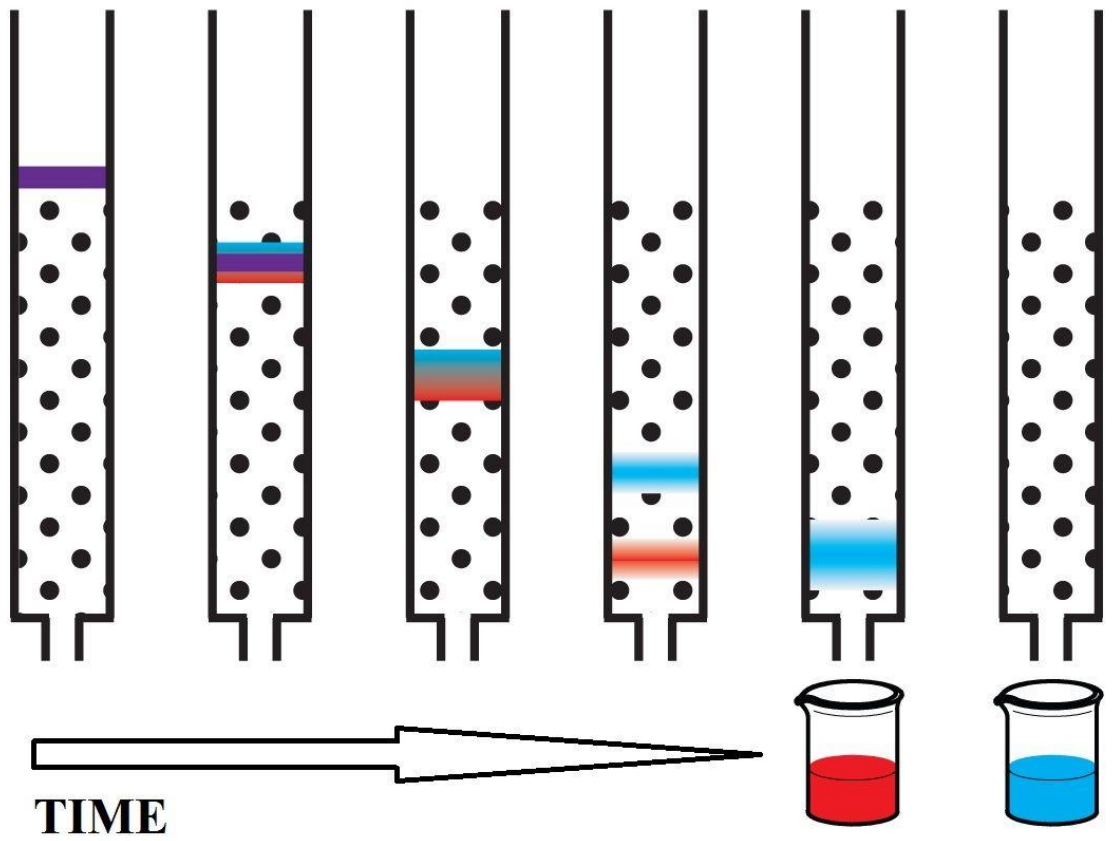
Chromatography

Retention and Separation factors, Theoretical plates, Instrumentation and uses of Gas Chromatography and High Performance Liquid Chromatography

References: Fundamentals of Analytical Chemistry, 9th Edition, Douglas A. Skoog, Donald M. West, F. James Holler, Stanley R. Crouch

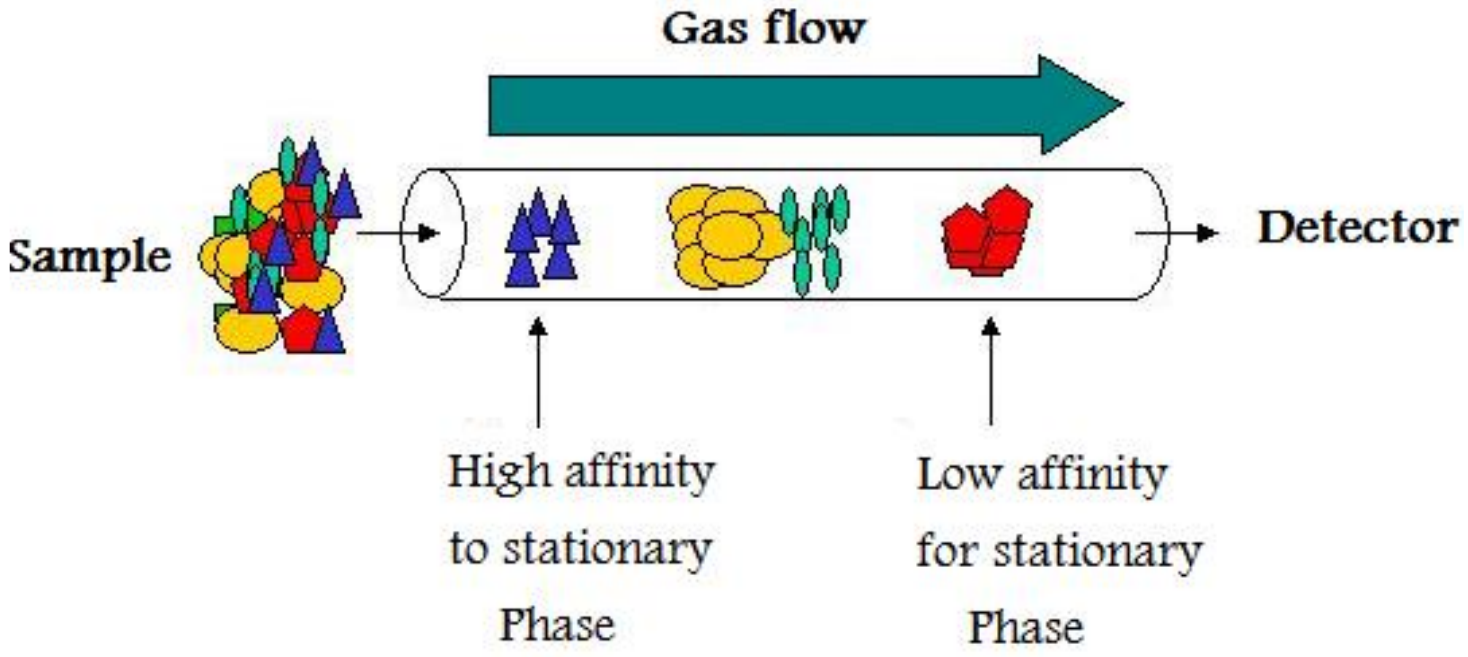

Coffee filter art


TIME

History of Chromatography

- Chromatography (colour writing) was first employed by **Mikhail Tswett in 1903**

- He worked on the separation of plant pigments such as **chlorophyll, carotenes**, and **xanthophylls** which are have different colours.



It is a physical separation method in which the components of a mixture are separated by differences in their distribution between two phases

Stationary phase

Mobile phase

Chromatography: Application

Purify – Separate components in order to isolate one of interest for further study

Identify – Determine the identity of a mixture or components based on known components

Quantify – Determine the amount of the a mixture and/or the components present in the sample

Analyze – Examine a mixture, its components and their relations to one another

❖The substances must interact with the stationary phase to be retained and separated by it.

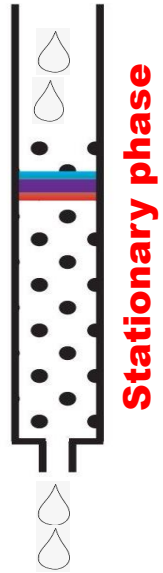
❖Components that strongly retained by the stationary phase move slowly by mobile phase.

❖Components that weakly held by the stationary phase move fast with the mobile phase.

Terminologies

Analyte:

The substance to be separated during chromatography



Chromatograph:

Equipment for performing chromatography

Chromatogram:

The visual output of the chromatograph

Retention time (R_T):

Is a measure of the time taken for a solute to pass through a chromatography column

It is calculated as the time from injection to detection

Flow rate (F_C):

Volume of mobile phase passed/minute (mL/min).

Eluent:

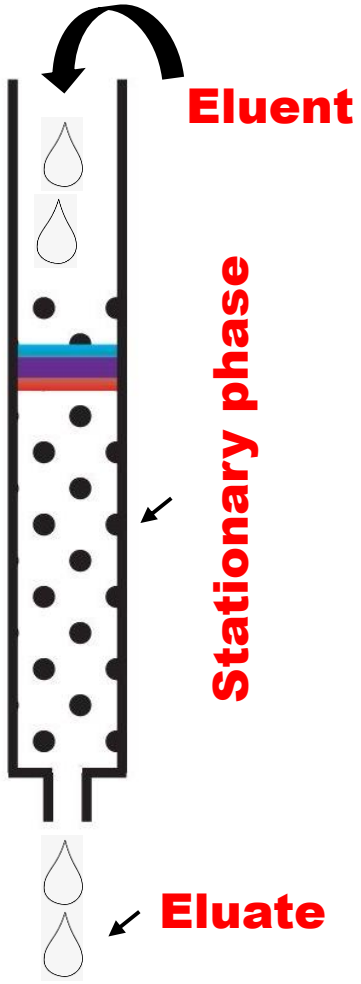
Is the solvent that carries the analyte.

Eluate:

Is the mobile phase leaving the column.

Elution/Development:

The process of passing the mobile phase through the column.



Stationary Phase

An immobilized phase in chromatography system through which the materials are to be separated

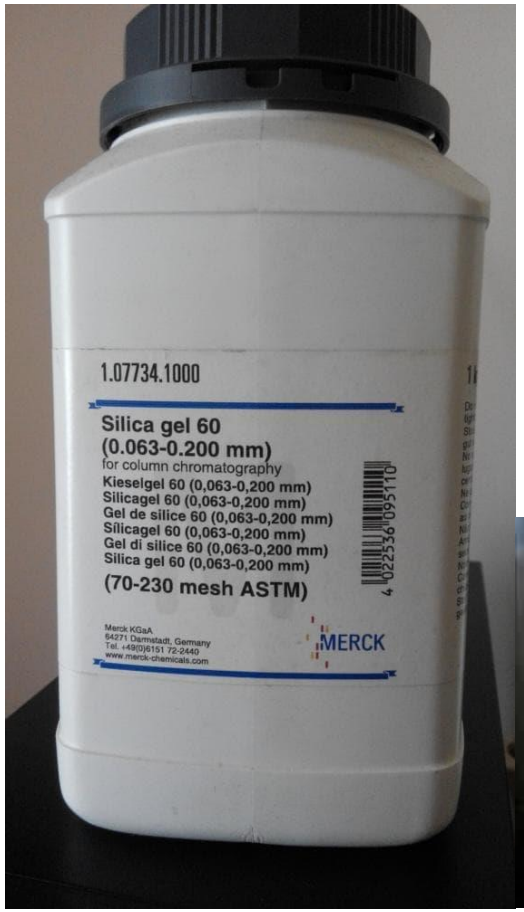
e.g. Silica, Alumina.

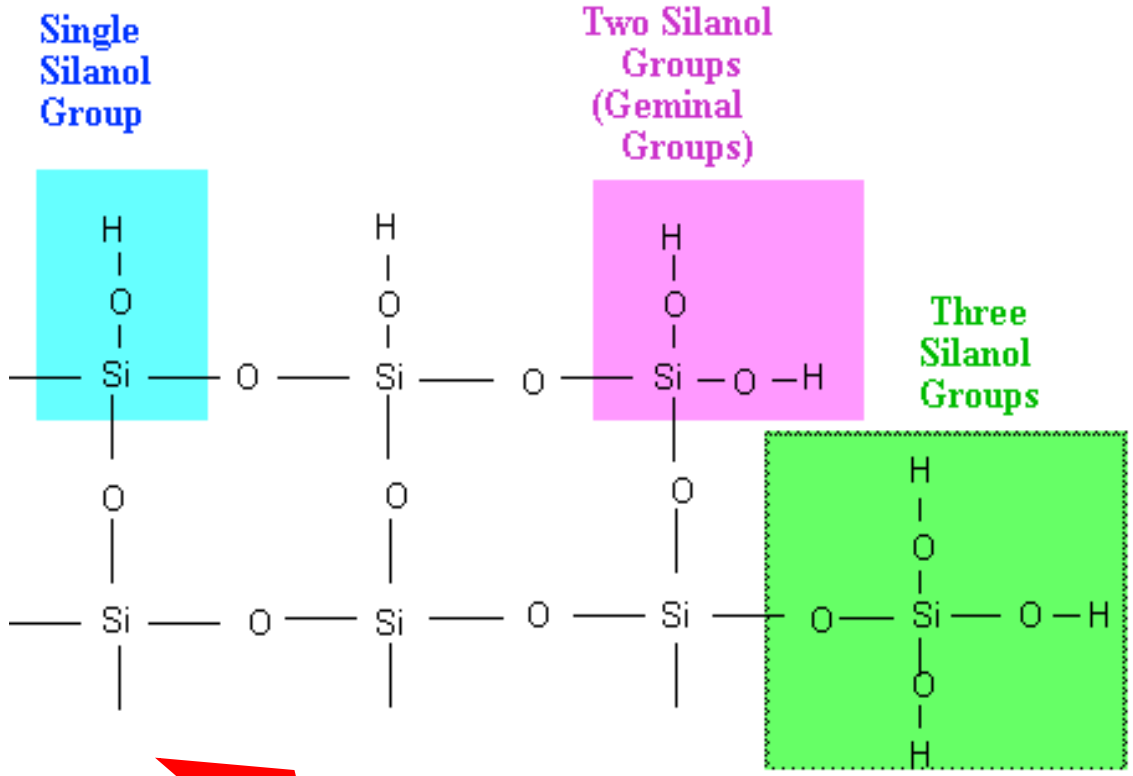
Characteristics:

- Spherical in shape & uniform in size
- Chemically inert and high mechanical stability
- Separating wide variety of compounds
- Easily available & inexpensive
- Particle size is in the range 50-200 µm

Stationary Phase

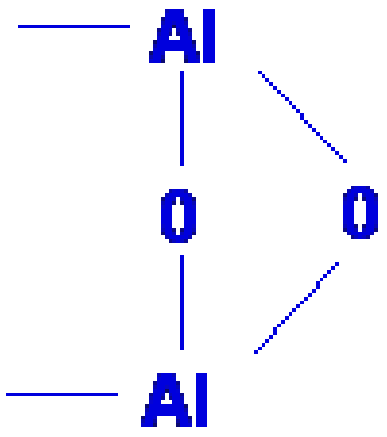
Silica Gel



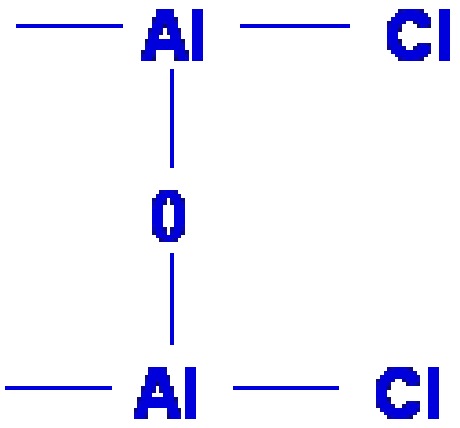


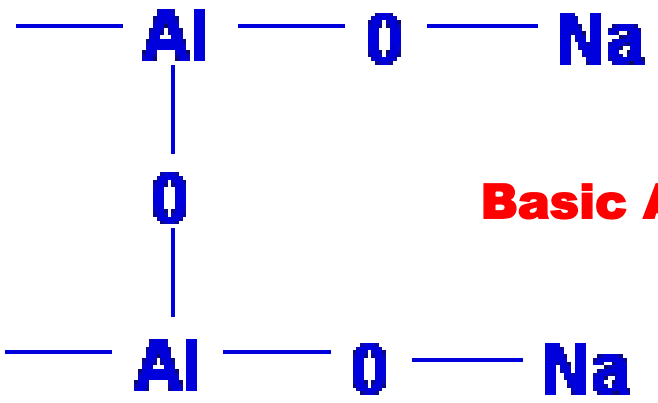
Stationary Phase

Neutral Alumina



Acidic Alumina





Basic Alumina

Neutral alumina - Useful for separation of aldehydes, ketones, quinones, esters and lactones *etc*.

Acidic alumina - Useful for separation of acid pigments and strong acids.

Basic alumina - Useful for basic and neutral compounds that are stable to alkali, as well as for amines, steroids, alkaloids and natural pigments.

Mobile Phase

Gas or liquid that carries the mixture of components through the stationary phase

The function of a mobile phase:

1. To introduce the mixture into the column – as solvent.
2. To remove or elute pure components out of the column – as eluent.
3. As color developing agent.

Selection of solvent as mobile phase

❖ The solvents should have sufficiently low boiling points to permit ready recovery of eluted material

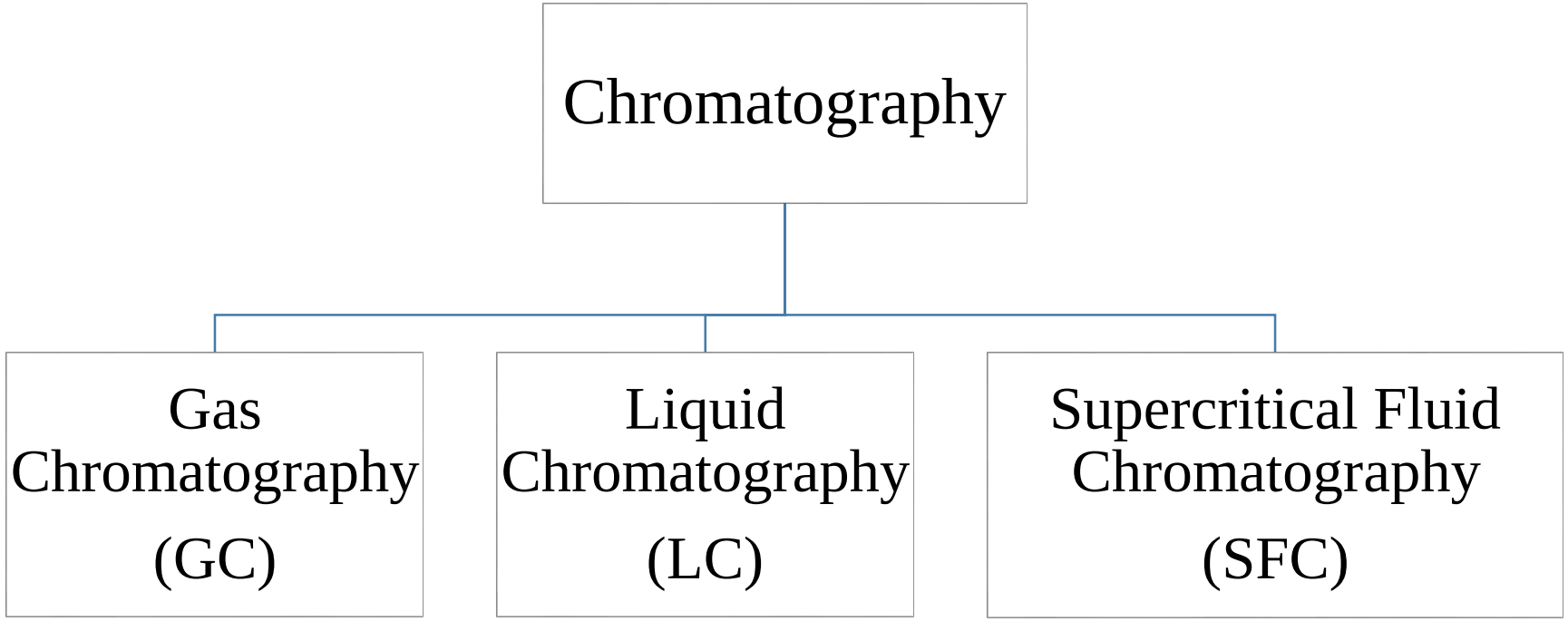
❖ However, polarity as seen the most important factor in adsorption chromatography

❖ It can be used in either pure form or as mixture of solvents

Polarity order of various solvents:

Pure water > Methanol > Ethanol > Propanol > Acetone > Ether > Chloroform > Dichloromethane > Toluene > Hexane > Pentane.

Classification: based on mobile and stationary phases



Classification: based on mobile and stationary phases

Gas Chromatography (GC)

1. **Gas-liquid Chromatography :**

Stationary Phase: Liquid bonded or adsorbed on solid surface

Type of equilibrium: Partition between gas and liquid

2. **Gas-solid Chromatography :**

Stationary Phase: Solid surface

Type of equilibrium: Adsorption

Classification: based on mobile and stationary phases

Liquid Chromatography (LC)

1. Liquid-liquid or Partition Chromatography :

Stationary Phase: Liquid bonded or adsorbed on solid surface

Type of equilibrium: Partition between immiscible liquid

2. Liquid-solid or Adsorption Chromatography :

Stationary Phase: Solid surface

Type of equilibrium: Adsorption

3. Ion Exchange Chromatography :

Stationary Phase: Ion exchange resin

Type of equilibrium: Ion exchange

Classification: based on mobile and stationary phases

4. Size Exclusion Chromatography :

Stationary Phase: Liquid in interstices of a polymeric solid.

Type of equilibrium: Partition or sieving

5. Affinity Chromatography:

Stationary Phase:

Solid surface tagged with some specific group

Type of equilibrium:

Partition between surface liquid and mobile liquid

Supercritical Fluid Chromatography:

Mobile Phase: Supercritical Fluid

Stationary Phase: Solid surface

Type of equilibrium:
Partition between supercritical fluid and solid surface

Adsorption chromatography

Stationary phase: The most common are Silica-gel and Alumina

Stationary phase interacted with solute molecules
due to OH groups present on their surface

More polar molecules are adsorbed more strongly
Hence, elute more slowly

Order of adsorption of polar groups on polar support :
-C=C- < $-OCH_3$ < -COOR < -C=O < -CHO < $-NH_2$ < -OH < -COOH
Olefins < Ethers < Esters < Aldehydes < Amines < Phenols < Acids

Partition Chromatography

❖ **Stationary phase:** In this type, the packing consists of a theoretically inert support material coated with a film of the liquid stationary phase

❖ **The division** into adsorption and partition is only of theoretical significance as in partition chromatography the adsorption effects of the support can also be felt

❖ **Partition coefficient / Distribution Constant (K):** The ratio of the concentrations of a solute in two immiscible or slightly miscible liquids

$$K = C_S/C_M$$

Where, C_S = Concentration of solute in stationary phase

C_M = Concentrations of solute in mobile phase

Distribution constant : partition chromatography

$$K = C_S/C_M$$

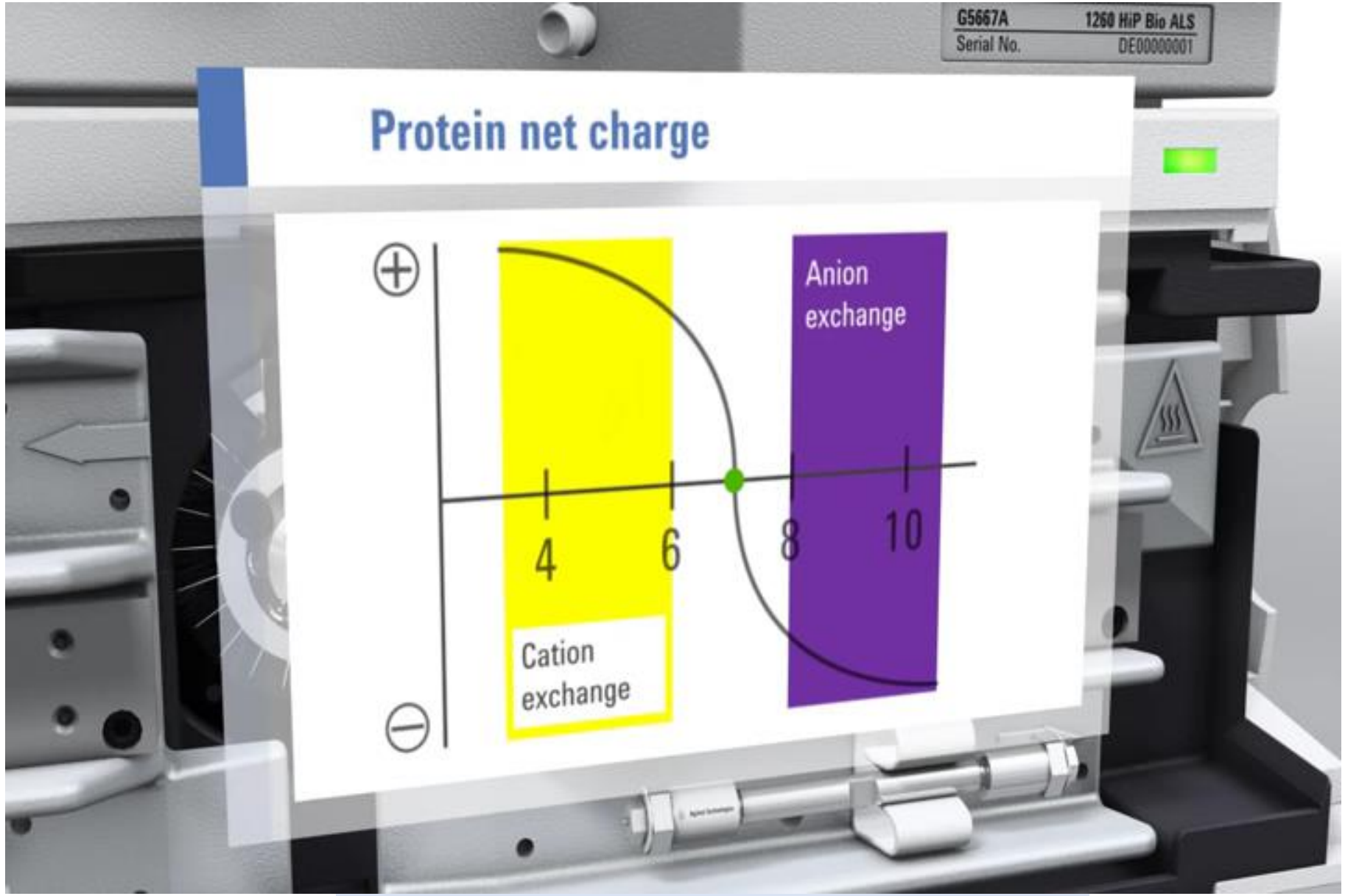
$$C_S = n_S/V_S$$

$$C_M = n_M/V_M$$

Therefore, $K = (n_S/V_S)/(n_M/V_M)$

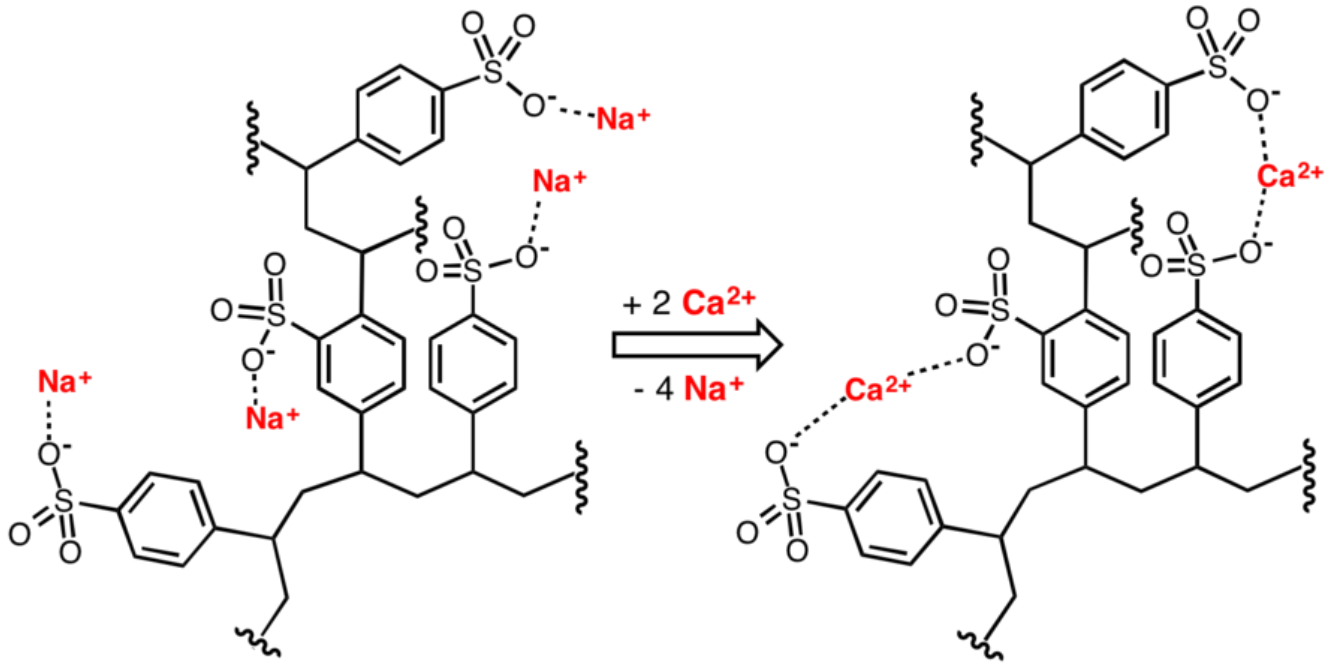
$= (n_S/n_M) \times (V_M/V_S)$

Ion-exchange Chromatography



Anion Exchange Cation Exchange

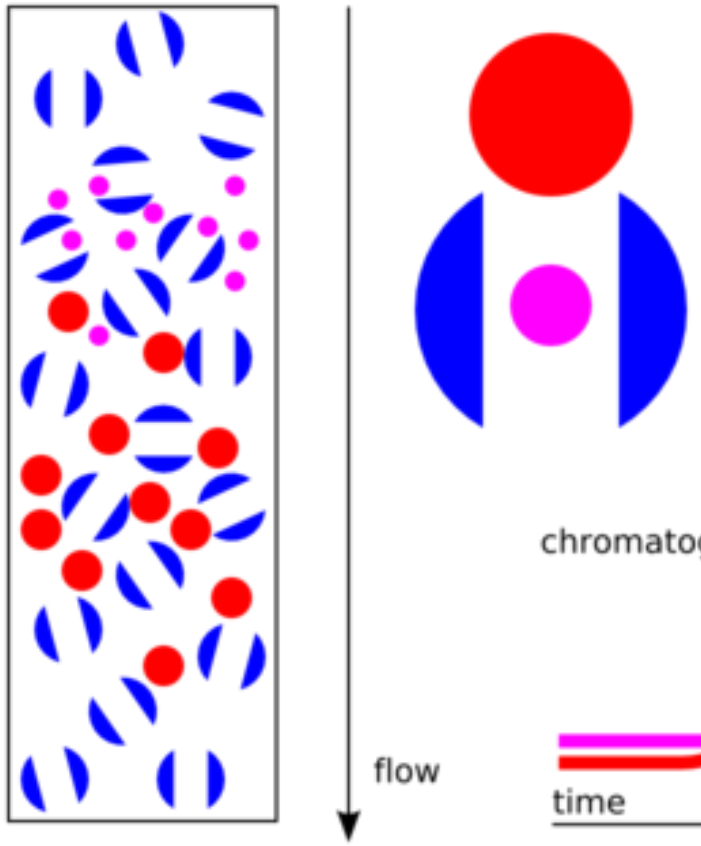
Ion-exchange Chromatography

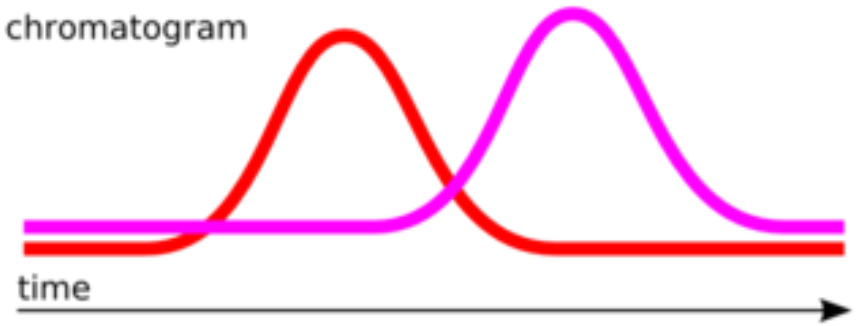


The water softening process involving replacement of calcium ions in water with sodium ions by a cation-exchange resign.

Size exclusion Chromatography

Useful for macromolecules, proteins and polymers separation





Large particles cannot enter into the gel and excluded They have less volume to traverse and elute sooner

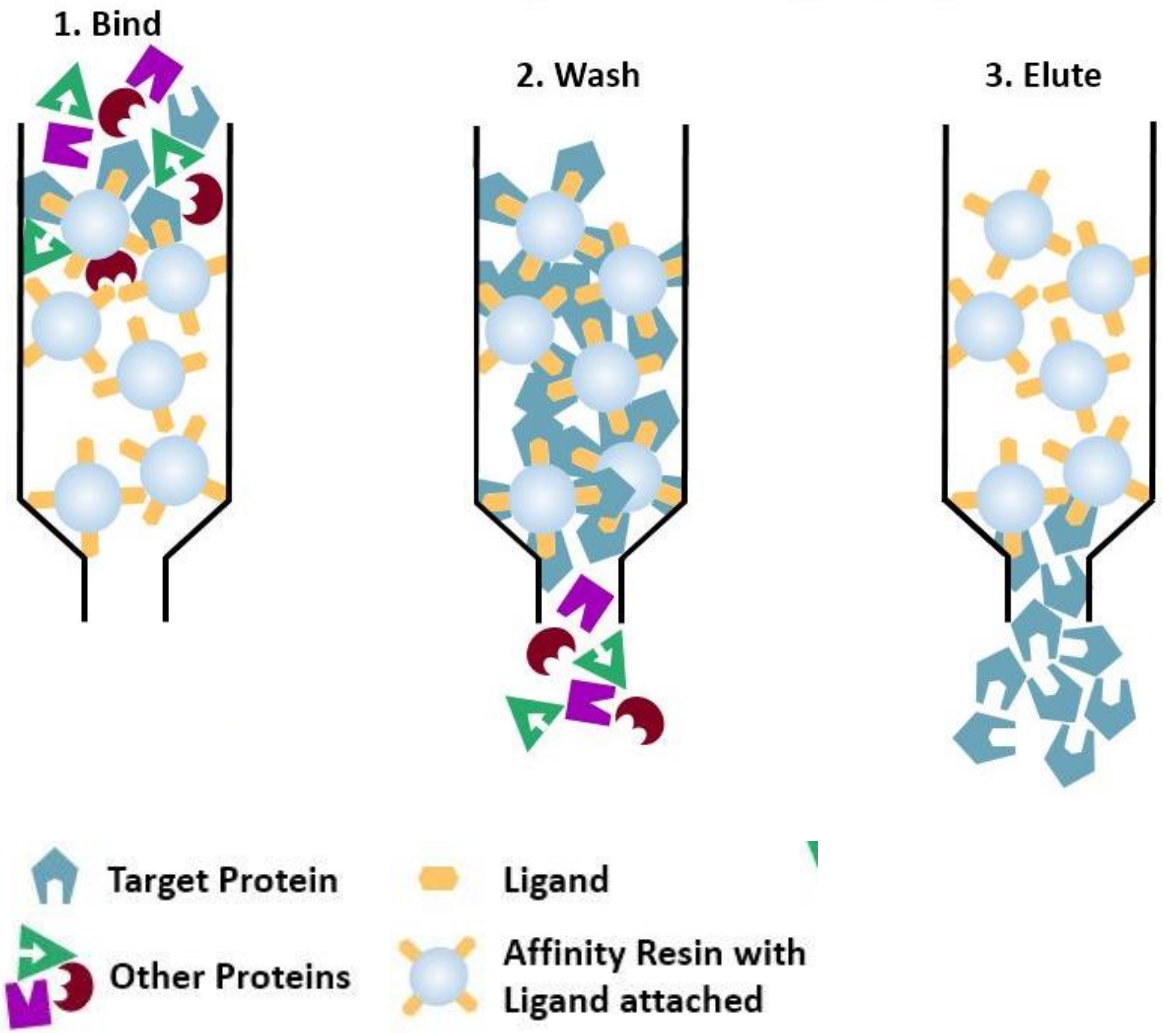
Small particles can enter into the gel and have less volume to traverse and elute latter

Size exclusion Chromatography

Gel Permeation Chromatography (GPC): Organic solvent can be used as mobile phase. Semi-rigid/rigid gels like polystyrenes, alkylated dextrons are used as stationary phase.

Gel Filtration Chromatography (GFC): Aqueous or buffer solution can be used as mobile phase. Dextran polymers (Sephadex), agarose (Sepharose), or polyacrylamide (Sephacryl or BioGel P) can be used as mobile phase.

Affinity Chromatography

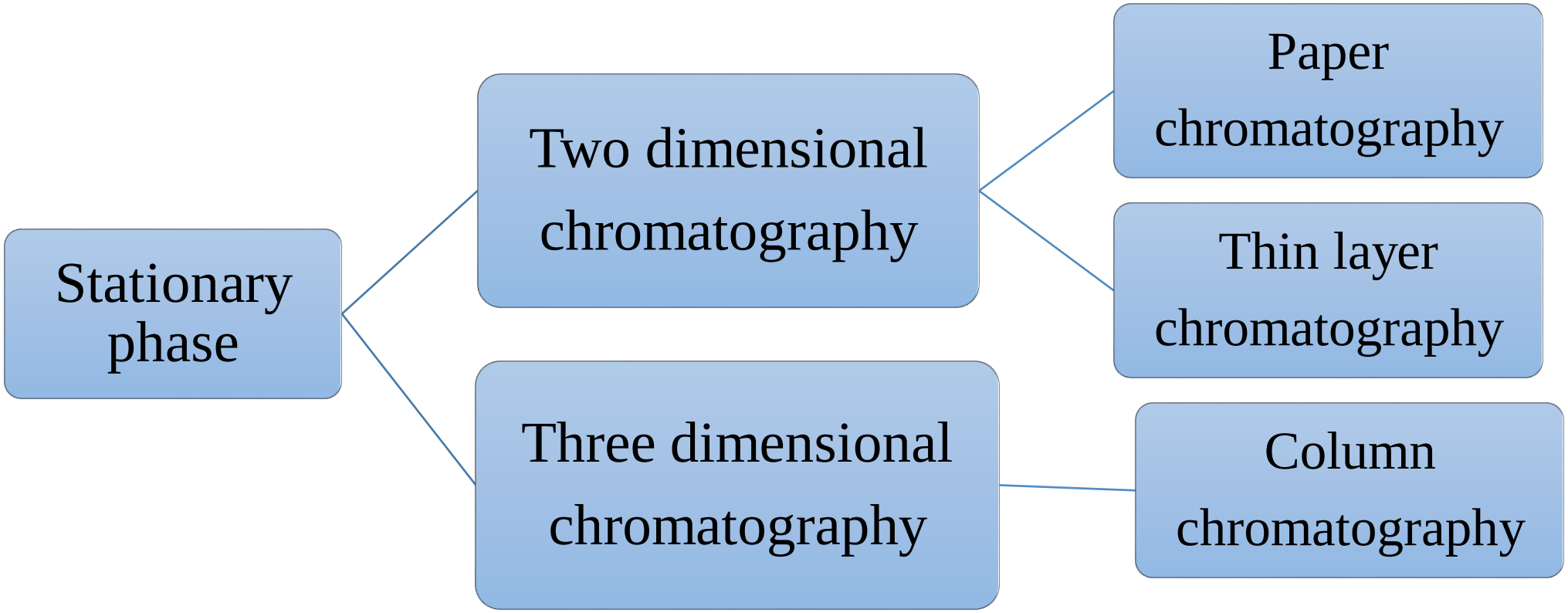


Affinity Chromatography

- Useful for separation of biochemical mixtures based on highly specific interaction between **antigen and antibody, enzyme and substrate, receptor and ligand** or **protein and nucleic acid**.

- Mostly used for the purification of proteins. It separates proteins on the basis of reversible interaction between protein (or group of proteins) and a specific ligand coupled to a chromatography matrix.

Classification based on the physical means (Methods holding the stationary phase)



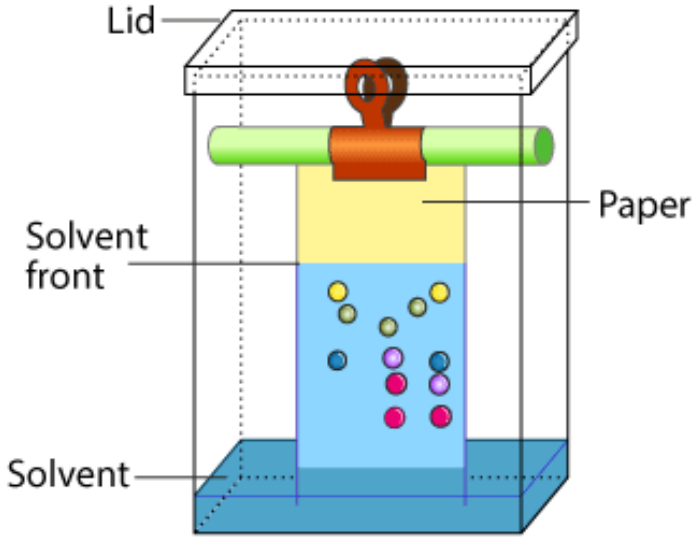
Classification based on the Technique

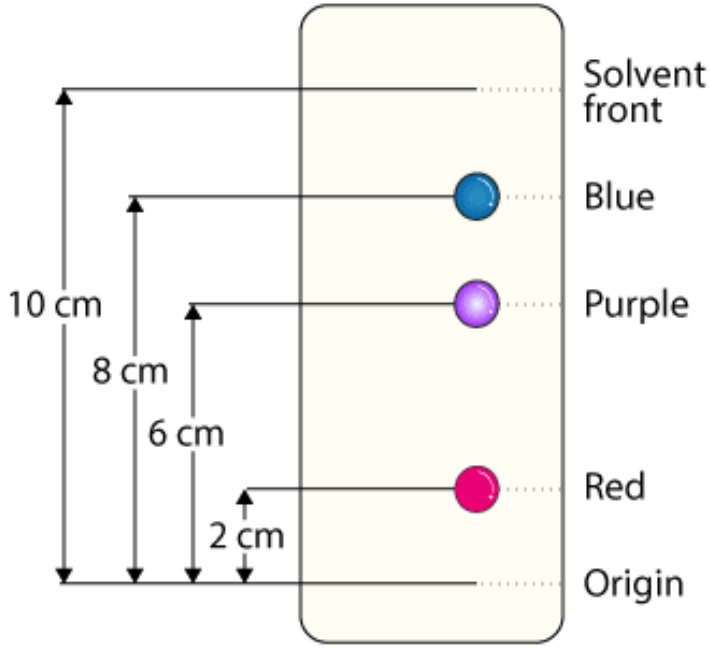
- **Thin Layer Chromatography (TLC):** The stationary phase is a thin layer supported on glass, plastic or aluminium plates.

- **Paper Chromatography (PC):** The stationary phase is a thin film of liquid supported on an inert support or paper.

Planar Chromatography

Planar Chromatography: Stationary phase is supported on a flat plate on which the mobile phase moves on it by capillary action





Planar Chromatography: Thin layer Chromatography (TLC)

Separations on TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase

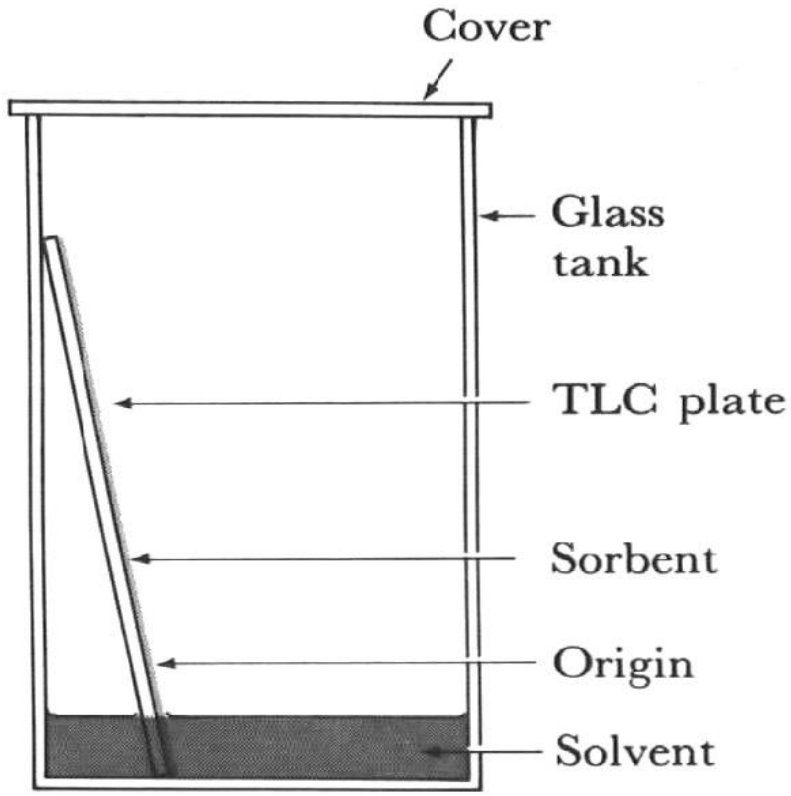
❖**The stationary phase:** Thin layer of adsorbent (usually silica gel or alumina) coated on a plate (Glass or Aluminium plate)

❖**The mobile phase:** It is a developing liquid which travels up the stationary phase, carrying the samples with it

Planar Chromatography: Thin layer Chromatography (TLC)

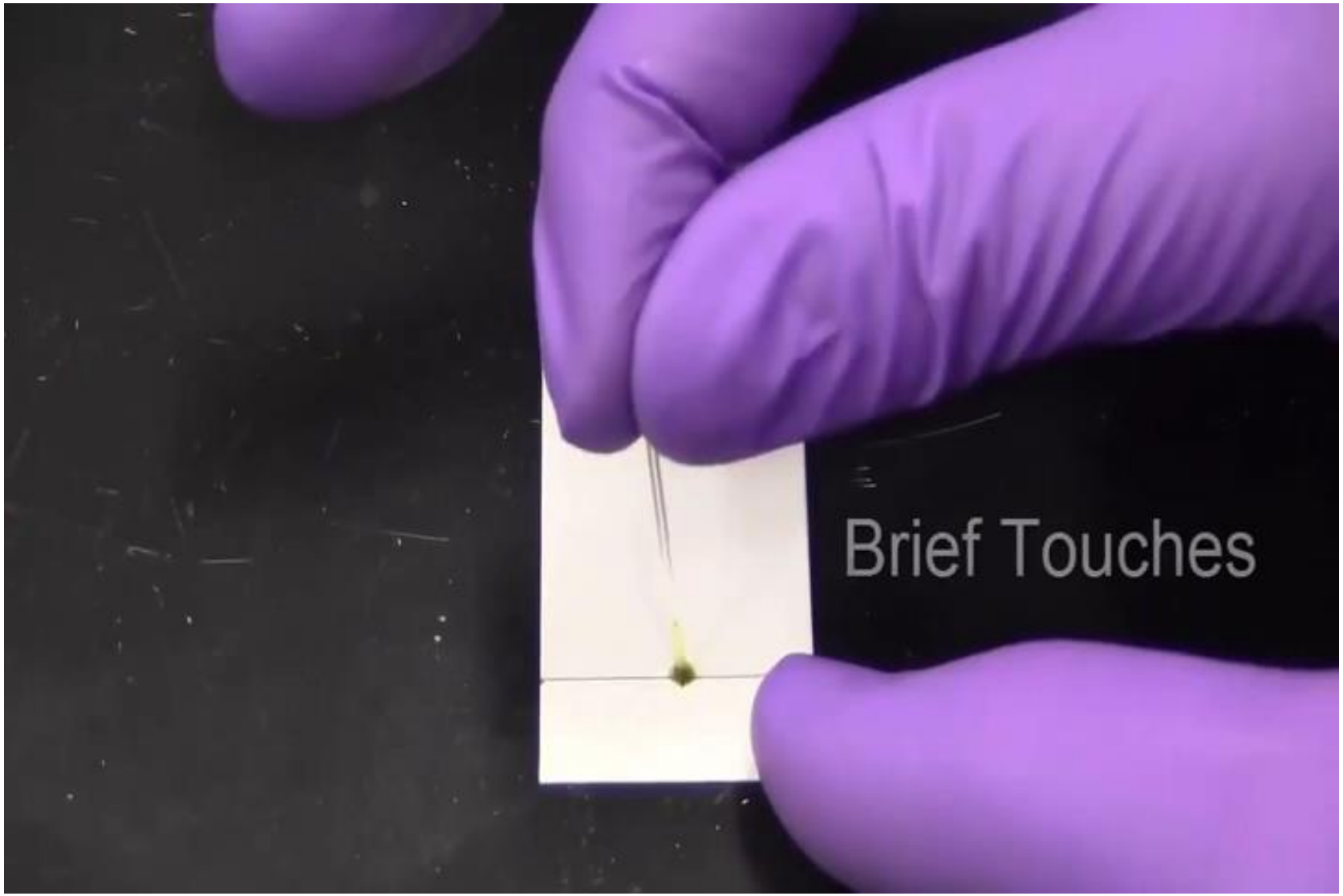
Components of the samples will separate on the stationary phase according to how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.

Thin layer chromatography (TLC): How to perform



When the plates are removed from the chamber, quickly trace the solvent front (the highest solvent level on the plate) with a pencil.

Thin layer Chromatography (TLC): How to Perform



Thin layer Chromatography (TLC): Identifying the Spots

The most common visualization technique is to hold the plate under a UV lamp

Visualizing Agents:

- Alkaloids: Dragendorff's reagent
- Cardiac glycosides: Antimony trichloride
- Sugar: Aniline phthalate
- Amino acids: Ninhydrin

Calculation for retention factor (R_f)

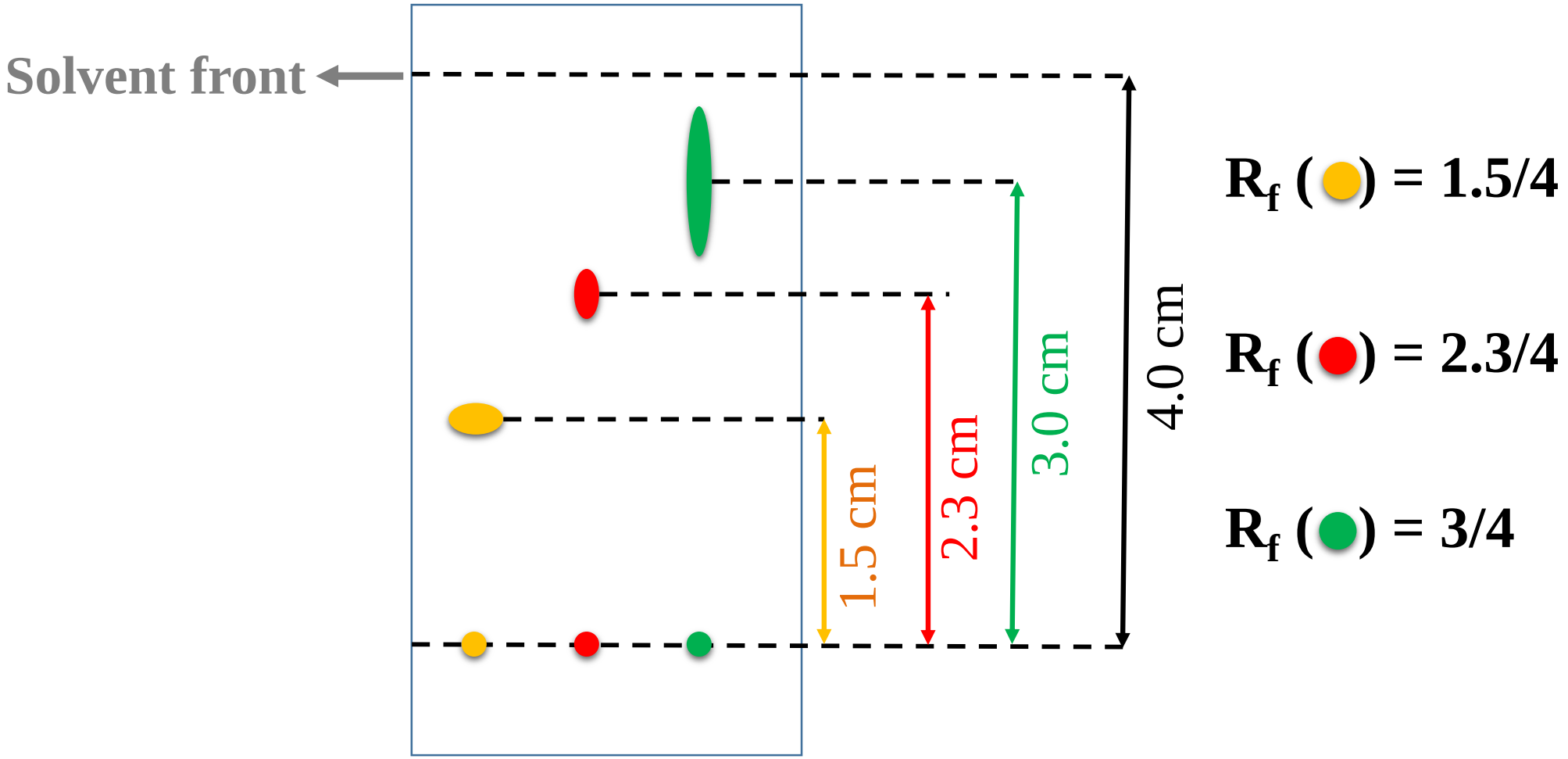
$$R_f = \frac{\text{distance travelled by the center of the spot}}{\text{distance simultaneously travelled by the mobile phase}}$$

OR

$$R_f = \frac{\text{distance travelled by the compound}}{\text{distance travelled by the solvent front}}$$

The R_f (retention factor) value for each spot should be calculated

Thin layer Chromatography (TLC): Interpreting the Data



Thin layer Chromatography (TLC): Retention Factor (R_f)

- It is characteristic for any given compound
  on the same stationary phase using the same mobile phase

- Hence, known R_f values can be compared to those of unknown substances to aid in their identifications

Classification Based on the Technique:

Column Chromatography (CC): Stationary phase is packed in a glass column

Column Chromatography (CC): Column Characteristics

- The main function of the column is to support the stationary phase
- Column is mostly good quality neutral glass and not affected by solvents
- Column dimensions - length and diameter ratio (10:1, 30:1 or 100:1)

Column Chromatography (CC): Column Packing

The stationary phase is packed into a column

Packing of column: Packing depends mainly on the density of the solid

Techniques used are the wet, dry and slurry methods

In all cases avoid inclusion of air bubbles

Sample addition: Apply evenly and in a concentrated solution to the top of the column which is protected from disturbance

Column Chromatography (CC): Types of Elution

Isocratic elution: Addition of solvent mixture of fixed composition during the whole process.

e.g. Single solvent or solvent mixture with fixed composition

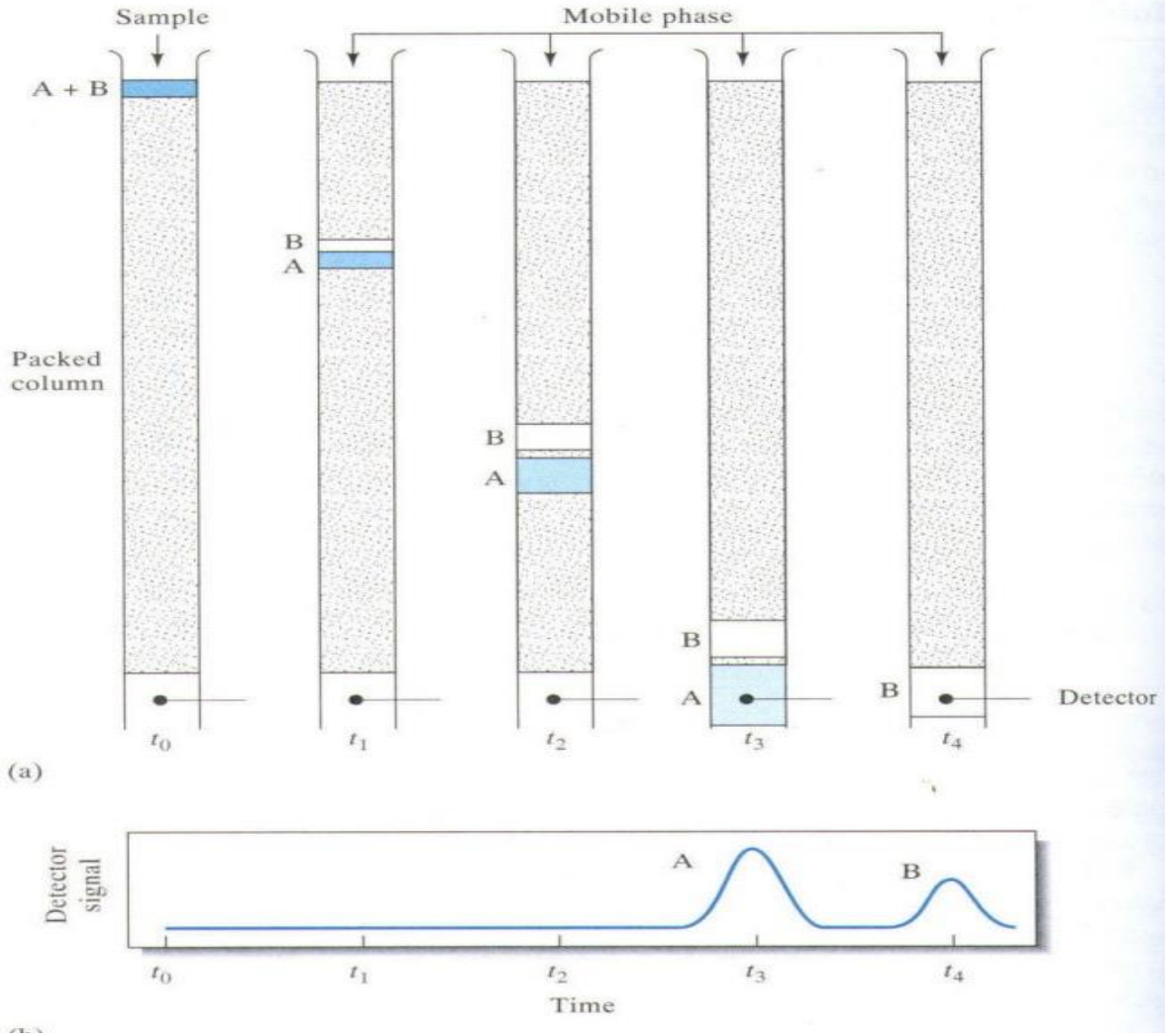
Gradient elution: Continuous change in the composition of the mobile phase over a period of time (*e.g.* polarity, pH or ionic strength).

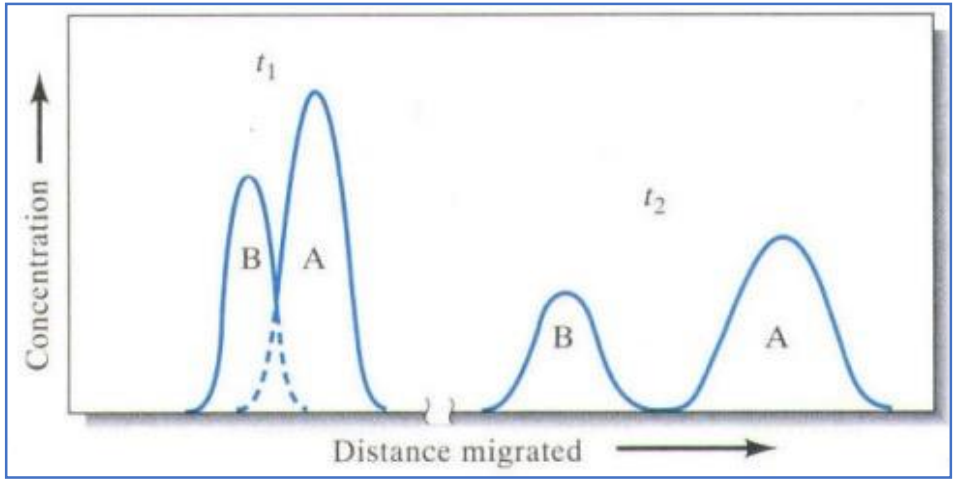
Usually mixture of solvents *e.g.* Ethyl acetate-hexane, Methanol-dichloromethane

Column Chromatography (CC): Detection

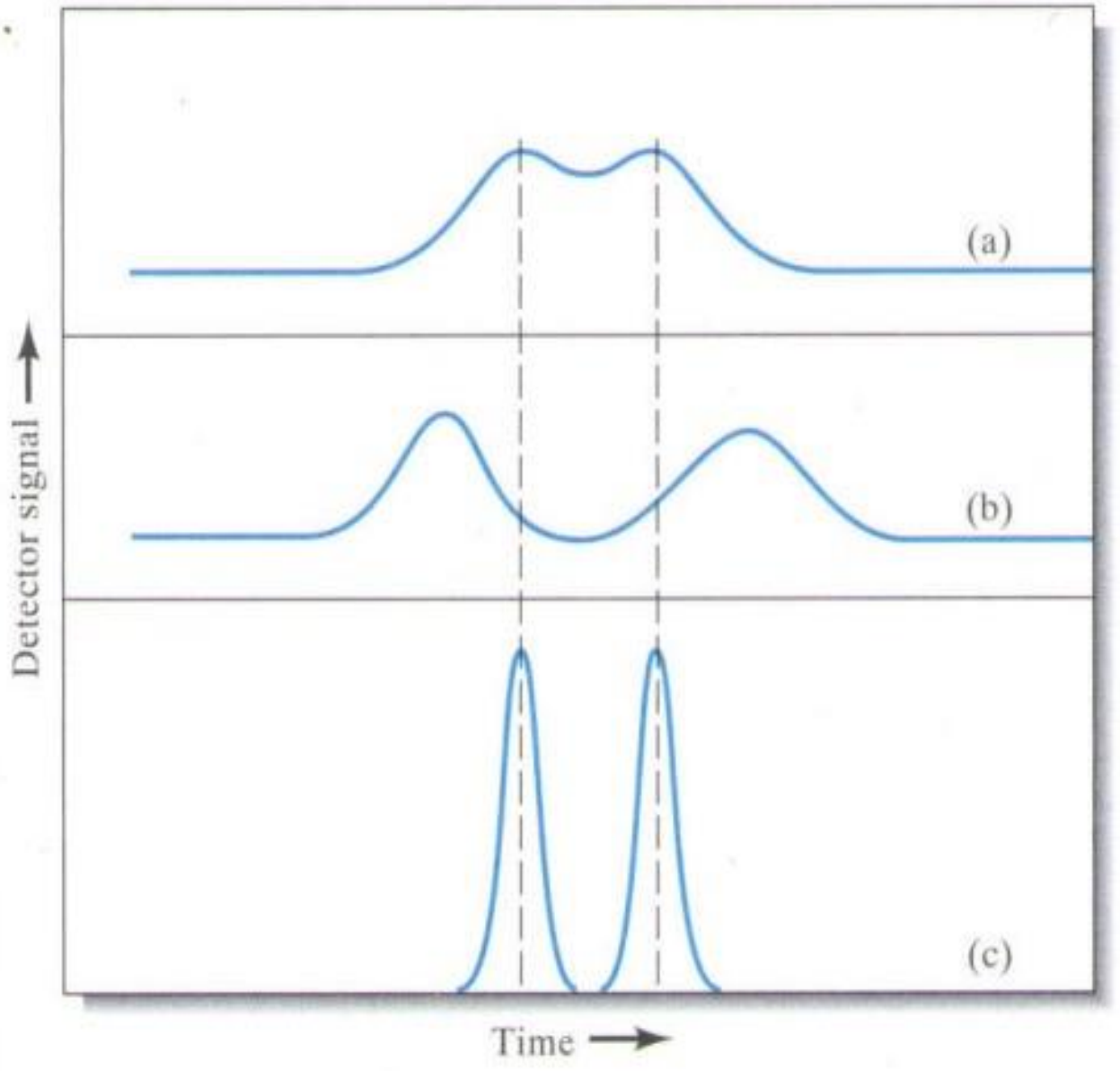
- On-column detection: For colored or fluorescent compounds directly after developing the chromatogram.
- Monitoring the eluted fractions with TLC or PC.
- Using special detectors: Connected to the column such as refractive index, UV detectors, etc.

Column Chromatography (CC): Detection





Column Chromatography (CC): Peak Resolution



Poor resolution

Good resolution

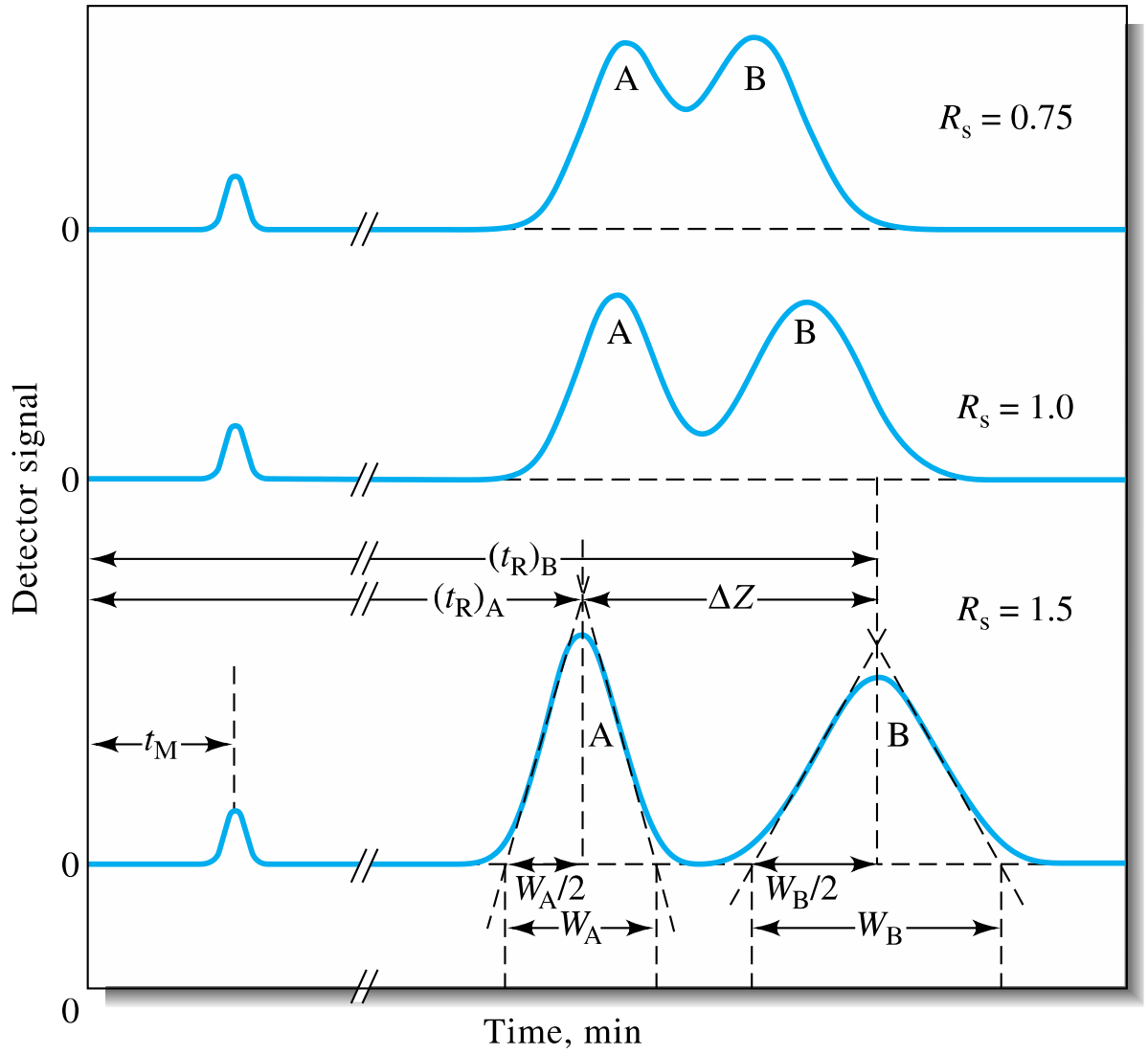
Column Resolution

The resolution (R_s) of the column tells us how far apart two bands are relative to their widths.

$$R_S = \frac{\Delta Z}{W_A/2 + W_B/2}$$

$$R_S = \frac{2\Delta Z}{W_A + W_B}$$

$$R_S = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$



Substances **A** and **B** have retention times of **10** and **20 min**, respectively, on a **25 cm** column. The peak widths at base for A and B are **1.4** and **1.6 min**, respectively. Calculate the column resolution

CC: Factors Affecting Column Efficiency

Particle size of solid stationary phase : Decrease of size improves separation

Column dimensions: Efficiency increases as ratio length / width increases

Uniformity of packing: Non uniform packing results in irregular movement of solutes through column and less uniform zone formation (i.e. band broading or tailing)

Column temperature: Increase in column temperature results in speed of elution but does not improve separation (tailing)

CC: Factors Affecting Column Efficiency

Eluting solvent: Solvents should be of low viscosity (to give efficient resolution) and high volatility (to get rapid recovery of the substances)

Solvent flow rate: Uniform and low flow rate gives better resolution

Continuity of flow: Discontinuous flow disturbs resolution

Condition of adsorbent: Deactivation of adsorbent decreases separation

Concentration of solutes: Substances of high concentration move slowly

Column Chromatography (CC): An Automated Machine

Monitor: To get the chromatogram

Column

Collected fractions

Column Chromatography (CC): Migration Rates of Solutes

All chromatographic separations are based on differences in the extent to which solutes are distributed between mobile and stationary phase

A (stationary) ⇌ A (mobile)

The equilibrium constant for this reaction is called as distribution constant.

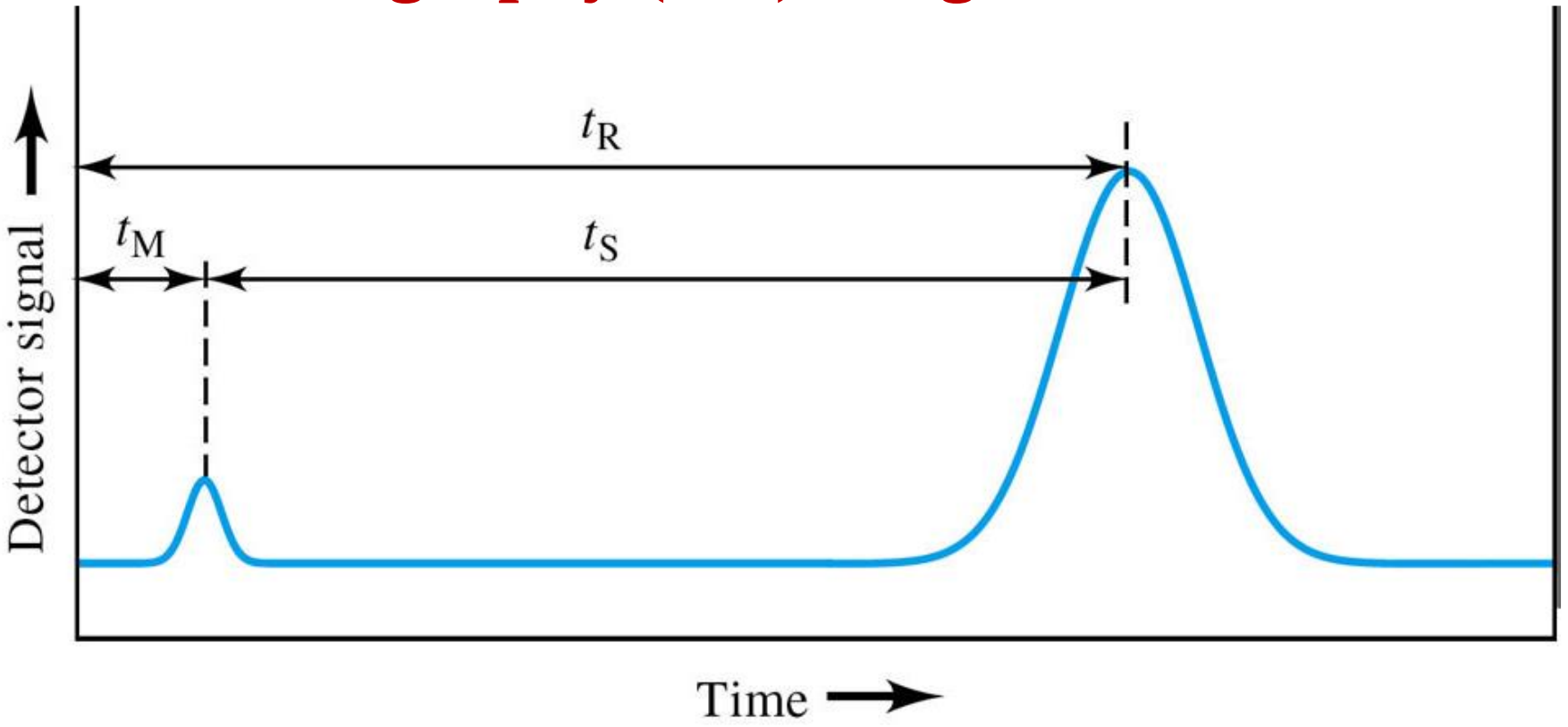
$$\mathbf{K_C = C_S/C_M}$$

K_C is equilibrium constant (distribution constant)
C_S = Concentration of solute in stationary phase
C_M = Concentrations of solute in mobile phase.

C_S is directly proportional to C_M

$$C_S = n_S/V_S, \quad C_M = n_M/V_M$$

Column Chromatography (CC): Migration Rates of Solutes



$$t_R = t_M + t_S,$$

t_S = time duration of the analyte retained in the stationary phase

Volumetric flow rate $F_C = V_R / t_R$

Column Chromatography (CC): Migration Rates of Solutes

- **Retention Volume (V_R)** - Volume of eluent needed to convey a solute band from the point of injection , through the column, to the detector

- **Retention time (t_R)** - Time needed for each component of the mixture after injection to reaches the detector

- **Dead or void time (t_M)** - Transit time of the un-retained solute to reach the detector, known as void time

Column Chromatography (CC): Velocities

Velocity = Distance/Time → Length of column/ Retention times

Velocity of Solute: $\bar{v} = \frac{L}{t_R}$ Velocity of Mobile Phase: $\mu = \frac{L}{t_M}$

Velocity of solute: $\bar{v} = \frac{L}{t_R} \times t_M/t_M$

$$\bar{v} = \mu \times t_M/t_R$$

$$\bar{v} = \mu \times \text{fraction of time solute spends in mobile phase}$$

$$\bar{v} = \mu \times \frac{\text{no. of moles of solute in mobile phase}}{\text{total no. of moles of solute}}$$

CC: Velocity, Distribution Constant and Retention Factor

$$\bar{v} = \mu \times \frac{c_M V_M}{c_M V_M + c_S V_S}$$

$$\bar{v} = \mu \times \frac{1}{1 + c_S V_S / c_M V_M}, K = \frac{c_S}{c_M}$$ Distribution Constant

$$\bar{v} = \mu \times \frac{1}{1 + K\, V_S / V_M} \qquad where \frac{K_A V_S}{V_M} = k_A$$ Retention factor for solute A

$$\bar{v} = \mu \times \frac{1}{1 + k_A}$$

$$\frac{L}{t_R} = \frac{L}{t_M} \times \frac{1}{1 + k_A}$$

$$k_A = \frac{t_R - t_M}{t_M} = \frac{t_S}{t_M}$$

$$\bar{v} = \frac{L}{t_R}$$

$$\mu = \frac{L}{t_M}$$

Ideally the retention factor for analytes in a sample between 1 and 5

CC: Selectivity factor/Separation factor (α)

In two component system; Can "A" be separated from "B"?

For example; B retained in the column more than A → **α >1**

$$\alpha = \frac{k_B}{k_A}$$

k_B and k_A are Retention factors

$$k_A = \frac{(t_R)_A - t_M}{t_M} \quad and \quad k_B = \frac{(t_R)_B - t_M}{t_M}$$

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$$

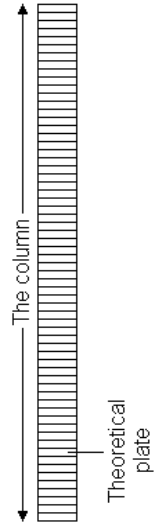
t_M , t_R Retention times.

Column Efficiency - Plate Theory

Assumption

The column is divided into a number of zones (theoretical plates)

Within each theoretical plates complete equilibration of analyte occurs between stationary and mobile phase



Greater separation occurs with:

Greater number of theoretical plates (N)

Height **E**quivalent to a **T**heoretical **P**late (**HETP**)

$$L = N \times H$$

$$H = L/N$$

Where, **L** - Length of the column

N - Number of plates

H - **H**eight **E**quivalent of **T**heoretical **P**lates

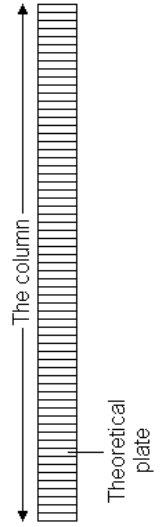
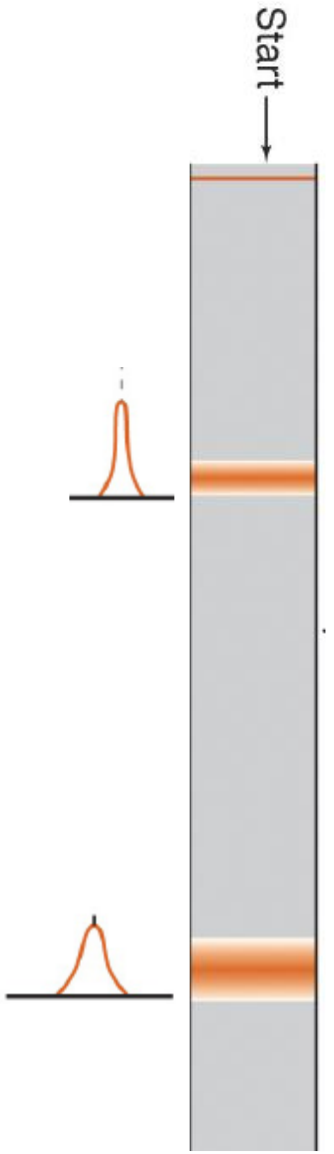


Plate Theory

Solute moving through a column spreads into a Gaussian shape with standard deviation σ, and the variance is σ^2.

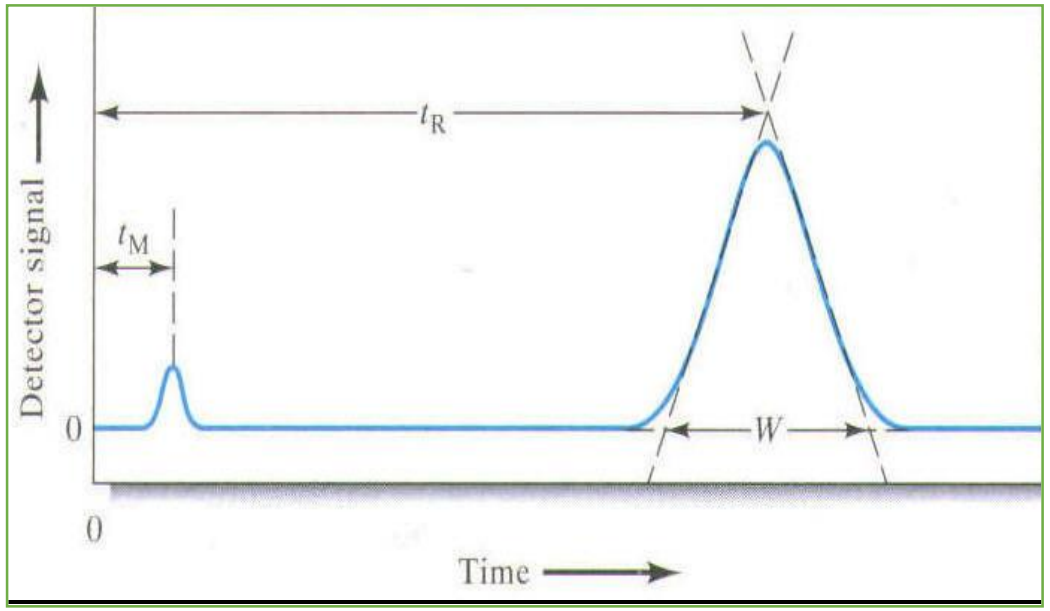
The variance per unit length of column is the measurement of column efficiency.

$$H = \frac{\sigma^2}{L}$$



Band spreading

The width of bands increases as their retention time (volume) increases



$$N = \text{L/H number of pates}$$

$$N = 16\left(\frac{t_R}{W}\right)^2$$

Common measures of breadth are:

The width **W** at the baseline between tangents drawn to the steepest parts of the peak (inflection points).

Q. Substances **A** and **B** have retention times of **13.01** and **14.31 min**, respectively, on a **25 cm** column. The peak widths at base for A and B are **1.45** and **1.61 min**, respectively.

Calculate (a) The column resolution, (b) The average number of plates in the column, (c) The average plate height

Ans: Given, $(t_R)_A = 13.01$ min; $(t_R)_B = 14.31$ min

$L = 25$ cm

$W_A = 1.45$ min; $W_B = 1.61$ min

(a) Resolution; $R_S = 2\,[(t_R)_B - (t_R)_A] / (W_A + W_B) = 0.85$

(b) Number of Plates $N_A = 16\,[(t_R)_A / W]^2 = 1288$

$N_B = 16\,[(t_R)_B / W]^2 = 1264$

Average number of plates; $N_{Average} = [N_A + N_B] / 2 = 1276$

(c) $L = H \times N$

$H = L / N_{average} = 25 / 1276 = 2.35 \times 10^{-2}$ cm

Q. Substances **A** and **B** have retention times of **16.40** and **17.63 min**, respectively, on a **30 cm** column. An unretained species passes through the column in **1.30 min**. The peak widths at base for A and B are **1.11** and **1.21 min**, respectively.

Calculate (a) The column resolution, (b) The average number of theoretical plates in the column, (c) The average plate height

Ans: Given, $(t_R)_A = 16.40$ min; $(t_R)_B = 17.63$ min; $t_M = 1.30$ min

$L = 30$ cm

$W_A = 1.11$ min; $W_B = 1.21$ min

(a) Resolution; $R_S = 2\,[(t_R)_B - (t_R)_A] / (W_A + W_B) = 1.06$

(b) Number of Plates $N_A = 16\,[(t_R)_A / W]^2 = 3493$

$N_B = 16\,[(t_R)_B / W]^2 = 3397$

Average number of plates; $N_{Average} = [N_A + N_B] / 2 = 3445$

(c) $L = H \times N$

$H = L / N_{average} = 30 / 3445 = 8.7 \times 10^{-3}$ cm

Q. Two components **A** and **B** are separated by elution with hexane from a column packed with silica-gel (water adsorbed on silica surface). Distribution coefficients ($K = C_{aq}/C_{org}$) of compounds A and B in water/hexane system are **5.99** and **6.16** respectively. The ratio V_S/V_M for the packing is **0.425**.

Calculate, (a) Retention factor for A and B, (b) Selectivity factor, (c) Length of the column if the number of theoretical plates and plate height of packing are 9.03×10^4 and 1.53×10^{-3} cm respectively.

Ans: Given, $K_A = 5.99$; $K_B = 6.16$; $V_S/V_M = 0.425$

(a) Retention factor; $k_A = K_A \times (V_S/V_M) = 2.546$

$k_B = K_B \times (V_S/V_M) = 2.618$

(b) Selectivity factor; $\alpha = k_B / k_A = 1.03$

(c) $L = HN = 9.03 \times 10^4 \times 1.53 \times 10^{-3} = 138.159$ cm

Q. The following data are for a liquid chromatographic column:
Length of packing: 24.7 cm, Flow rate: 0.313 mL/min, V_M: 1.37 mL, V_S: 0.164 mL

A chromatogram of a mixture of species A, B, C and D provided the following data:

	Retention Time, min	Width of Peak Base (W), min
Nonretained	3.1	—
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16
D	21.6	1.72

Calculate

a) The number of plates from each peak

b) Average plate height

c) Standard deviation

Ans:

- $N_A = 16\,[(t_R)_A / W]^2$
- $H = L / N$
- $H = \sigma^2/L$

Q. The following data are for a liquid chromatographic column:
Length of packing: 24.7 cm, Flow rate: 0.313 mL/min, V_M: 1.37 mL, V_S: 0.164 mL

A chromatogram of a mixture of species A, B, C and D provided the following data:

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Nonretained	3.1	—
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16
D	21.6	1.72

From the above data Calculate

(a) Retention factor

(b) The distribution constant.

Ans. (a) Retention factor; $k_A = [(t_R)_A - t_M]/t_M = t_S/t_M$

Similarly, k_B, k_C, k_D can be calculated

(b) Distribution constant; $K_A = k_A (V_M/V_S)$

Similarly, K_B, K_C, K_D can be calculated

Q. The following data were obtained by gas-liquid chromatography on a 40-cm packed column:

Compound	t_R, min	W, min
Air	1.9	—
Methylcyclohexane	10.0	0.76
Methylcyclohexene	10.9	0.82
Toluene	13.4	1.06

Calculate

(a) an average number of plates from the data.

(b) the standard deviation for the average in (a).

(c) an average plate height for the column.

Ans.

- $N_A = 16\,[(t_R)_A / W]^2$
- $L = H \times N$
- $H = \sigma^2/L$

Q. The following data were obtained by gas-liquid chromatography on a 40-cm packed column:

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Air	1.9	—
Methylcyclohexane	10.0	0.76
Methylcyclohexene	10.9	0.82
Toluene	13.4	1.06

Calculate

(a) an average number of plates from the data.

(b) the standard deviation for the average in (a).

(c) an average plate height for the column.

From the above data; Calculate the resolution for

(a) Methylcyclohexene and methylcyclohexane

(b) Methylcyclohexene and toluene

(c) Methylcyclohexane and toluene

Ans. Resolution;

$$R_S = \frac{\Delta Z}{W_A/2 + W_B/2}$$

$$R_S = \frac{2\Delta Z}{W_A + W_B}$$

$$R_S = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$$

9. If V_S and V_M for the column in the problem 7 are **19.6** and **62.6 mL,** respectively, and a non retained air peak appears after **1.9 min,** Calculate

(a) The retention factor for each compound

(b) The distribution constant for each compound

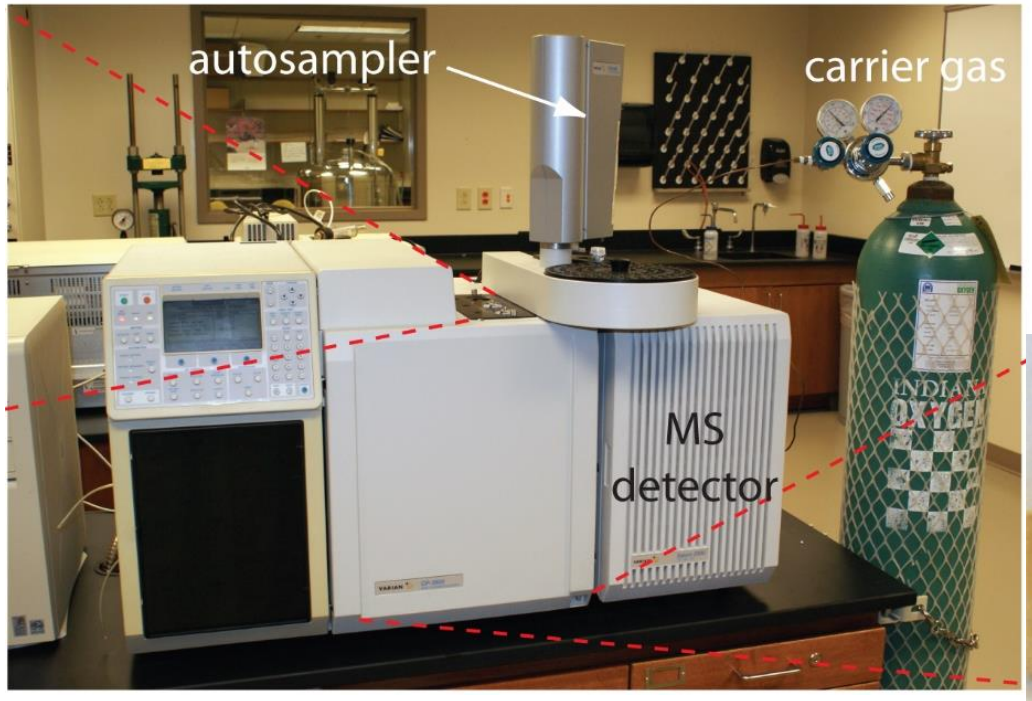
(c) The selectivity factor for methylcyclohexane and methylcyclohexene

Ans. (a) Retention factor, $k_A = \frac{t_R - t_M}{t_M} = \frac{t_S}{t_M}$

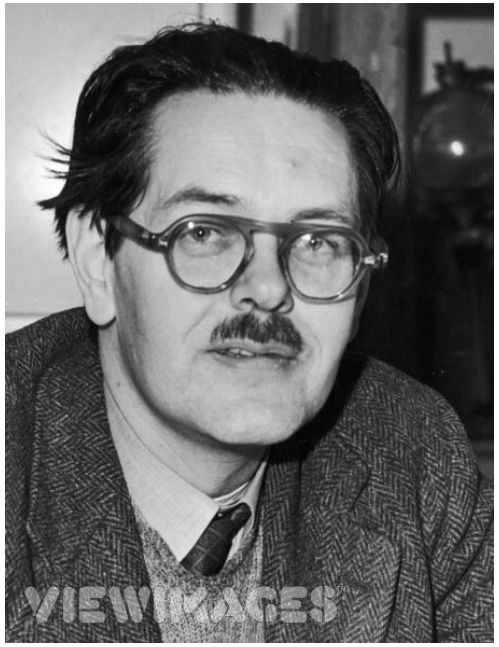
(b) Distribution constant, $k_A = K_A \times (V_S/V_M)$

(c) Selectivity factor, $a = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$

Gas Chromatography



capillary
column



John Porter Martin developed first gas-liquid chromatograph in 1950

Gas Chromatography

Separation of gaseous and volatile substances

The mobile phase only transport the analyte

The mobile phase do not interact with the analyte

Inert gas (e.g. He, N_2, Argon etc) are used as mobile phase

Type of GC

GSC (Gas-solid chromatography)

GLC (Gas-liquid chromatography

GSC (Gas-solid chromatography)

- ❖ **GSC** principle is **ADSORPTION**
- ✓ not used because of limited no. of Stationary phase
- ✓ useful for separation of low molecular mass gaseous like air, H_2S, CO, CO_2, CS_2 and rare gases

GLC (Gas-liquid chromatography

- ❖ **GLC** principle is **PARTITION**

Criteria for compounds to be analyzed by G.C

- ✓ Volatility
- ✓ Thermostability

Advantages of Gas Chromatography

Strong separation power

Complex mixture can be resolved into constituents

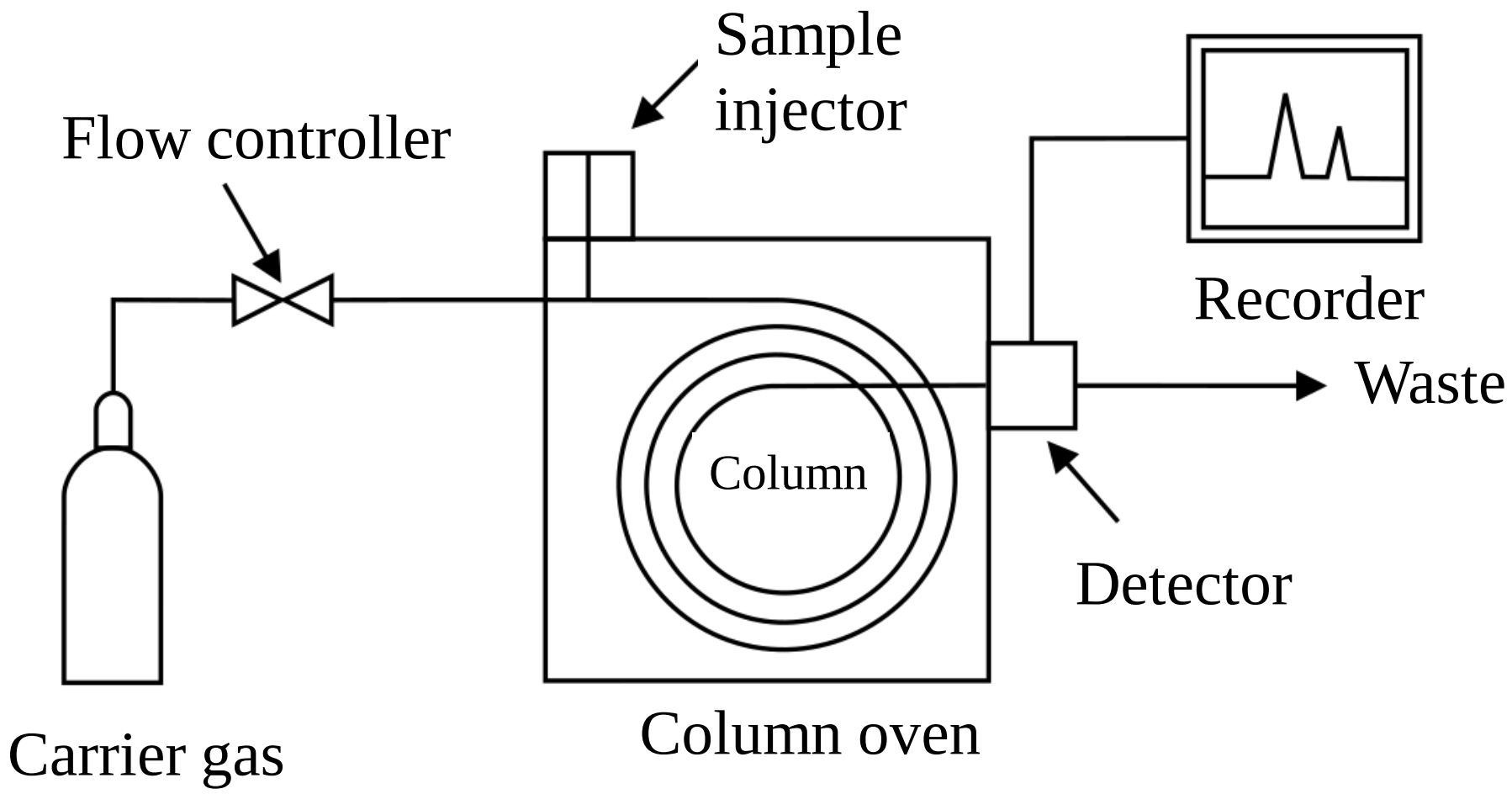
Good precision and accuracy

Separation completed in a short time

Low cost of instrument with generally long lifetime

Suitable for routine analysis

Schematic diagram of a gas chromatograph



How a GC machine works

✓Smple is injected onto the machine.

✓ Sample converted into vapor and mixed with gaseous mobile phase

✓ The sample moves through the column along with the flow of inert gas and separation occurs

✓Components are separated according to their Partition coefficient

✓Components reaches the detector and recorded as a sequence of peaks as they leave the column

Components of Gas chromatography

Carrier gas

✓ He (common), N_2, H_2 and Argon

Sample injection port

✓ micro syringe

Columns

✓ 2-50 m coiled stainless steel/glass/Teflon

Detectors

✓Flame ionization (FID)
✓Thermal conductivity (TCD)
✓Electron capture (ECD)
✓Flame photometric (FPD)
✓Photo-ionization (PID)

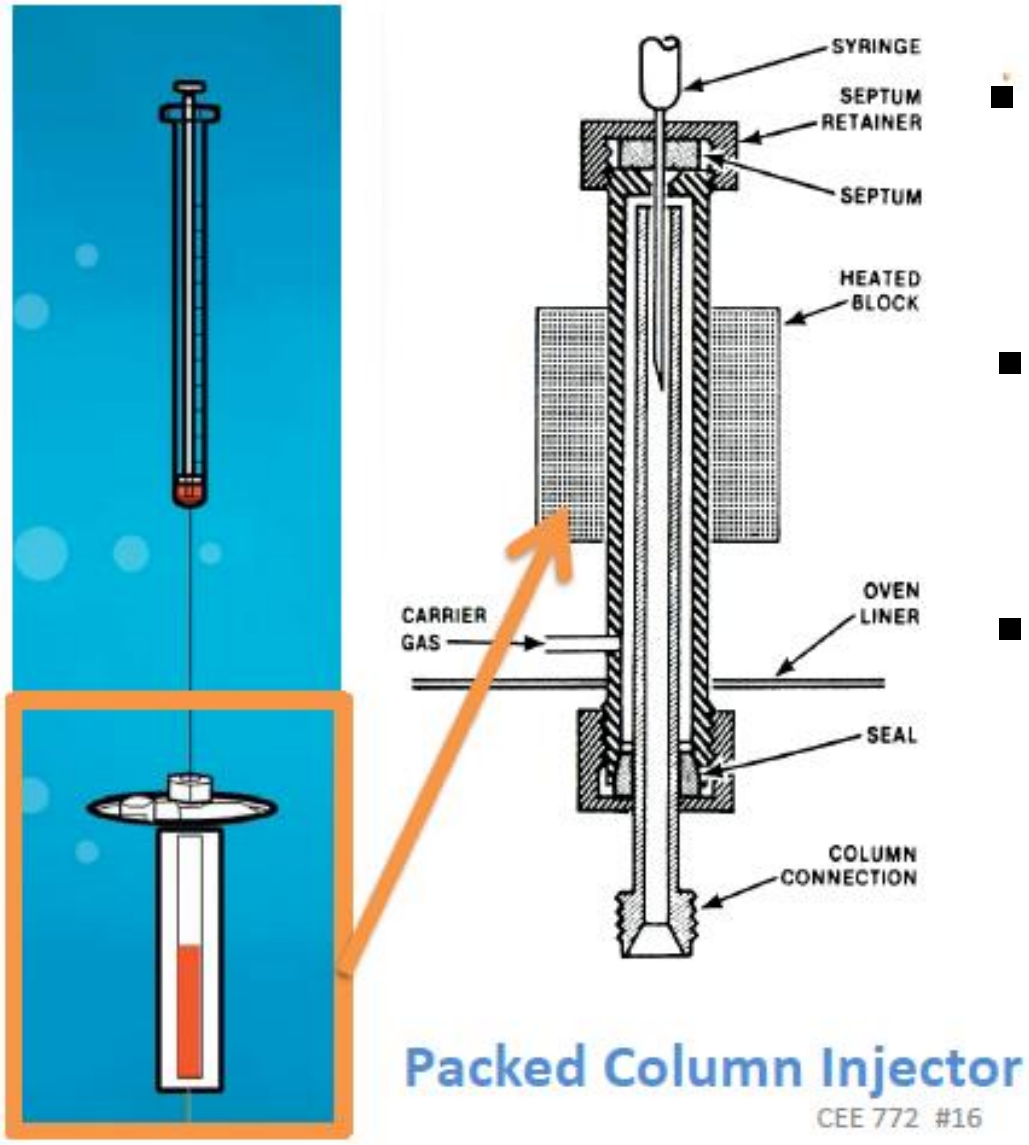
Carrier gas

- ✓ Must be chemically inert
- ✓Commonly used gases include nitrogen, helium, argon, and carbon dioxide
- ✓The choice of carrier gas is often dependant upon the type of detector which is used
- ✓The carrier gas system also contains a molecular sieve to remove water and other impurities
- ✓Pressure, P = inlet 10-50 psi (pound per inches^2)

  Flow, F = 25-150 mL/min (packed column)

  F = 1-25 mL/min (open tubular column)

Sample injection- Direct Injection



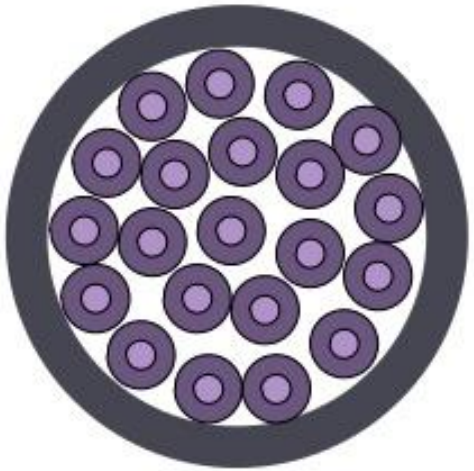
- Direct injection into heated port (>T oven) using micro syringe
- Slow injection or oversized samples cause band spreading and poor resolution
- The sample port is usually at about 50 ^{o}C

Column types

Packed column:

contain a finely divided, inert, solid support material coated with liquid stationary phase.

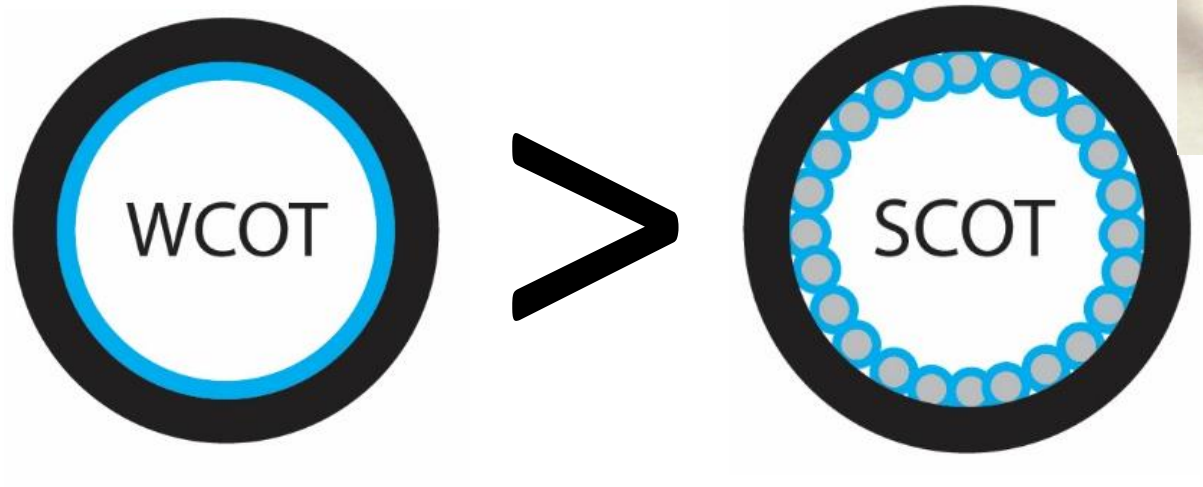
Most packed columns are 1.5 – 10 m in length
and
have an internal diameter of 2 – 4 mm.



Column types

Capillary columns are of two types

- ✓ Wall coated open tubular (WCOT)
- ✓ Support coated open tubular (SCOT)



➢ The stationary phase (liquid) coated with a thin layer of *0.05 to 1 μm.*

Gas Chromatography - Detectors

Detectors can be grouped into *concentration dependent detectors* and *mass flow dependent detectors*

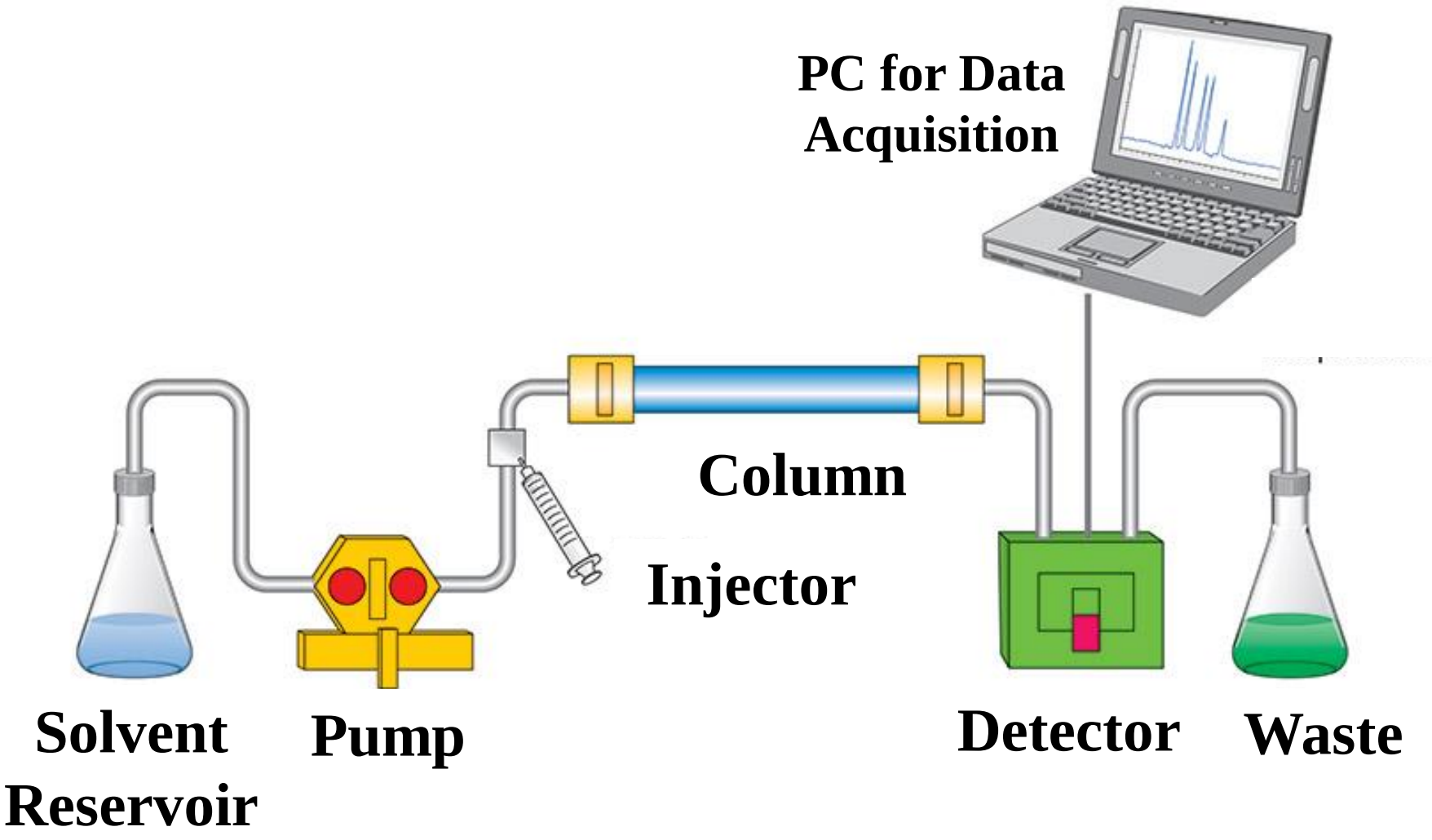
Concentration dependent detector

- ✓ signal is related to the concentration of solute in the detector
- ✓ does not usually destroy the sample

Mass flow dependent detectors

- ✓signal is related to the rate at which solute molecules enter the detector
- ✓usually destroy the sample

High performance liquid chromatography (HPLC)



Components of HPLC

Solvent Reservoir :

- The mobile phase, or solvent, in HPLC is usually a mixture of polar and non-polar liquid.

Pump :

- pump extracts mobile phase from the reservoir
- forces it through the system's column and detector

Sample Injector:

- single injection or
- automated injection system

Columns :

- usually between 50 and 300 mm long
- have an internal diameter of between 2 and 5 mm.
- commonly filled with a stationary phase with a particle size of 3–10 μm.

Detectors:

- UV or fluorescence spectroscopy
- Mass-spectrometric

HPLC : Basic principle

Separation of a sample into its constituent parts because of the difference in the relative affinities of different molecules for the mobile phase and the stationary phase used in the separation.

- ✓ HPLC is basically a highly improved form of column liquid chromatography
- ✓ Instead of a solvent being allowed to drip through a column under gravity, it is forced through under high pressures of up to 400 atmospheres.
- ✓ It is much faster.

Types of HPLC

Normal Phase HPLC:

✓ NP-HPLC uses polar stationary phase and non-polar mobile phase

✓ the stationary phase is usually silica

✓ mobile phases are hexane, methylene chloride, chloroform, diethyl ether, and mixtures of these

✓ polar samples are retained on the polar surface of column packing longer than less polar materials.

Types of HPLC

Reverse Phase HPLC:

✓The stationary phase is non-polar (hydrophobic) in nature

✓ mobile phase is a polar liquid, such as mixtures of water and methanol or acetonitrile.

✓works on the principle of hydrophobic interactions hence the more nonpolar the material will be retained longer

Applications of HPLC

Pharmaceutical Applications

1. Pharmaceutical quality control.

Environmental Applications

1. Detection of phenolic compounds in drinking water.
2. Bio-monitoring of pollutants.

Applications in Forensics

1. Determination and quantification of drugs in blood, urine etc.

Food Safety

1. Measurement of Quality of soft drinks and water.
2. Sugar analysis in fruit juices.