Heterogeneous phase enzyme reactions

Advantages/disadvatages:

Advantages:

- homogeneity of the system,
- enzyme does not need previous preparation - (over isolation and purification)

Economic disadvatages:

- Enzymes are expensive, 1-10- $/mg
- can be used only once, after reaction they are to be discarded…

Technological disadvatage:

- Proteins contaminate products

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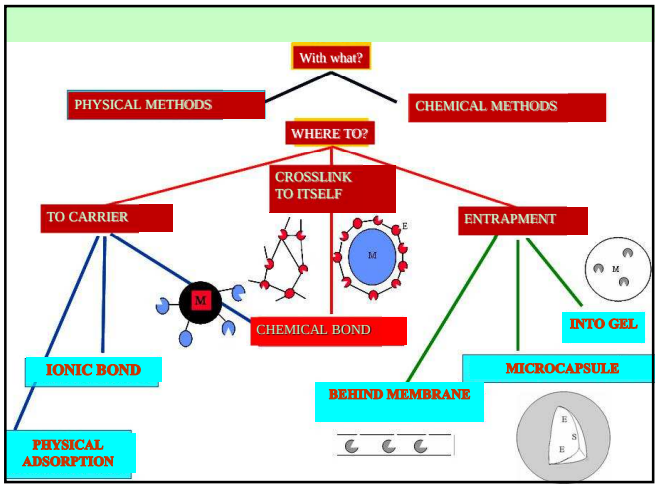
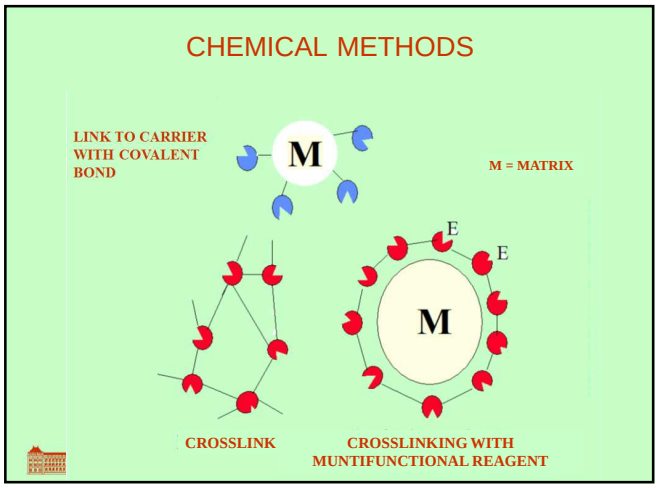


CHEMICAL METHODS



CHEMICAL METHODS

Covalent bond between non essential amino acid sidechain(!) and water insoluble matrix with function groups

CARRIERS :

natural polymers: *agar, agarose, chitin, cellulose, collagene,...*,
synthetic polymer: *polyurethane, polystyrene, nylon*, ...,
inorganics: *glass, aluminium, silicagel, magnetit*,...

CHEMICAL METHODS

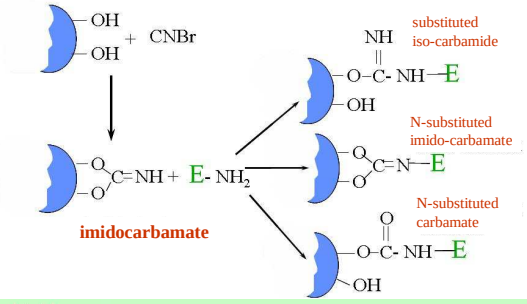
Building of covalent bond:
free α-, β- or γ-COOH , α-, β –NH_2 groups
phenyl-, OH-, SH- imidazole-groups

STEPS:
1. Activation of carrier (arm and reactive X-group),
2. Creating covalent bond between enzyme and activated carrier.

Protection of the active sites: S or analog

MATRIX: vicinal –OH goups like:

cellulose, Sepharose, Sephadex



Origin of carbohydrate matrix

Glucose → dextrane → Sephadex®

Alga → agar(ose) → Sepharose ®

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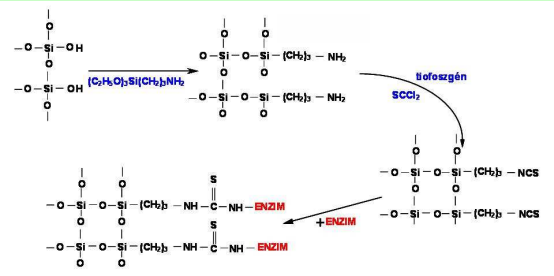
Immobilization onto glass surface



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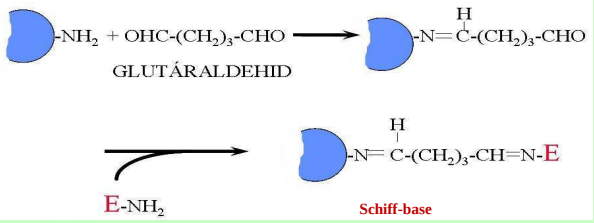
Chemical methods: bifunctional molecules

MATRIX: –NH_2 goups like:

AE-cellulose, DEAE-cellulose, collagen, chitin, nylon…



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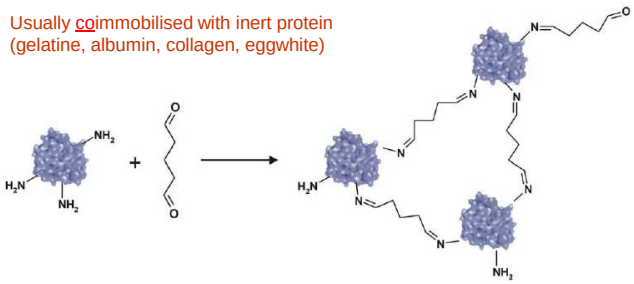
Chemical methods: crosslinking

Usually coimmobilised with inert protein
(gelatine, albumin, collagen, eggwhite)



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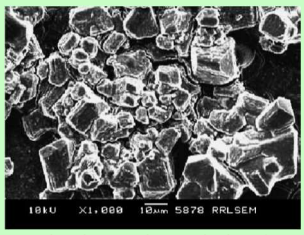
CLEC = Cross-Linked Enzyme Crystals

Cross-linked Enzyme crystal of PNP
(purine nucleoside phosphorylase)



Scanning electron microscopic view of CLEC laccase
Surface area (m^2/g) 2.456

Preparation and characterization of cross-linked enzyme crystals of laccase, J. J. Roy, T. E. Abraham Journal of Molecular Catalysis B: Enzymatic 38 (2006) 31–36

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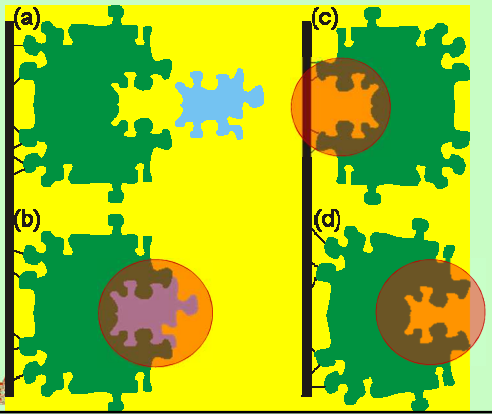
Possible effect of chemical immobilisation: Specific activity loss



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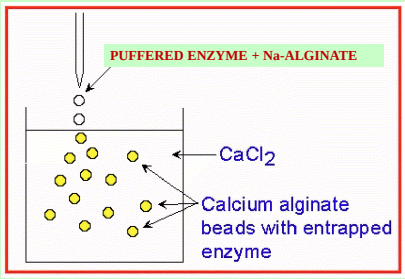
PHYSICAL METHODS

1. Adsorption e.g. on *ionexchanger resins* – nonspecific, easily desorps (pH)
2. Gel entrapment
3. Microencapsulation
4. Closing behind membrane

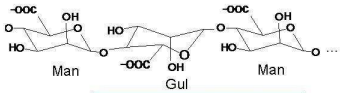
ALGINATE GEL ENTRAPMENT



ALGINATE: poly-β D-mannuronic acid (1→ 4), …..-guluronic acid
Hydrophyl colloid, linear polymer ***Macrocystis pyrifera***

Gel forming polysaccharides

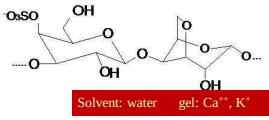
Alginate: heteropolymer of mannuronic acid and guluronic acid, 1,4-bonds



polyanionic

Solvent: water gel:Ca^{++}, Zn^{++}, Al^{3+}

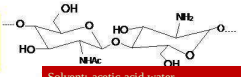
κ-carragenan: helical bio-polymer of 3,6 anhydro-galactose



polyanionic

Solvent: water gel: Ca^{++}, K^{+}

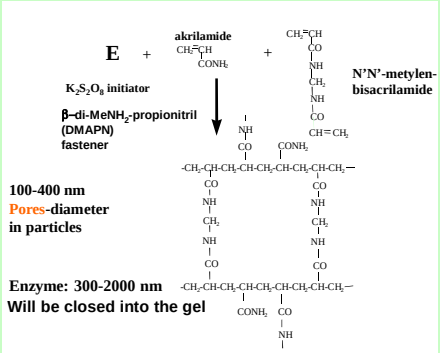
chitosan: partially deacylated N-acetyl-glucoseamin polymer



polycationic

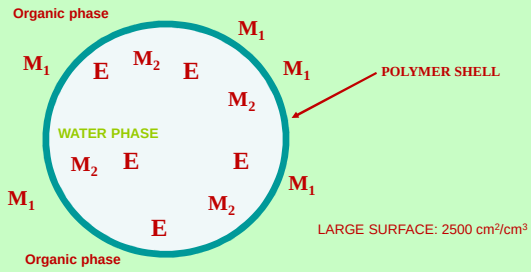
Solvent: acetic acid,water
gel:ploy phosphates, pH-change

Poly-acrylamide gel entrapment



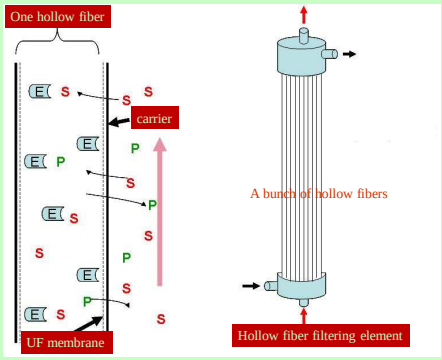
Physical methods: microencapsulation

stable polymeric membranes

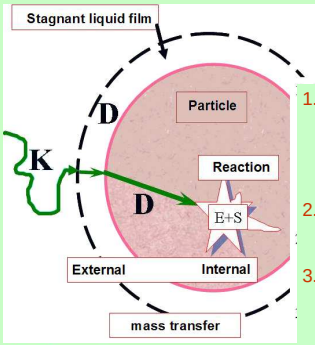


LARGE SURFACE: 2500 cm²/cm³

Ultrafiltration membrane



Kinetics of immobilised enzymes



1. Convective transport from the bulk liquid to the liquid film: no transport barrier K
2. Diffusion through the liquid film. D
3. Diffusion into the inner space of the particle to the enzyme D

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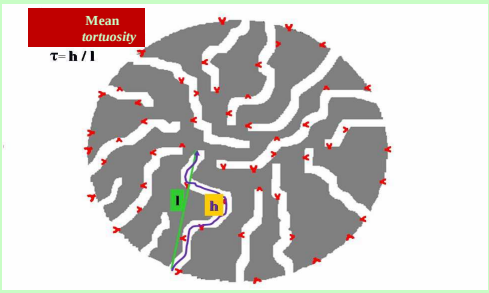
Tortuosity



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Pros/cons about immobilised enzymes

Dissoved enzymes

Advantages
- homogeneous system
- no preparation needed
- no mass transfer limitation

Disadvantages
- expensive (1-10-50 $/mg)
- discarded after use
- contamination of product
- only batch technology

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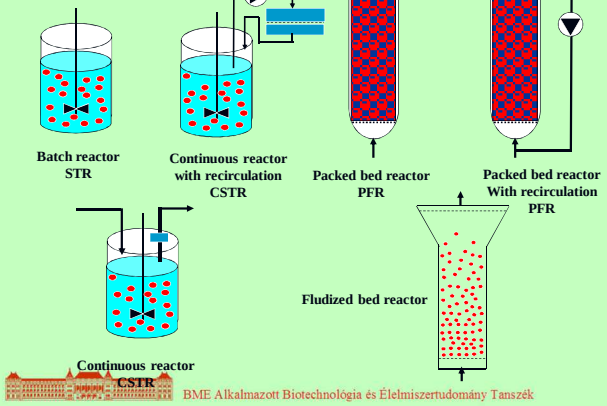
Pros/cons about immobilised enzymes

Immobilised enzymes

Advantages
- No contamination of product
- Easily separable
- Possible reuse
- Also continuous technologies
- Easy termination
- Increasing stability

Disadvantages
- Expensine preparation need
- Loss in enzyme activity
- Diffusion barrier



Industrial application of immobilised enzymes

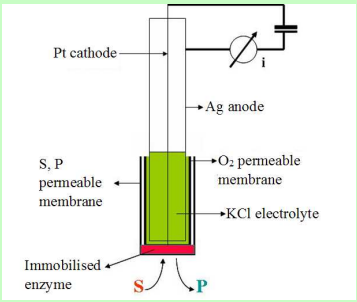
Aminoacilase	resolvation of D,L-amino acids
Glucose-isomerase	conversion of glucose to glucose+fructose 1:1 mixture
Penicillin-amidase	preparation of 6-amino-penicilloic acid
β-galactosidase	hydrolysis of lactose to glucose+galactose
Lipase	hydrolysis and transesterification of lipids
Thermolysin	Preparation of aspartame

Enzyme electrode

Based on an amperometric electrode for dissolved oxygen measurement. It is covered with an enzyme producing or consuming oxygen.
Eg. glucose oxydase + catalase.
The electrode reaction:

Ag anode: $4Ag + 4Cl^- \rightarrow 4AgCl + 4e^-$

Pt cathode: $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$



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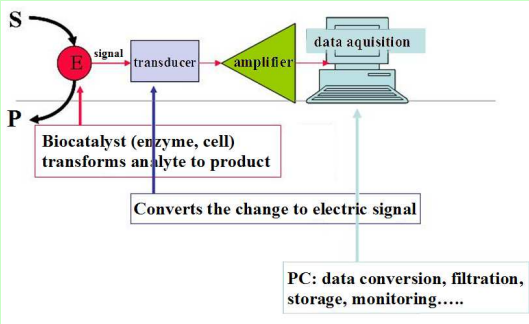
BIOSENSOR



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Analytical enzyme applications

In these cases not the activity of enzyme is measured but the concentration of an analyt molecule.

1. Determination of S
2. Determination of I
3. Marker reactions (eg. in immunoassays)
   Enzyme Linked Immunosorbent Assay (ELISA)
   diagnostical, research purposes

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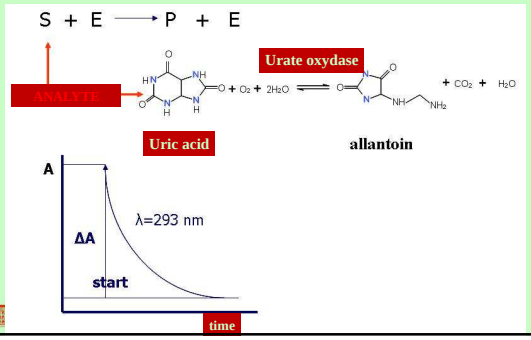
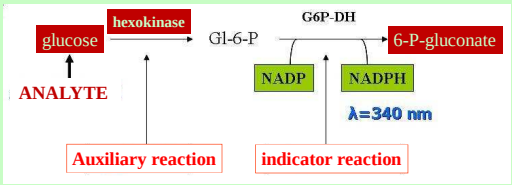
End-point measurement of substrate

The whole amount of substrate is converted – change is measured



Indicator reaction

If S and P are not observable → an enzymatic indicator reaction makes it measurable.



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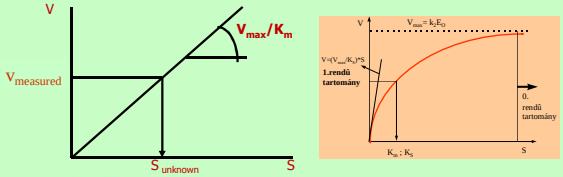
Kinetic measurement of S

At small substrate concentrations the reaction rate changes linearly with S concentration (M-M kinetics).

If $S \ll K_m$ → $V \sim V_{max}/K_m \cdot S$ → $-dS/dt$, dP/dt



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