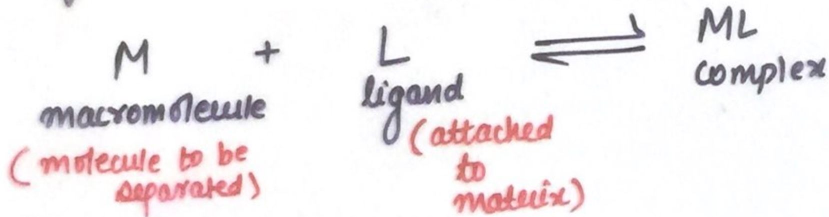
AFFINITY CHROMATOGRAPHY.

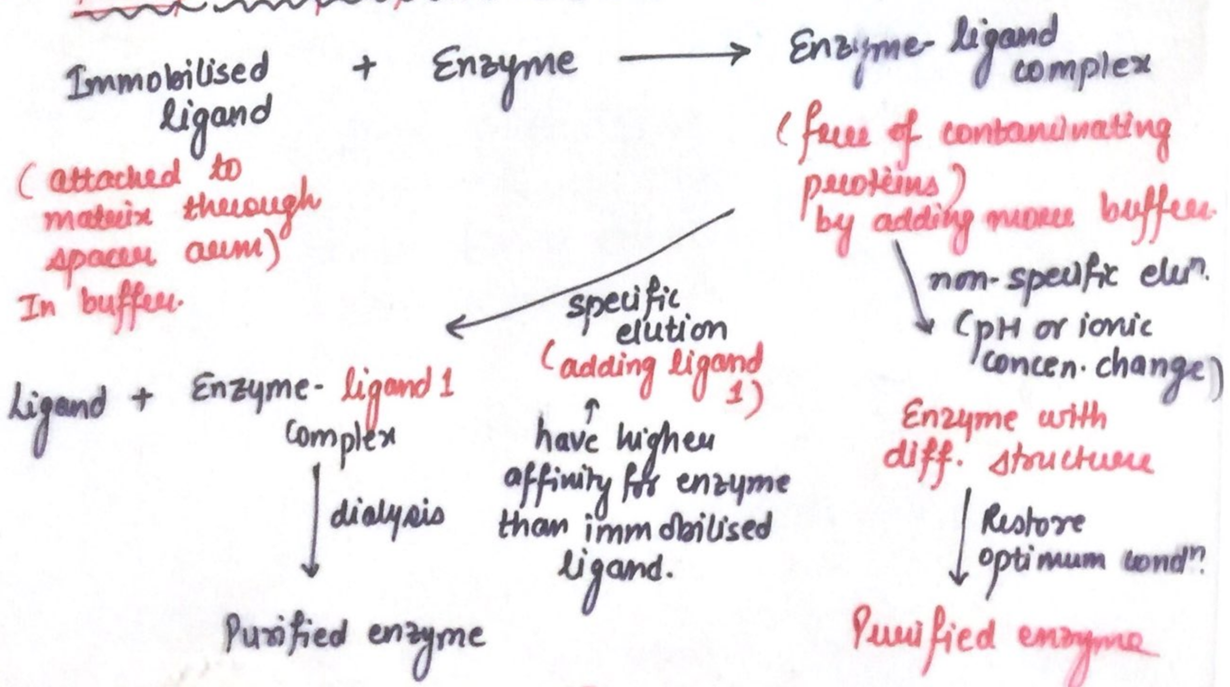
Separation is based on the specific biological interaction. Interaction is b/w a stationary immobilised ligand and a mobile liquid phase.

The technique requires that the material to be separated is capable of binding reversibly to a specific ligand that is attached to an insoluble matrix.



In the case of an enzyme, the ligand may be substrate, a competitive inhibitor or an allosteric activator. The condn. chosen would normally be those that are optimal for enzyme-ligand binding.

* Principle of purification of an Enzyme.



- pH change by using dil. acetic acid & NH_4OH resulting in the change in state of ionisation of groups in the ligand or the macromolecule that are critical to ligand-macromolecule binding.

* Characteristics of matrix for affinity chromatography

- Possess suitable and sufficient chemical groups to which the ligand may be covalently coupled.
- interact only weakly with other macromolecules to minimise non-specific adsorption.
- broad range of physical, chemical and thermal stability.
- exhibit good flow properties.

Commonly used matrix materials :- Cross-linked dextrans and aga polyacrylamide, polymethacrylate, polystyrene, cellulose, porous glass & s

Agarose and polyacrylamide are useful matrix bu they exhibit minimal adsorp^n, maintain good flow pro after coupling & tolerate the extremes of pH & ionic strength as well as change in concen. of denaturants are often needed for successful elution.

→ Polyacrylamide - is not as generally useful as agarose it fails to work properly for molecules or having a high molecular wt.

Ligand

Ligand displays absolute specificity & group specificity.

absolute specificity (it will bind exclusively to 1 particular compd.)

group specificity (it will bind to a clo related grp. of a compo possess a similar chemic

→ Examples of group-specific ligands

ligand	affinity
5' AMP	NAD^+ dependent dehydrogenases, some kinases.
2', 5'-ADP	$NADP^+$ dependent dehydrogenases
Heparin	Lipoproteins, lipases, coagulation factors, DNA polymerases.
Proteins A and G.	Immunoglobulins.
Poly (A)	RNA containing poly (U) sequences, RNA-specific proteins.
Lysine	rRNA
Cibacron-blue	coagulation factors, kinases, dehydrogenases, DNA polymerase, nitrate reductase.

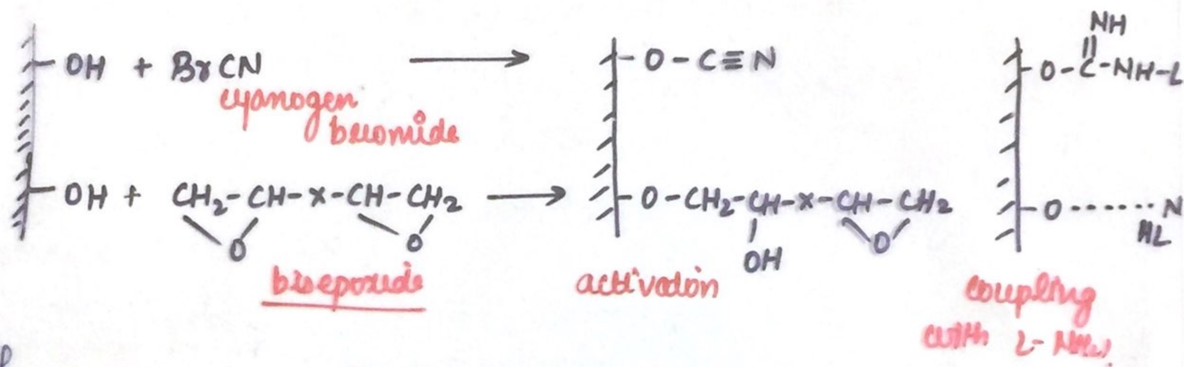
→ It is essential that the ligand possess a suitable chemical group which can be used to attach the ligand to the matrix (not in binding of macromolecule). The most common of such groups are $-NH_2$, $-COOH$, $-SH$ and $-OH$.

→ In case of some small ligands, steric interference occurs bcoz ligand is too near the matrix surface. This problem is usually eliminated by inserting a 6-C atom spacer b/w matrix & the ligand. This is called spacer — arm.

e.g. 6-amino hexanoic acid (having free -COO grp.), 1,6 diaminohexane (having free $-NH_2$ group) and 1,4 bis (2,3-epoxyproxy) butane. (contains free oxirane groups which allow linkage of sugars, carbohydrates or any ligand containing a $-OH$, $-NH_2$ or thiol group).

→ Methods of attachment of the ligand to the matrix.

by activating matrix done by treatment with CNBr, bis-epoxides, dichloro triazines, sulphonyl chloride, sodium periodate, N,N'- disubstituted carbodiimides.



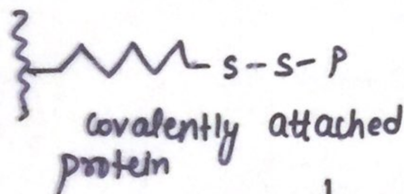
functional hydroxyl grp. in matrix + activating reagent → activated matrix. contains reactive groups or good leaving groups. → Immobilised ligand L.

* Covalent chromatography.

used to separate thiol-containing proteins by interaction with immobilised ligand containing a disulphide group attached to an agarose matrix.

Ligand used is disulphide 2' pyridyl group.

↓ P−SH
thiol containing protein



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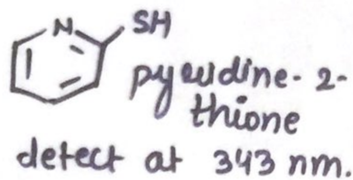

covalently attached protein

pyridine-2-thione
detect at 343 nm.

- non thiol containing contaminants r eluted
- Remove unreacted thiopyridyl grp. by using 4mm dithiothreitol or mercaptoethanol.
- displace bound protein with 20-50mm DTT.