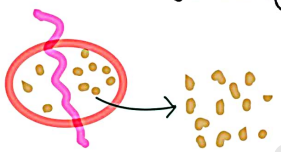
Affinity Chromatography. 1.

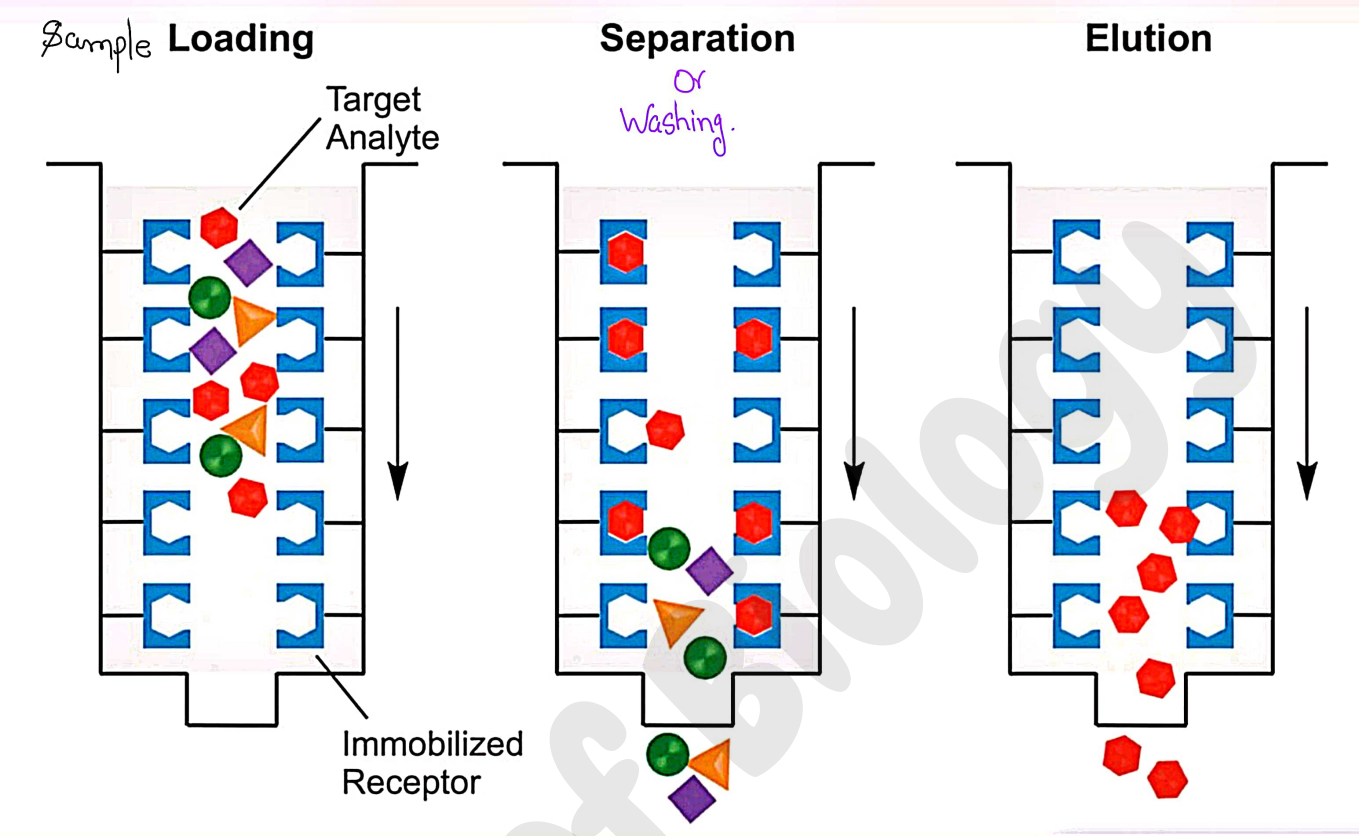


- Sample is Cell Lysate.
- Cell Lysate (After breaking of Cell we recieved a mixture Contain DNA, RNA, Protein etc.).
- Here Purification is done on the basis of Interaction b/w molecules.
- Here Stationary Phase is → Modified Resin (Solid).
  Mobile Phase → Liquid (Solvent/Buffer).

☆ Choose Stationary Phase on the basis of Sample.

→ If we want to Isolate Antibody then our stationary Phase must be (Antigen – Resin) → small beads like structure.
→ Modified Resin.

Or, for the Isolation of His-Tag protein we Use Nickle bound resin (Ni-Resin).



Antibody – Resin – Antigen

Stationary Phase Ligand	Purified Protein
Protein A	IgG
Antigen Peptide	Antibody (IgG, IgY, etc.)
Antibody	Antigen
gelatin	fibronectin
GST	GST-Tagged Protein
lectin	Sugars/glycoproteins
heparin	growth factors
Amino acid (aa)	aa binding proteins

⇒ After Selection of Resin / S.Phase next step is Column Packing. 2.

⇒ Column Packing Resin / S.P filled in Column properly.

⇒ Purification is done Smoothly when Column is packed properly.

⇒ Equilibrate ⟶

- We run Equilibration buffer in Column.
- It equilibrate Column & also ensure that our Column is ready for Purification Process.

⇒ Sample Loading ⟶ Sample is Mixed with Mobile Phase. & run into Column.

- Sample Contain different Component.
- Molecules / Components of Sample which shows affinity towards Modified resin bind with it. (Reversible Adsorption).
- Remaining Molecules is float in Column.

⇒ Washing → Low Salt Conc. Solution is passed through 3.. Column. With the help of this the Unwanted molecule which is float in Column are easily Washout.

• This Washout molecule Collected Separately in Collecting Tube.

Now, after Washing our Sample left which is bind with Modified Resin.

⇒ Elution → High Salt Conc. Solution is passed through Column, it leads to break the Interaction b/w Modified Resin & Sample. & Our Sample is eluted & Collected in Collecting Tube.

Affinity chromatography has various applications in:

1. _Protein purification_: Purifies specific proteins by exploiting their unique biological interactions.
2. _Antibody purification_: Purifies antibodies for research, diagnostic, and therapeutic applications.
3. _Enzyme purification_: Purifies enzymes for industrial, research, and diagnostic applications.
4. _Vaccine development_: Purifies vaccine components, like antigens and antibodies.
5. _Biomarker discovery_: Identifies and purifies biomarkers for disease diagnosis and monitoring.
6. _Protein-protein interaction studies_: Studies protein interactions, like protein-ligand binding.
7. _Epitope mapping_: Maps antibody epitopes for vaccine development and immunotherapy.
8. _Single-chain antibody production_: Produces single-chain antibodies for research and therapeutic applications.
9. _Recombinant protein production_: Purifies recombinant proteins for research, diagnostic, and therapeutic applications.
10. _Diagnostic assays_: Develops diagnostic assays, like ELISA and Western blotting.
11. _Therapeutic protein production_: Purifies therapeutic proteins, like insulin and growth factors.
12. _Cell therapy_: Purifies cells for cell therapy applications, like stem cell therapy.