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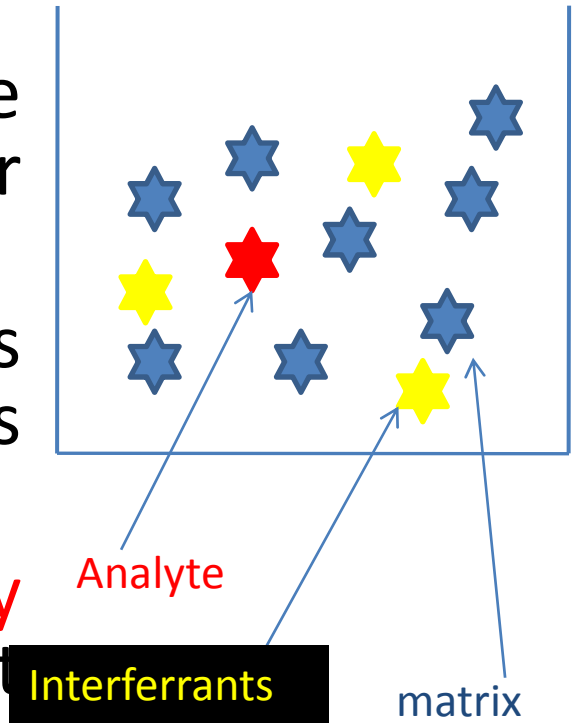
Semester VI (CHB-601)

Separation of molecules by chromatographic techniques

Dr Meenakshi Singh
Professor of Chemistry
Mahila Mahavidyalaya
Banaras Hindu University
Varanasi, INDIA

Analytical Separations

- Separations isolate the analyte from potentially interfering constituents
- Interfering constituents might be molecules similar to analyte or other matrix constituents.
- In the separation process, material is transported while its components are spatially redistributed.
- Separation always requires energy because reverse process, mixing at constant volume is spontaneous, being accompanied by an increase in entropy.



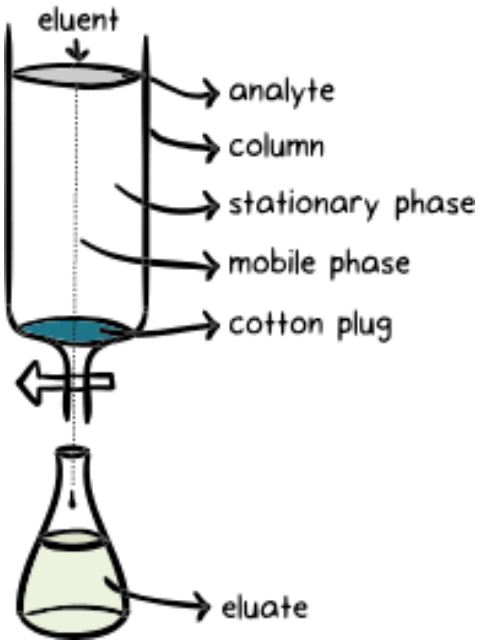
- Separations can be preparative or analytical
- Analytical separations usually eliminate or reduce interferences

	Method	Basis of Method
1	Mechanical phase separation a. Precipitation and Filtration b. Distillation c. Extraction d. Ion exchange	 Difference in solubility of compounds formed Difference in volatility of compounds Difference in solubility in two immiscible liquids Difference in interaction of reactants with ion-exchange resin
2	Chromatography	Difference in rate of movement of a solute through a stationary phase
3	Electrophoresis	Difference in migration rate of charged species in an electric field
4	Field-flow fractionation	Difference in interaction with a field or gradient applied perpendicular to transport direction

Chromatography

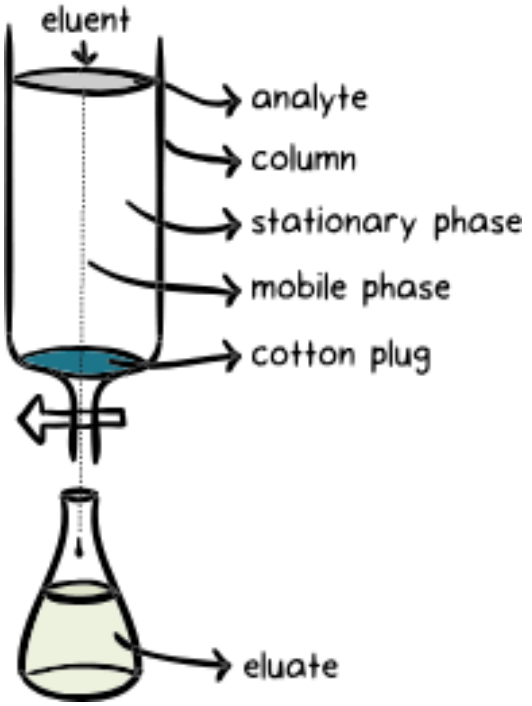
- It is used for
  - separation
  - identification
  - determination

  of the chemical components in complex mixtures.
- None other method is as powerful and as widely applicable as chromatography
- 12 Nobel prizes between 1937 - 1972 were awarded to the works in which chromatography played a vital role.



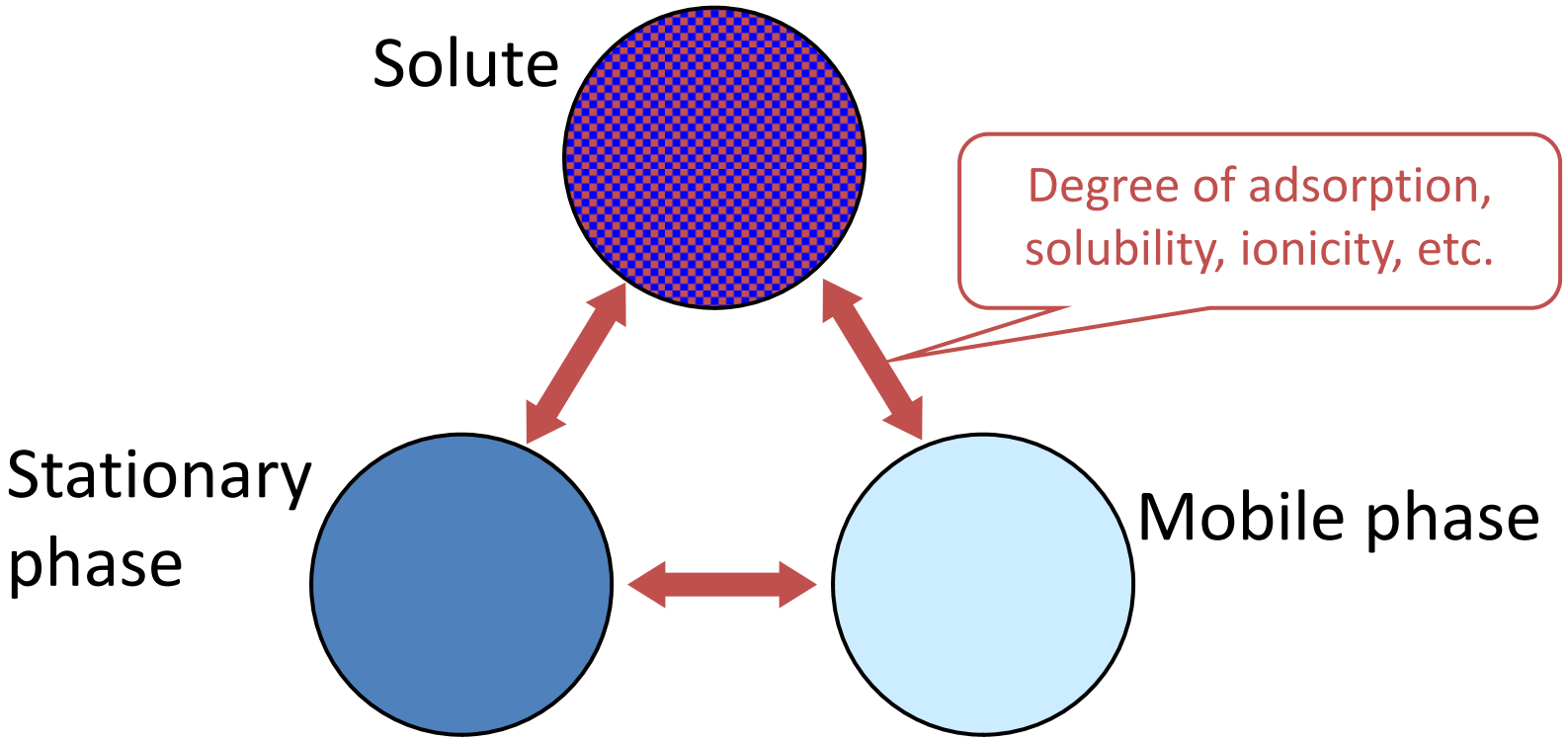
Chromatography

- Chromatography embraces a variety of processes which are based on differential distributions of the sample components between two phases.
- All chromatographic methods have in common the use of a stationary phase and a mobile phase.
- Movement of the mobile phase causes a differential migration of the sample components.

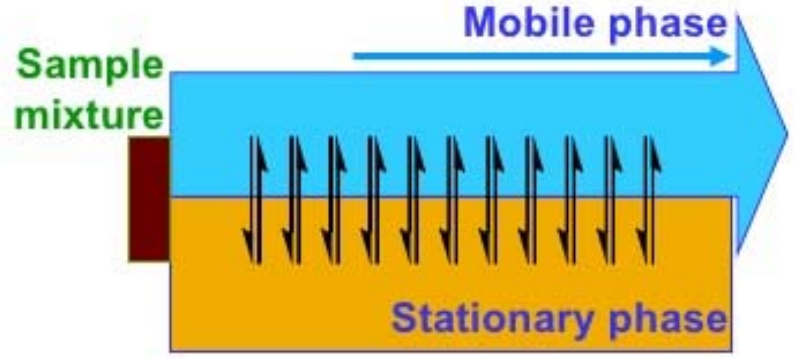


Interaction Between Solutes, Stationary Phase, and Mobile Phase

- Mechanisms responsible for the differential migration are diverse.
- Differences in the interactions between the solutes and stationary and mobile phases enable separation.



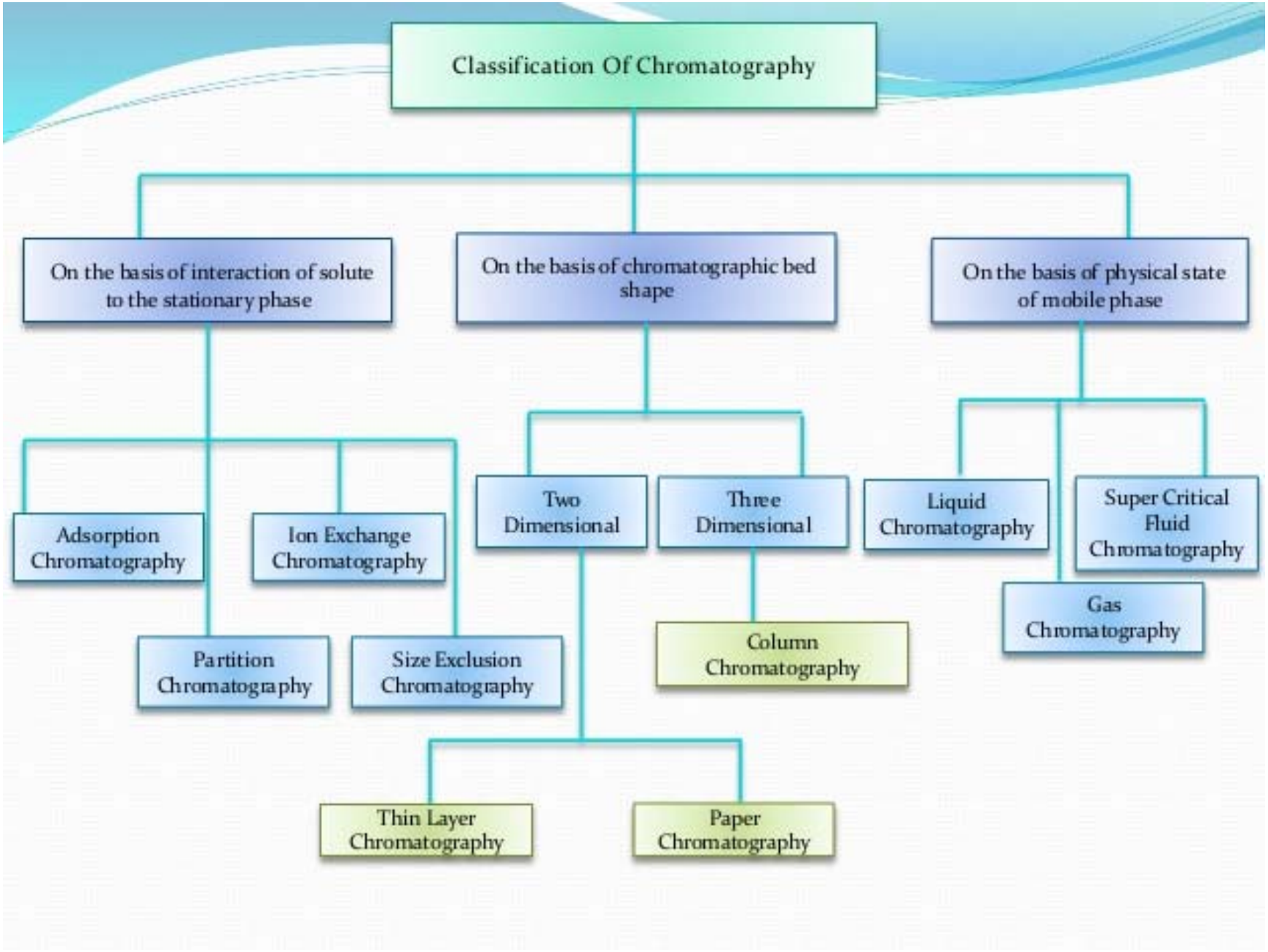
Chromatographic principle



Equilibrium establishes at each point (ideally)

The molecules of the mixture interact with the molecules of the Mobile and Stationary Phase → Retardation of rate of movement of molecules

Each molecule interacts differently with MP and SP → Different distribution coefficients and different net rates of migration


Classification Of Chromatography
On the basis of interaction of solute to the stationary phase
On the basis of chromatographic bed shape
On the basis of physical state of mobile phase
Adsorption Chromatography
Ion Exchange Chromatography
Partition Chromatography
Size Exclusion Chromatography
Two Dimensional
Three Dimensional
Thin Layer Chromatography
Paper Chromatography
Column Chromatography
Liquid Chromatography
Super Critical Fluid Chromatography
Gas Chromatography