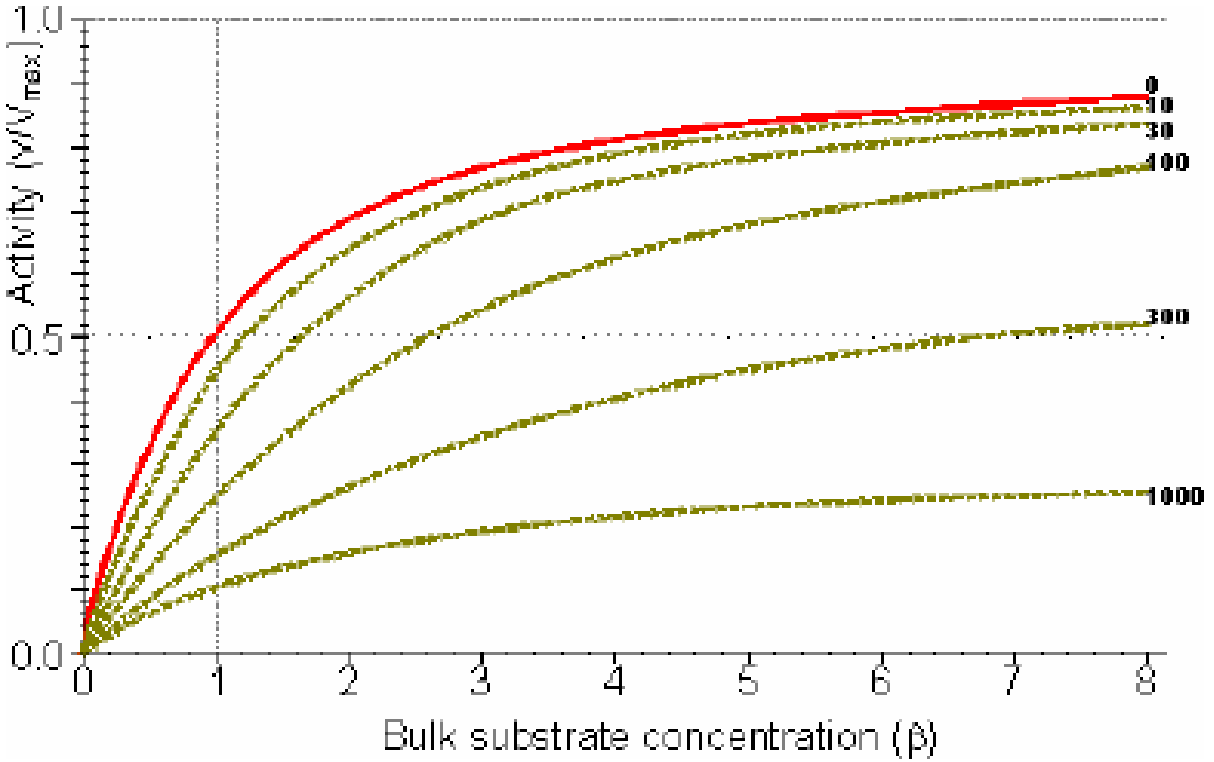
Michaelis–Menten eqn is defined in moles per unit time per unit volume

- Defining dimensionless numbers

$$\frac{d^2\bar{S}}{d\bar{r}^2} + \frac{2}{\bar{r}}\frac{d\bar{S}}{d\bar{r}} = \frac{R^2 V_m}{[S_0]D_e}\frac{\bar{S}}{\beta + \bar{S}} = \phi^2\frac{\bar{S}}{\beta + \bar{S}}$$

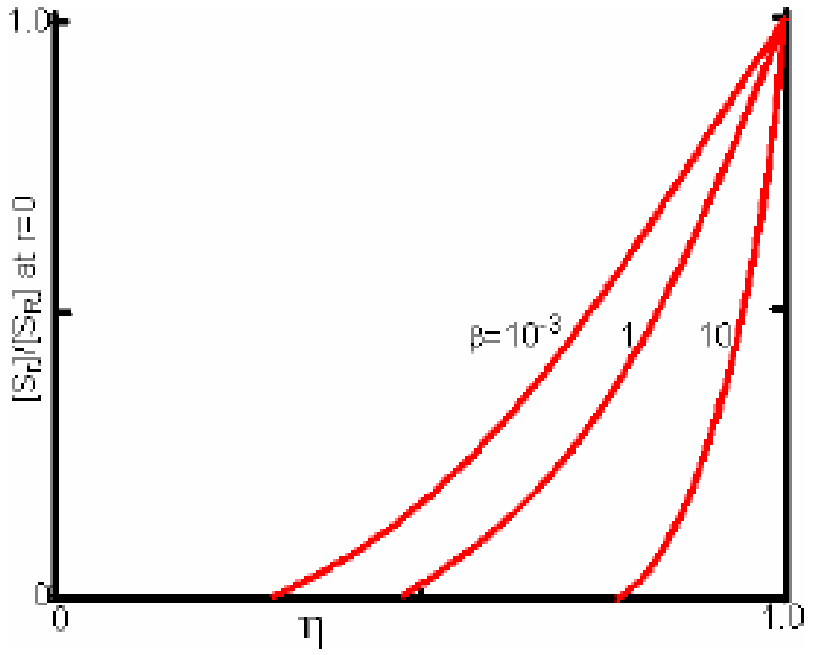
Where $\bar{S} = \frac{[S]}{[S_0]}, \bar{r} = \frac{r}{R}, \beta = \frac{K_m}{[S_0]}$

Kinetics of immobilized enzyme in porous matrix



Kinetics of immobilized enzyme in porous matrix

η (effectiveness factor) is defined as the rate with diffusion limitation versus the rate w/o diffusion limitation



Kinetics of immobilized enzyme in porous matrix