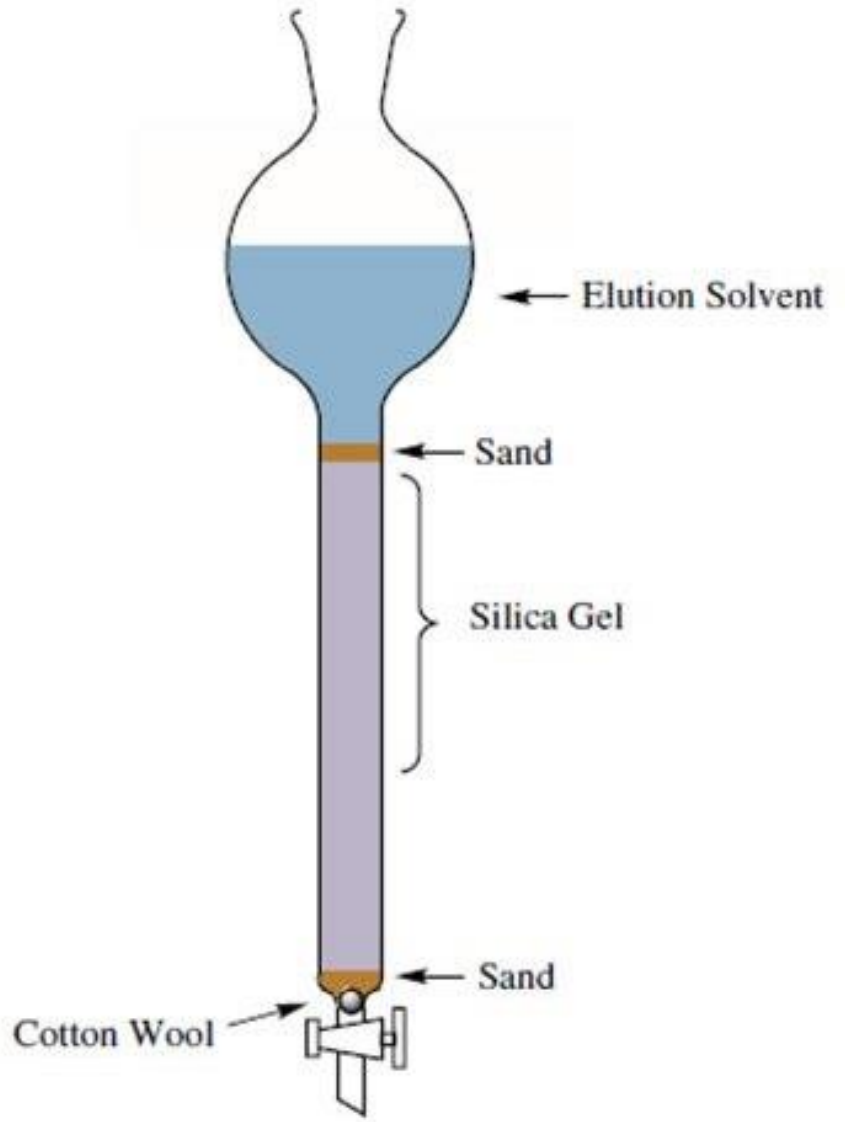
Instrumental Technique

FLASH CHROMATOGRAPHY



CHROMATOGRAPHY

- **Chromatography** is a Greek word chroma “colour” and graphein “to write”.

- And chromatography is a family of analytical chemistry techniques for the separation of mixtures.

- It was the Russian botanist “Mikhail Tsvet” who invented the first chromatography technique in 1901.

FLASH CHROMATOGRAPHY

- Differs from the conventional technique in 2 ways:

  - ✓ Slightly smaller silica gel particles are used

  - ✓ Due to restricted flow of solvent caused by the small gel particles, pressurized gas (*10*-15 psi) used to drive the solvent through the column of stationary phase

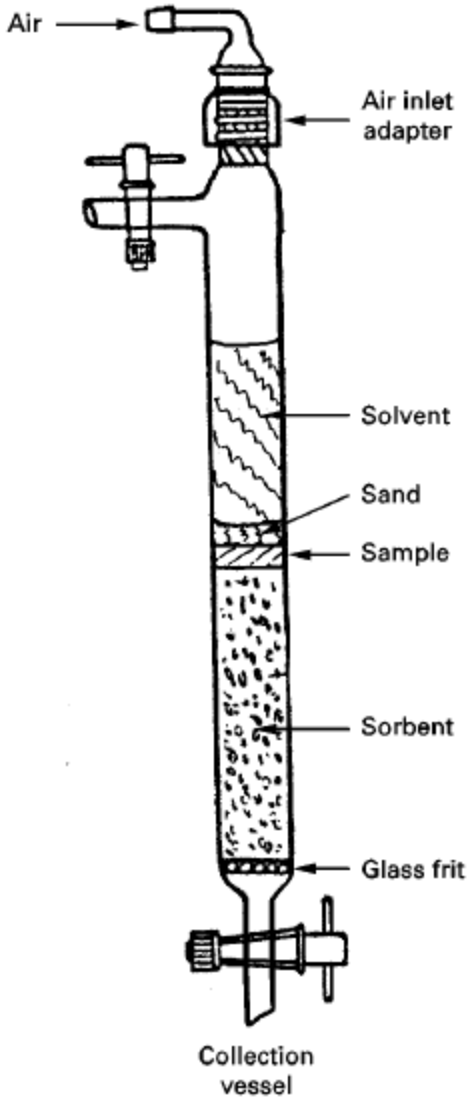
The net result is a rapid "**over in a flash**" and high resolution chromatography.

PRINCIPLE OF FLASH CHROMATOGRAPHY

- Chromatography exploits the differences in partitioning behaviour between a mobile phase and a stationary phase to separate the components in a mixture.

- Compounds of the mixture interact with the stationary phase based on charge, relative solubility or adsorption.

- The retention is a measure of the speed at which a substance moves in a chromatographic system.



- 1903 - Michael Tswett reported separation of plant pigments using glass columns packed with calcium carbonate.
- 1978 - Clark Still reported fast flow glass column chromatography – flash chromatography
  - Do-by-yourselves style
  - Significant labor involved in packing, unpacking and washing columns
  - Safety risks in shattering glass columns
  - Limited flow rate and pressure
  - Reproducibility concerns

(1994-Present)

1994 –disposable cartridges for flash chromatography were introduced

1. Disposable plastic cartridges – time and reproducibility
2. Cartridges of different size – easy scale-up
3. Solid sample module and injection valve – easy sample loading
4. Pressure up to 100 psi – fast separation
5. Narrow particle size distribution - Low backpressure and higher efficiency

Selection of solvent system

Solvent system

- Compound should have TLC R_f *of 0.15 to 0.20* in the solvent system

- Binary solvent system –
  - Polarity can be adjusted
  - Rate of elution can be determined
  - Common solvents used :
    - dichloromethane/hexane, ether/hexane, hexane/ethyl acetate, and dichloromethane/methanol

High polarity of solvents increase the rate of elution of all compounds.

Quantity of silica gel required

- 40-63 µm silica gel particles are used

- Amount depends on 2 factors :
  - R_f difference of the compounds to be separated
  - Amount of sample

- ↑ silica gel -↑ the length of time for chromatography

- For,
  - Easier separations, ratios closer to 30 : 1 are effective
  - Difficult separations, more silica gel is often required

Advantage

- Large quantities of the sample can be separated (0.5-2g)
- Fast (1o to 15 minutes)
- Cost efficient
- Elaborate equipment and the purchase of expensive equipment is not necessary
- If high resolution is required, flash chromatography is carried out before HPLC to avoid contamination of the expensive plates

Applications

- Purification of various peptides, antibiotics
- Separation of closely related organic compounds
- Purification of closely related drug intermediates
- High speed fractionation of natural products – tocopherols, alkaloids, lignans, xanthones, stilbenes, flavonoids
- Drug discovery
- Agrochemistry
- Petrochemistry


Thank you