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UNIT –V

Ion exchange chromatography- Introduction, classification, ion exchange resins, properties, mechanism of ion exchange process, factors affecting ion exchange, methodology and applications

Gel chromatography- Introduction, theory, instrumentation and applications

Affinity chromatography- Introduction, theory, instrumentation and applications

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Gel Chromatography.

1.

- Also known as * Size Exclusion Chromatography.
- Here purification/separation is done on the basis of size **
- Also known as Gel filtration Chromatography.
Gel Permeation Chromatography.
Molecular Sieve Chromatography.
- This type of Chromatography performs either Manually or Automatic.

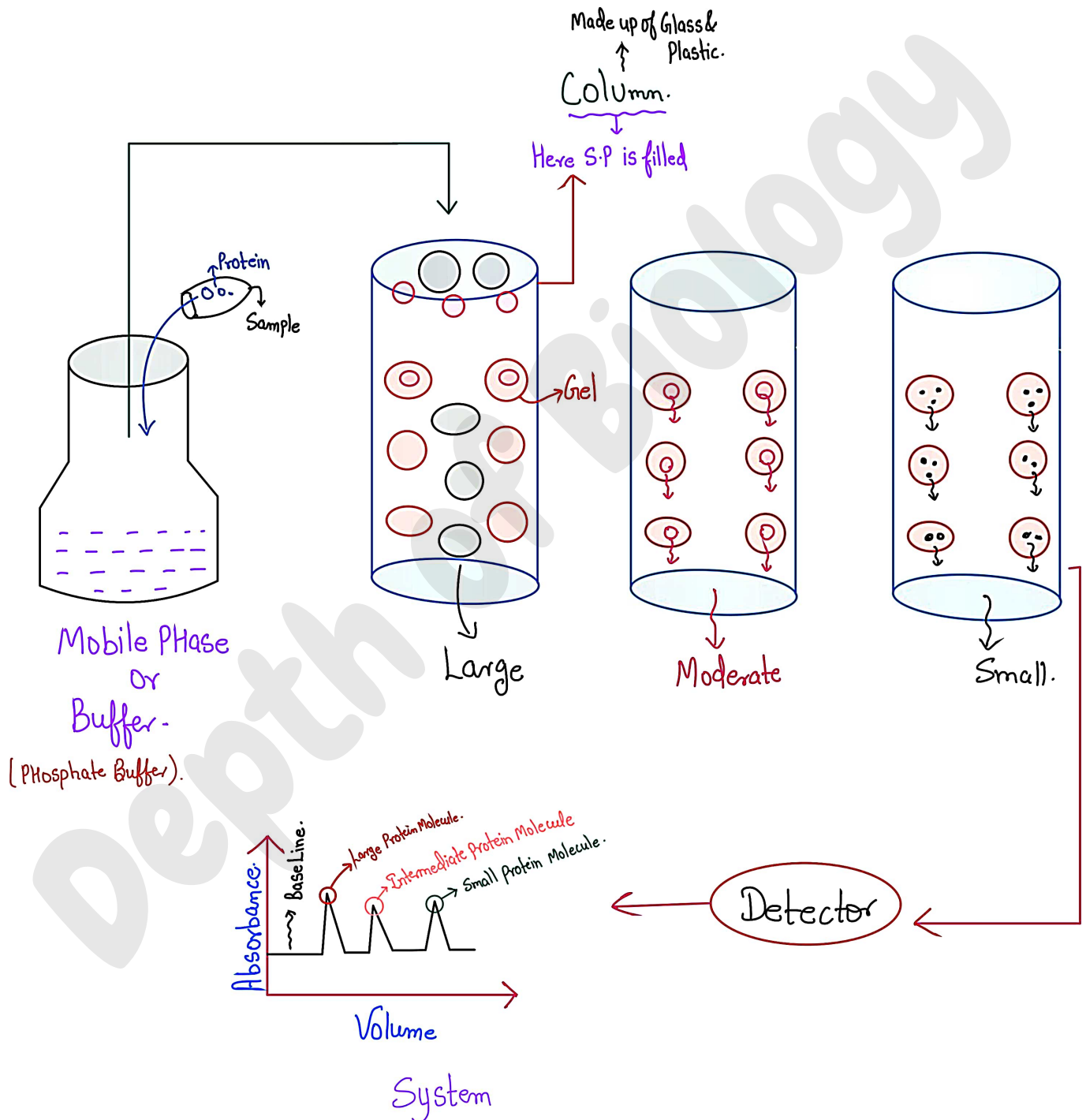
Instrumentation

- Here Mobile Phase is Liquid which is filled in bottle
- This bottle is Connected with Column (Column is made up of Glass/Plastic)
- Column Contain Stationary Phase.
- Column is attach with a device Called detector.

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Instrumentation



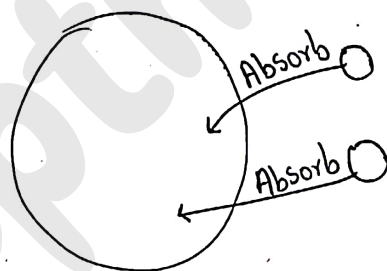
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- Detector detect the Elute. & send signal to system.²
- System Generate a graph
- Detector also attach with Collecting Tube. (where elute is collected).

★ Now, first of all we have to select the Stationary Phase & Stationary Phase is Selected on the basis of Sample.

* SoP example  Dextran. (sephdex G-50).
Agarose.



○ (Protein) → Sample

(Resin) Beads (SoP Phase)

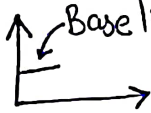
But, if Protein is larger in size than Stationary Phase (Beads) then Protein is not absorb in resin & elute out from the Column.

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- Mobile Phase/Buffer. generally Used Phosphate Buffer.^{3.}
- Column must be properly Packed. Then we start the Process.

1.

- Equilibration → In which only Buffer/Mobile Phase is run in the Column. & finally detector detect the buffer & straight line/Base line formed in the graph. 

2. Sample Loading →

- Sample is mixed with Mobile Phase or Buffer. Then load into Column.
- Large Size molecule not absorbed in Beads/Resin/S.p & they pass out from the Column.
- And Small Size molecule enter into Resin & stuck into resin & slowly move in Column.

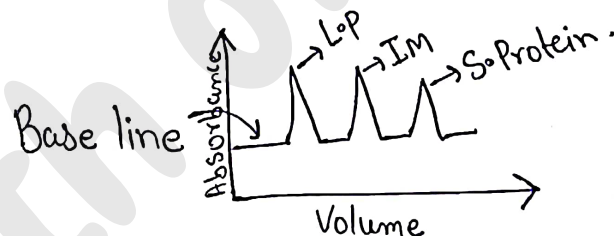
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- So, Large Size molecule easily pass from the Column⁴.
& then detect by detector. & first peak form on the system.
& large size sample is collected in Collecting Tube.



- Then Intermediate size sample/molecule/protein elute
& after this very small or small size protein eluted.



& all are collected in Collecting Tube.

$V_{be} \rightarrow$ Vol. of Beads & $V_o =$ Vol. of Void.

$$V_t = V_{be} + V_o$$

Total
Vol.

$V_e =$ amount of solvent req. to elute
solute.

$$\frac{V_e}{V_o} = \text{Relative elution Volume.}$$

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Gel chromatography, also known as size exclusion chromatography (SEC), has various applications in:

1. ***Protein purification***: Separates proteins based on size, useful for isolating specific proteins from complex mixtures.
2. ***Molecular weight determination***: Determines the molecular weight of proteins, polymers, and other molecules.
3. ***Biopolymer analysis***: Analyzes the size and distribution of biopolymers like DNA, RNA, and polysaccharides.
4. ***Pharmaceutical industry***: Purifies and characterizes pharmaceuticals, vaccines, and antibodies.
5. ***Food industry***: Analyzes food components, detects contaminants, and characterizes food additives.
6. ***Biotechnology***: Separates and purifies biomolecules, like enzymes, hormones, and nucleic acids.
7. ***Environmental monitoring***: Analyzes water and air samples for pollutants and contaminants.
8. ***Cosmetics industry***: Characterizes and purifies cosmetic ingredients, like proteins and polymers.
9. ***Research and development***: Used in various research applications, such as studying protein-protein interactions and characterizing new materials.
10. ***Quality control***: Ensures the quality and consistency of products in various industries.