PRINCIPLES AND APPLICATION OF CHROMATOGRAPHY

Lecture for:
M.Sc.Pharmceutical Chemistry/Chemistry: II sem
Subject:Introduction to Chromatographic techniques
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CHROMATOGRAPHY

- Laboratory technique for the Separation of mixtures
- *Chroma* -"color" and *graphein* - "to write".
- Colour bands - separation of individual compounds
- Measured or analysed.

PURPOSE OF CHROMATOGRAPHY

- **Analytical**

✓ Determine Chemical composition of a sample

- **Preparative**

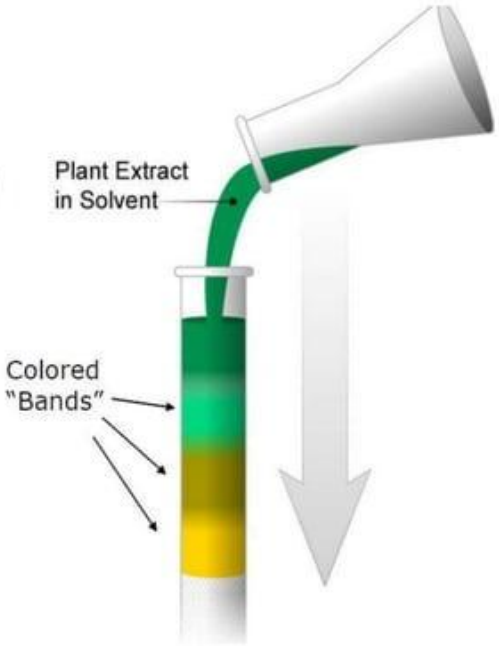
✓ Used to purify sufficient quantities of a substance

TSWETT EXPERIMENT

- Tall glass open column filled with sand-like particles
- Ground-up plant extract
- Poured into the column and saw colored "bands" develop as the extract percolated down thru the column
- Different compounds had separated

Greek

Chroma -- color
Graphy -- writing/study of



CHROMATOGRAPHY TERMS

- **Chromatograph** - equipment that enables a sophisticated separation

  EX. Gas chromatography or Liquid chromatography
- **Eluent** - Fluid entering column/ solvent that carries the analyte.
- **Eluate** - Mobile phase leaving the column.
- **Stationary phase - Immobilized phase**

❖ Immobilized on the support particles or on the inner wall of the column tubing.

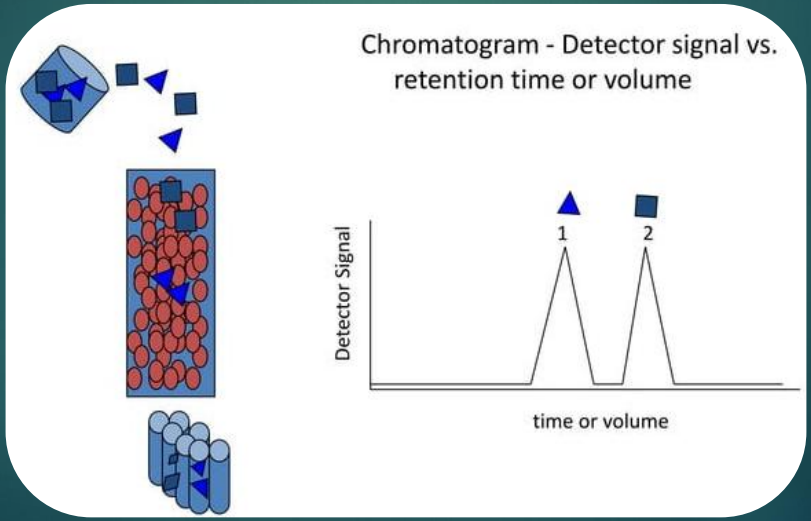
❖ Examples : Silica layer - Thin Layer Chromatography

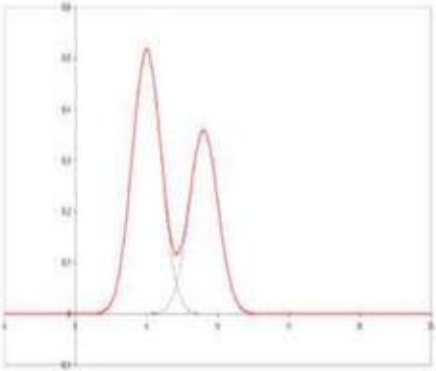
- **Mobile phase**

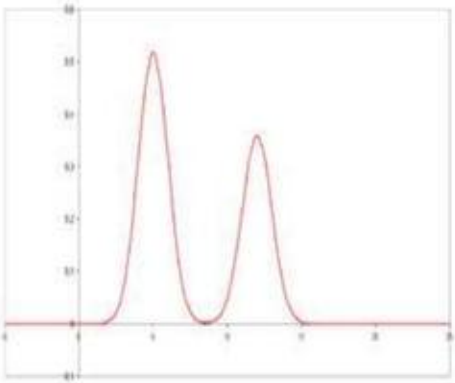
  Moves in a definite direction. Liquid (LC), Gas (GC).
- The mobile phase moves through the chromatography column (the stationary phase) where the sample interacts with the stationary phase and is separated.
- **Retention time :** Time takes for a particular analyte to pass through the system (from the column inlet to the detector) under set conditions.
- **Sample (Anylate)** :Substance analyzed in chromatography.
- **Solvent** : Any substance capable of solubilizing another substance.

❑ Chromatogram

- Visual output of the chromatograph.
- Separation - Different peaks or patterns on the chromatogram correspond to different components of the separated mixture.







- X- axis - Retention time
- Y-axis - Signal
- Signal is proportional to the concentration of the specific analyte separated.

HOW TO DESCRIBE A CHROMATOGRAM

Chromatogram - response of a detector vs time. - shows when various components come off a column

Retention time t_r - The time at which a component elutes from a column.

Theoretical Plates

Assume a chromatographic peak has a Gaussian shape

H is height of peak

$w_{1/2}$ is with at ½ height

(If true Gaussian $w_{1/2} = 2.35\sigma$

Where σ= standard deviation)

Width at baseline should be 4σ

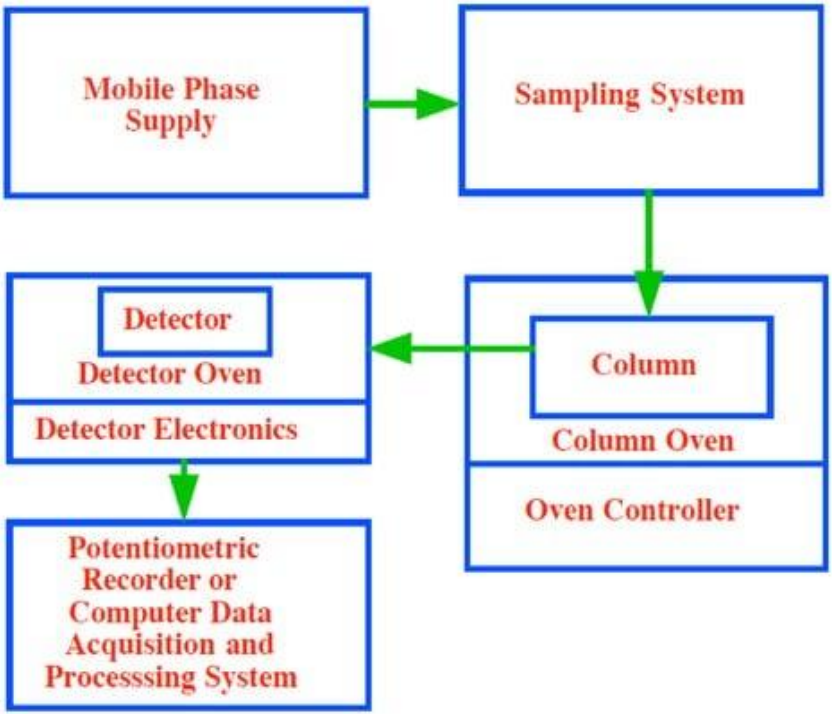
For chromatography we retain the name Theoretical Plate, but calculate it using the retention time and the width at ½ height

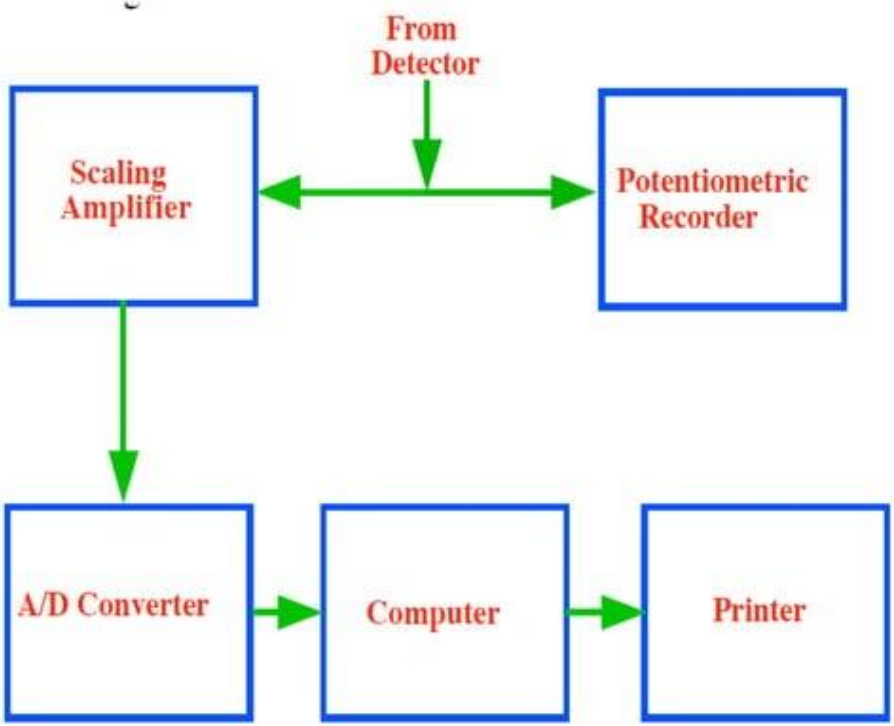
$$N = \frac{5.55 t_r^2}{w_{1/2}^2}$$

N is number of Theoretical plates. Again the bigger the N the better, because that means the width of the peak is small compared to its retention time.

PRICNIPLES OF CHROMATOGRAPGHY

- Physical method of separation that distributes components to separate between two phases moves in a definite direction.
- Substances are separated based on their differential distribution between two phases
- Substances will move with the mobile phase at different rate depending upon their Partition or Distribution co-efficients.


Mobile Phase Supply
Sampling System
Detector
Detector Oven
Detector Electronics
Column
Column Oven
Oven Controller
Potentiometric Recorder or Computer Data Acquisition and Processsing System


From
Detector
Scaling
Amplifier
Potentiometric
Recorder
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Computer
Printer

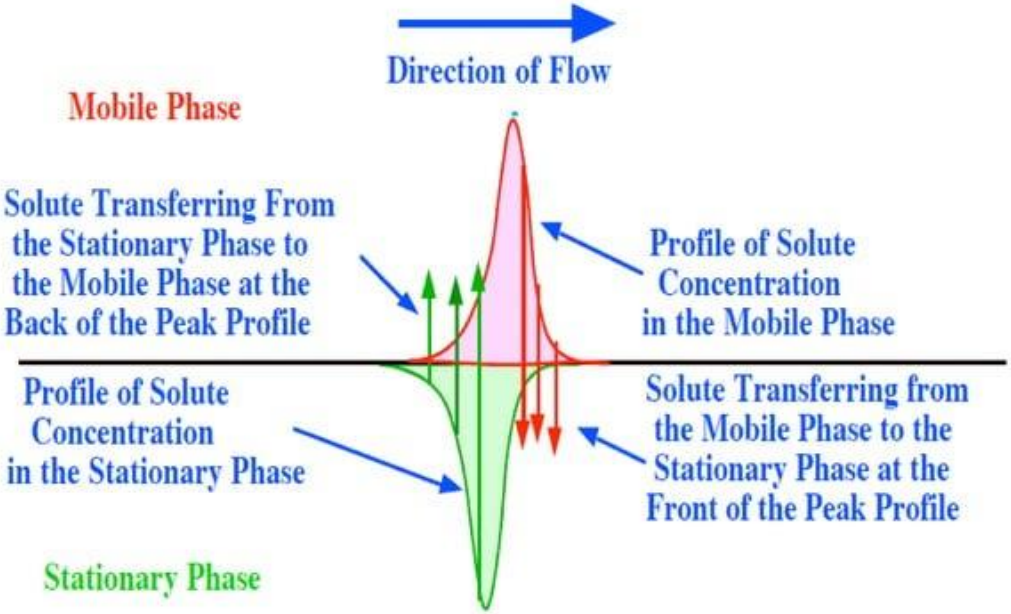

Figure 1. The Elution of a Solute Through a Chromatographic System

Thanks

- For Further Study :