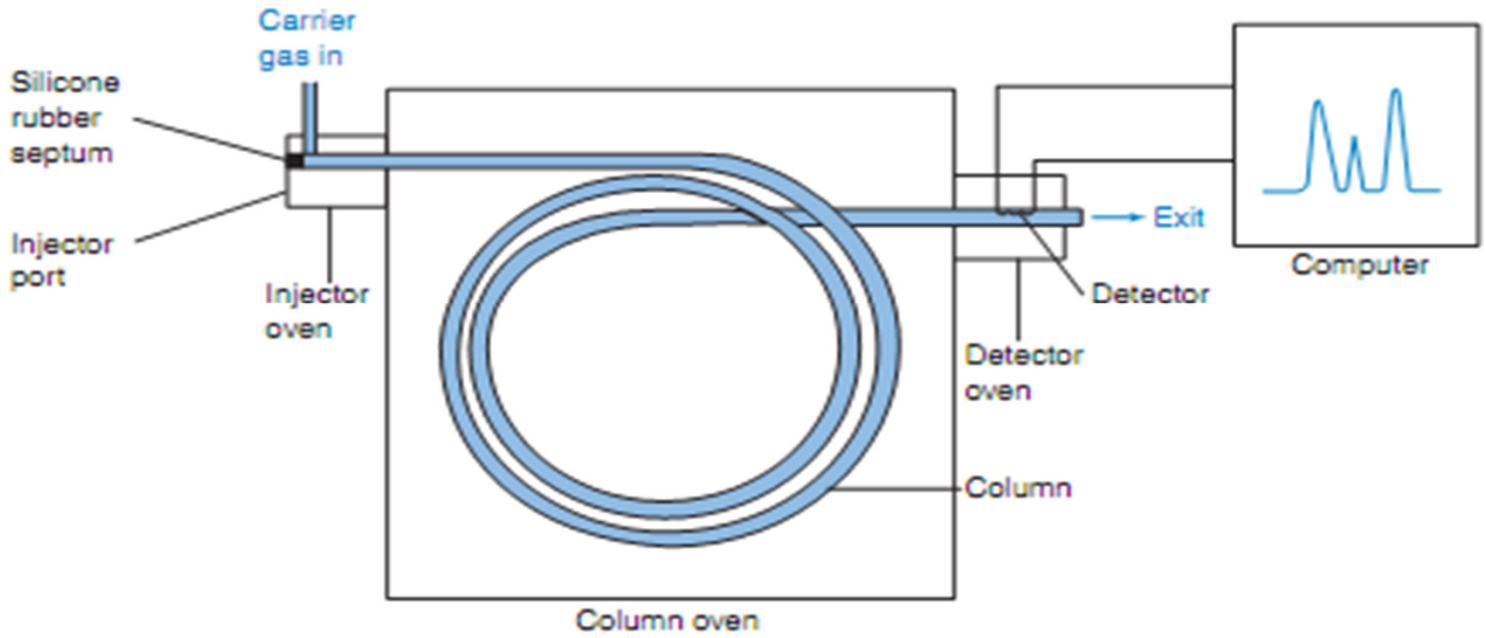
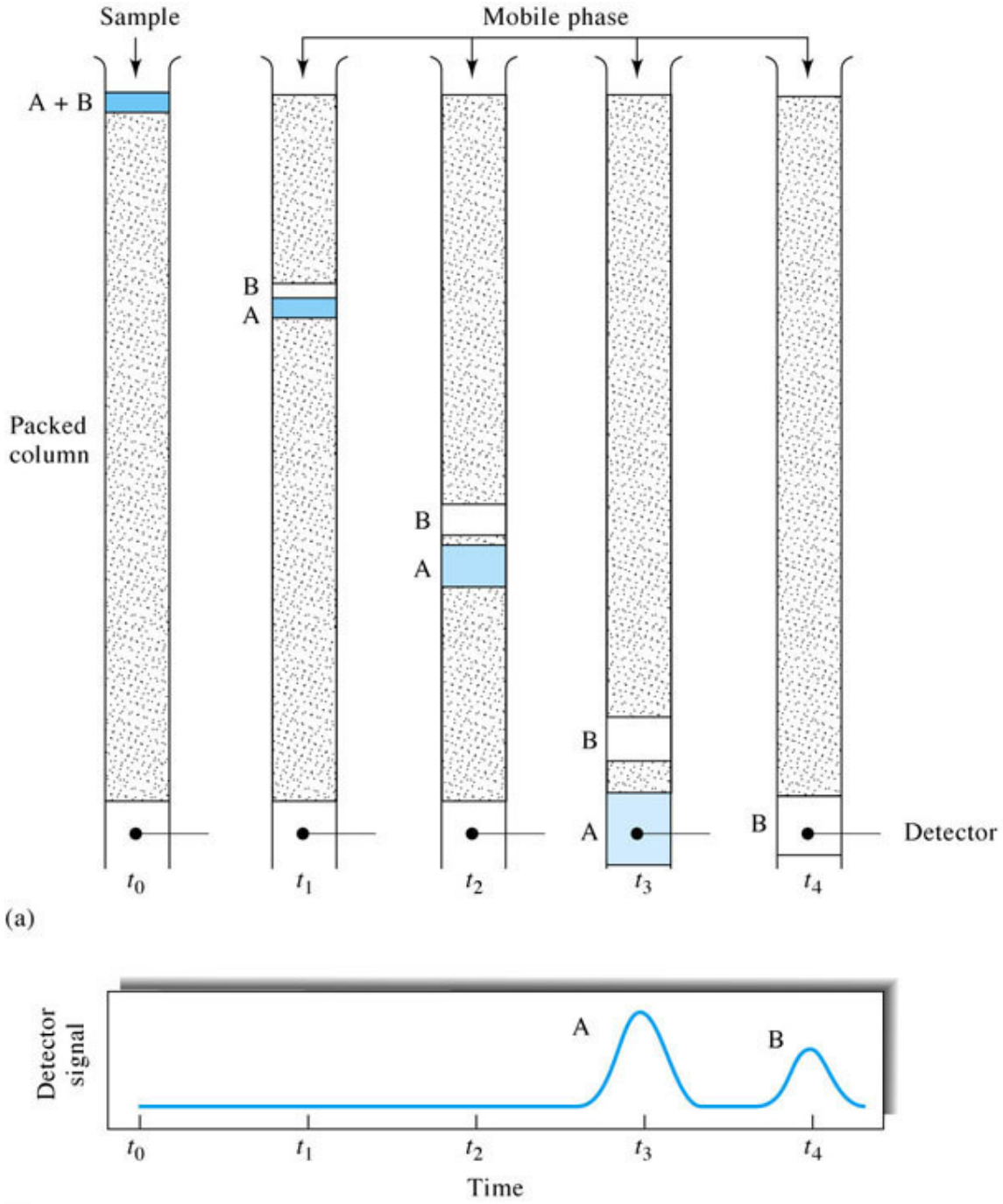
GAS CHROMATOGRAPHY

* Dr. FOZIA BATOOL



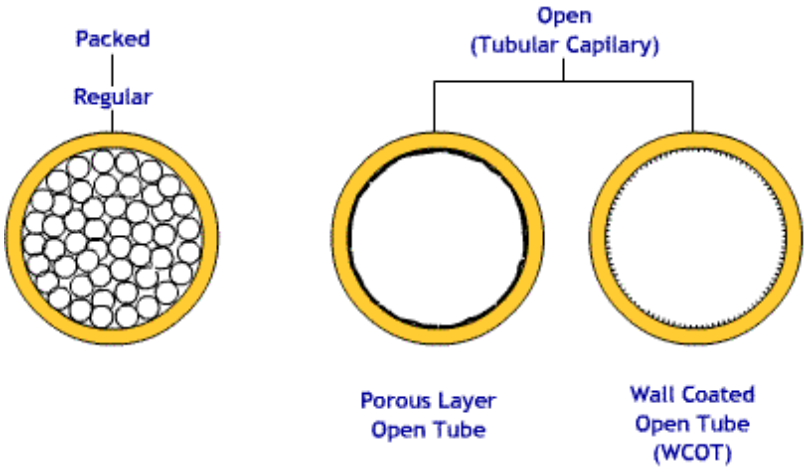


The analytes interacting most strongly with the stationary phase will take longer to pass through the system than those with weaker interactions.

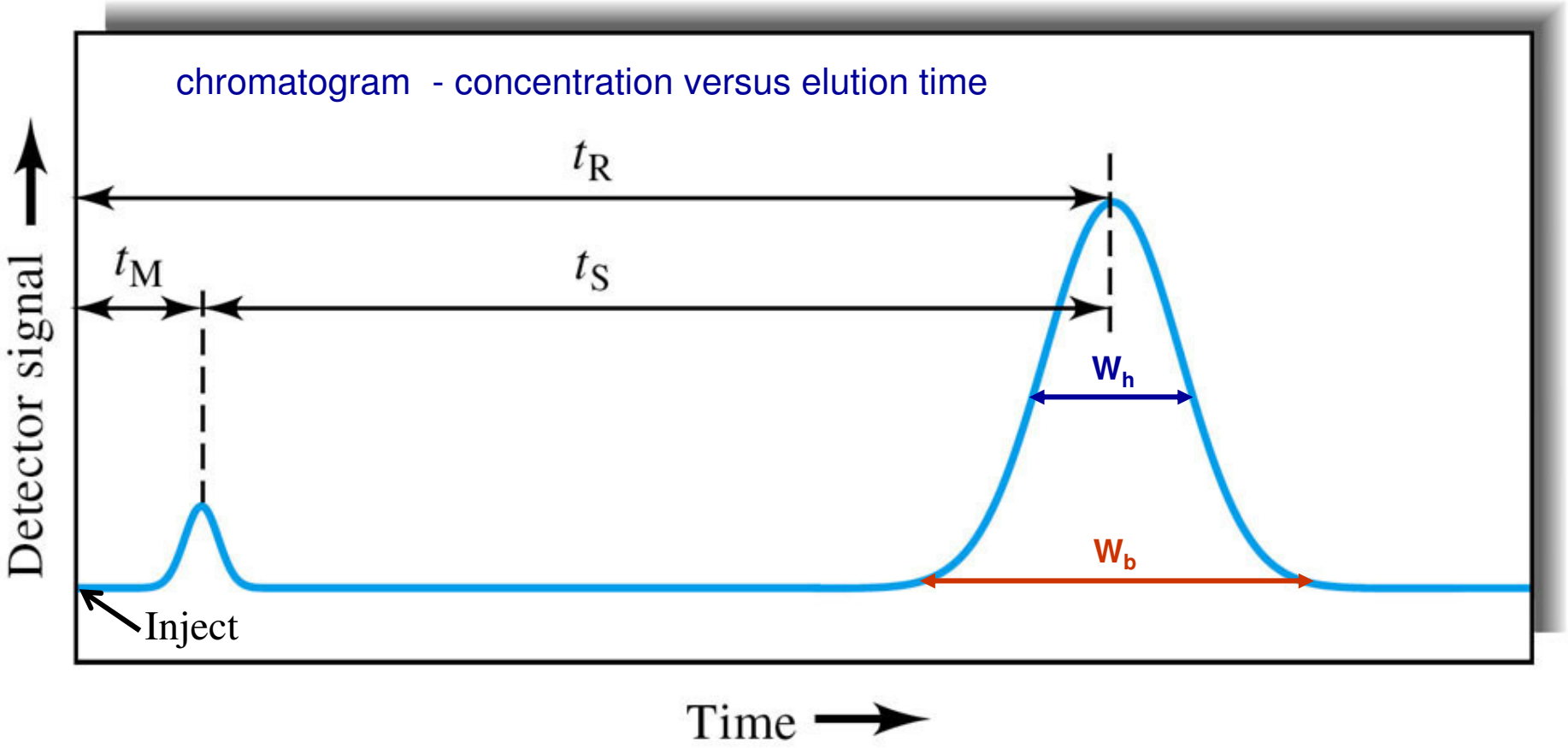
These interactions are usually chemical in nature, but in some cases physical interactions can also be used.

3.) Chromatographic techniques may also be classified based on the type of support material used in the system:

Packed bed (column) chromatography
Open tubular (capillary) chromatography
Open bed (planar) chromatography



1.) Typical response obtained by chromatography (i.e., a chromatogram):



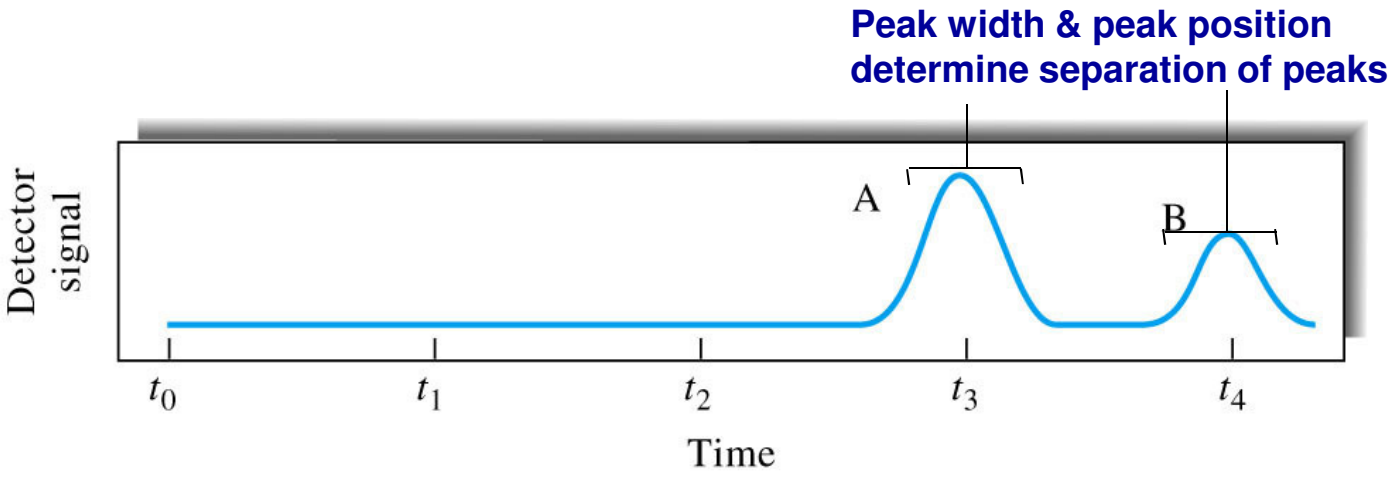
Where:

t_R = retention time
t_M = void time
W_b = baseline width of the peak in time units
W_h = half-height width of the peak in time units

Note: The separation of solutes in chromatography depends on two factors:

(a) a difference in the retention of solutes (i.e., a difference in their time or volume of elution

(b) a sufficiently narrow width of the solute peaks (i.e, good efficiency for the separation system)



A similar plot can be made in terms of elution volume instead of elution time. If volumes are used, the volume of the mobile phase that it takes to elute a peak off of the column is referred to as the *retention volume* (V_R) and the amount of mobile phase that it takes to elute a non-retained component is referred to as the *void volume* (V_M).

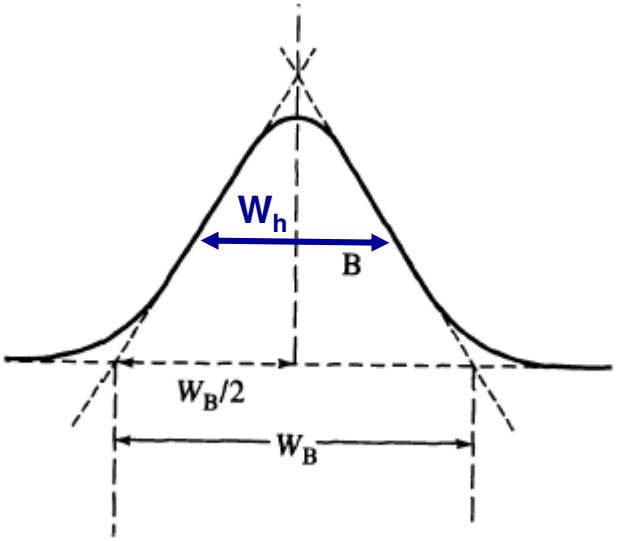
3.) Efficiency:

Efficiency is related experimentally to a solute's peak width.

- an efficient system will produce narrow peaks
- narrow peaks → smaller difference in interactions in order to separate two solutes

Efficiency is related theoretically to the various kinetic processes that are involved in solute retention and transport in the column

- determine the width or standard deviation (σ) of peaks



Dependent on the amount of time that a solute spends in the column (k' or t_R)

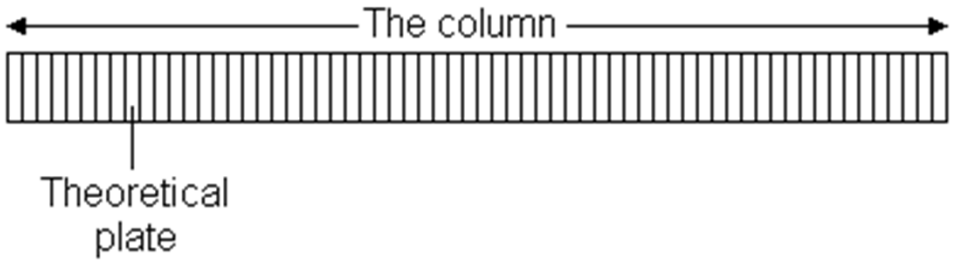
Number of theoretical plates (N): compare efficiencies of a system for solutes that have different retention times

or for a Gaussian shaped peak

$$N = 16\,(t_R/W_b)^2$$

The larger the value of N is for a column, the better the column will be able to separate two compounds.

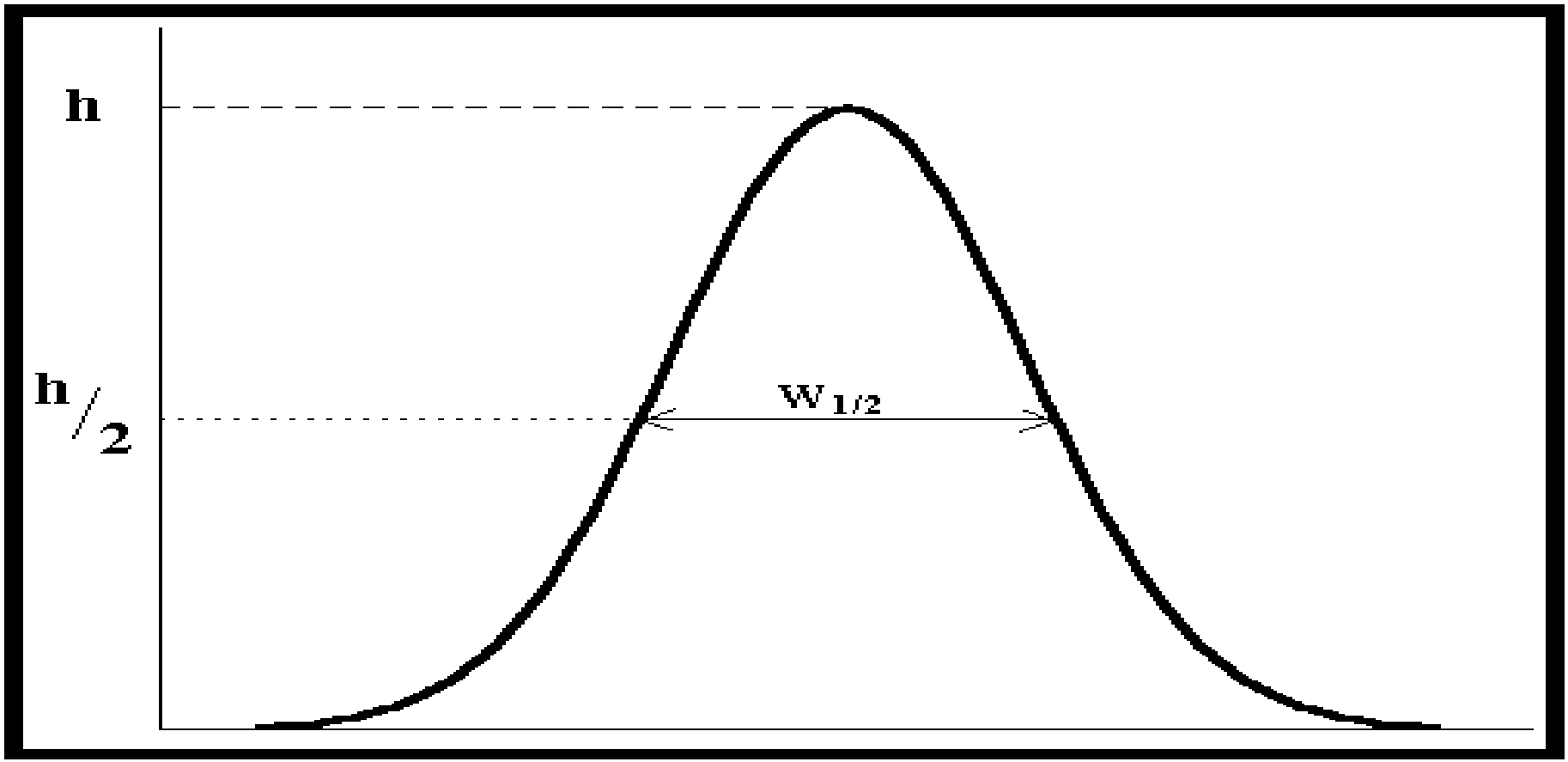
- the better the ability to resolve solutes that have small differences in retention
- N is independent of solute retention
- N is dependent on the length of the column



Number of Theoretical Plates

* H = L/N
* H= Plate height
* L= Length of column
* N= Number of theoretical plates
* N = L/H

GAUSSION SHAPE PEEK



VAN DEEMTER EQUATION

Van Deemter Equation

- $H = A + B/v + Cv$

  $H = L/N$

  H: Height equivalent of theoretical plates

  L: Column length

  N: Number of theoretical plates

Van Deemter Equation

$$H = A + \frac{B}{u} + Cu$$

- H = height of a theoretical plate
- u = average linear velocity of the mobile phase
- *A* = eddy diffusion term
- *B* = longitudinal or ordinary diffusion term
- *C* = nonequilibrium or resistance to mass transfer term

VAN DEEMTER EQUATION

* A Term = Multiple path term
* B Term = Longitudinal Diffusion
* C Term = Mass Transfer term

Plate height or height equivalent of a theoretical plate (H or HETP): compare efficiencies of columns with different lengths:

$$H = L/N$$

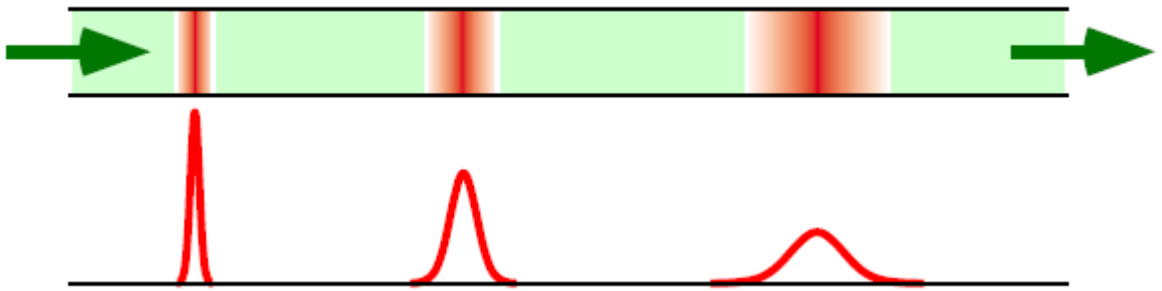
where: L = column length
N = number of theoretical plates for the column

Note: H simply gives the length of the column that corresponds to one theoretical plate

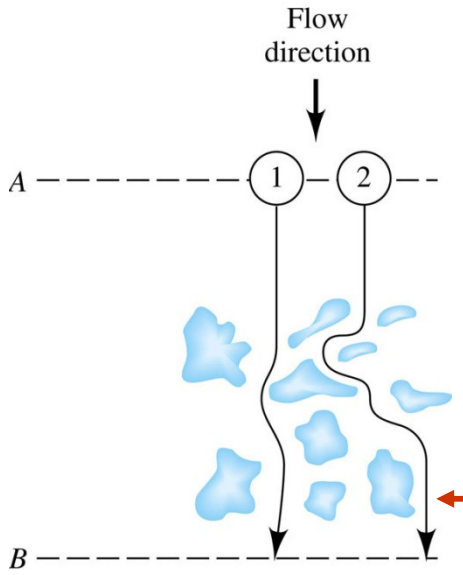
H can be also used to relate various chromatographic parameters (e.g., flow rate, particle size, etc.) to the kinetic processes that give rise to peak broadening:

Why Do Bands Spread?

a. Eddy diffusion
b. Mobile phase mass transfer
c. Stagnant mobile phase mass transfer
d. Stationary phase mass transfer
e. Longitudinal diffusion

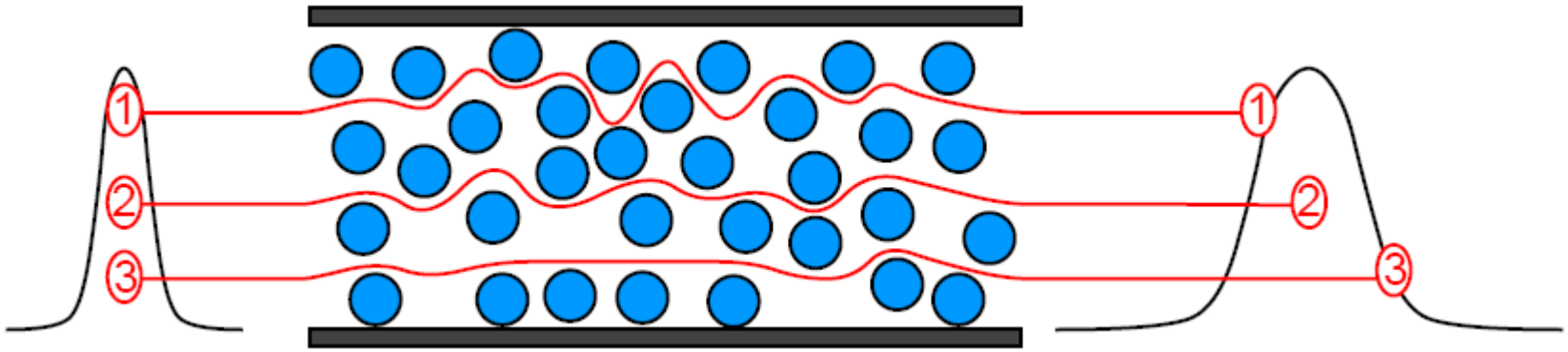


a.) *Eddy diffusion* – a process that leads to peak (band) broadening due to the presence of multiple flow paths through a packed column.

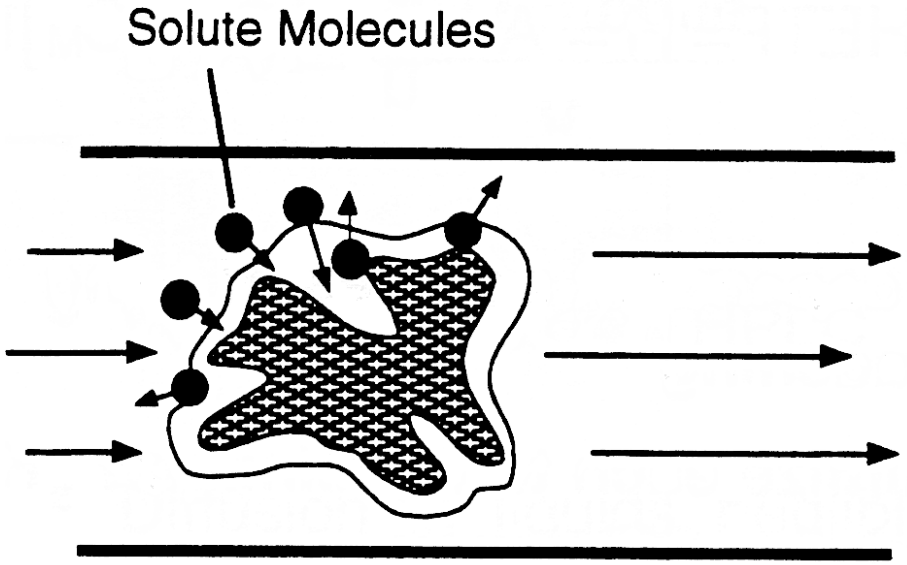


As solute molecules travel through the column, some arrive at the end sooner then others simply due to the different path traveled around the support particles in the column that result in different travel distances.

Longer path arrives at end of column after (1).



CTerm= Mass transfer Term – band-broadening due to the movement of solute between the stagnant phase and the stationary phase.

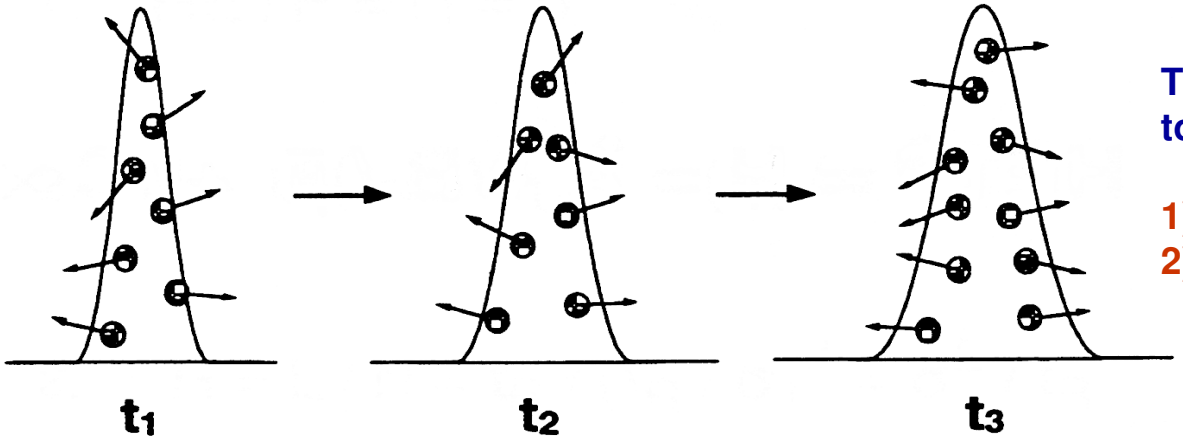


Since different solute molecules spend different lengths of time in the stationary phase, they also spend different amounts of time on the column, giving rise to band-broadening.

The degree of band-broadening due to stationary phase mass transfer depends on:

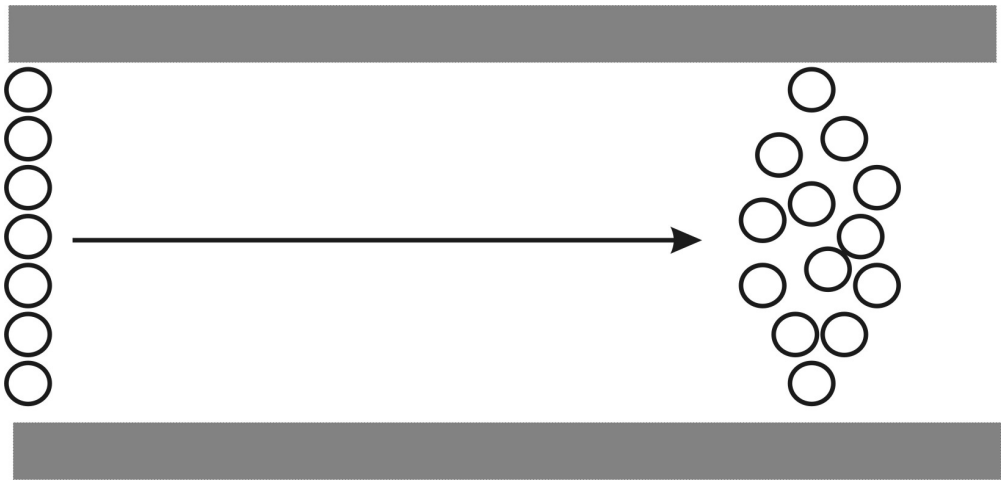
1) the retention and diffusion of the solute
2) the flow-rate of the solute through the column
3) the kinetics of interaction between the solute and the stationary phase

B Term=Longitudinal diffusion – band-broadening due to the diffusion of the solute along the length of the column in the flowing mobile phase.

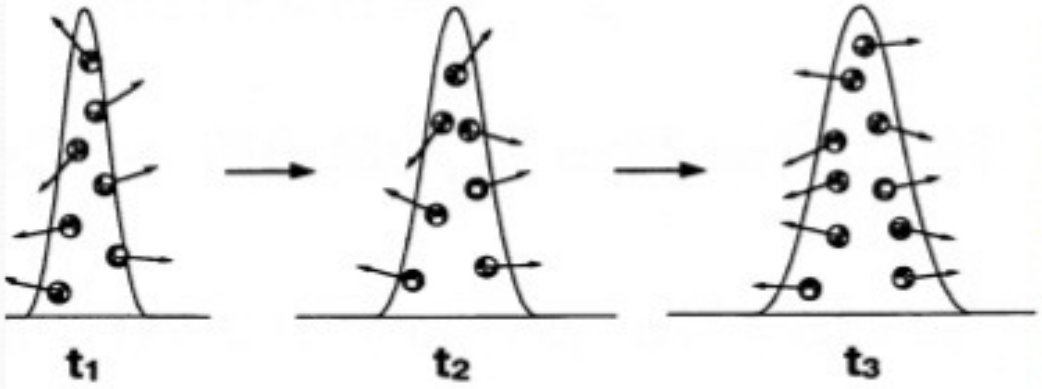


The degree of band-broadening due to longitudinal diffusion depends on:

1) the diffusion of the solute
2) the flow-rate of the solute through the column

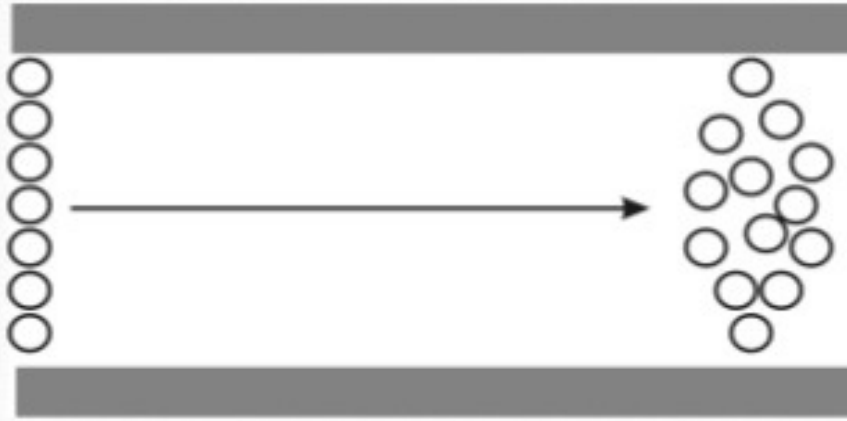


c.) Longitudinal diffusion: band-broadening due to the diffusion of the solute along the length of the column in the flowing mobile phase.



The degree of band-broadening due to longitudinal diffusion depends on:

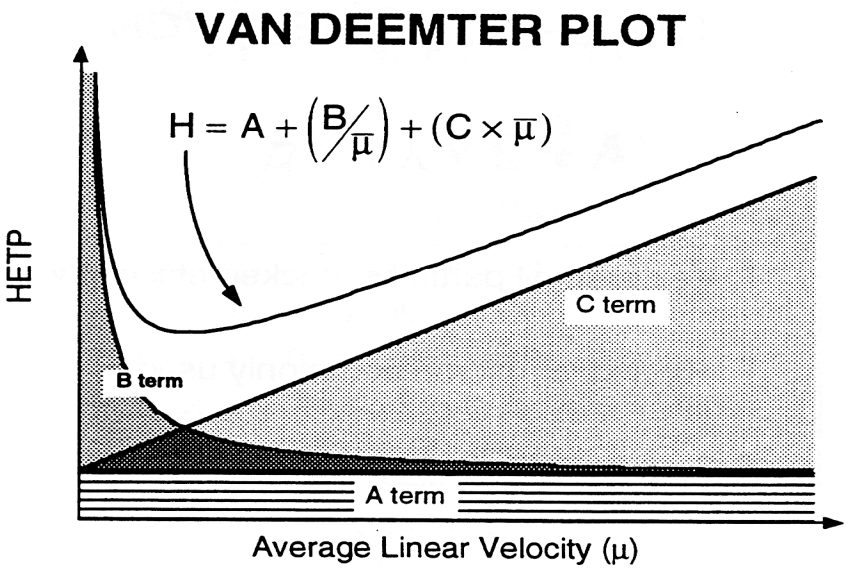
1) the diffusion of the solute
2) the flow-rate of the solute through the column



A solute in the center of the channel moves more quickly than solute at the edges, it will tend to reach the end of the channel first leading to band-broadening

Van Deemter equation: relates flow-rate or linear velocity to H:

$$H = A + B/\mu + C\mu$$



where:

μ = linear velocity (flow-rate x V_m/L)
H = total plate height of the column
A = constant representing eddy diffusion & mobile phase mass transfer
B = constant representing longitudinal diffusion
C = constant representing stagnant mobile phase & stationary phase mass transfer

One use of plate height (H) is to relate these kinetic process to band broadening to a parameter of the chromatographic system (e.g., flow-rate).

This relationship is used to predict what the resulting effect would be of varying this parameter on the overall efficiency of the chromatographic system.

Number of theoretical plates(N) $(N) = 5.54\ (t_R/W_h)^2$ *peak width (W_h)*

$$H = L/N$$

Numerical Problems

5. The following data was obtained by gas chromatography on a 65-cm packed column:

Compound	t_R (min.)	W (min)
air	1.61	-
hexane	2.46	0.23
heptane	3.30	0.31
octane	4.62	0.43

A. Calculate the **average** number of plates and plate height for the column.
B. Calculate the resolution between hexane and heptane.
C. Calculate the retention time for octane on a 100-cm column packed with the same stationary phase and separated under the same conditions (flow rate, temperature, etc.).

Solution

* $N=L/H$
* $H = 16tr^2/W_{av}$

Tr= retention time

Wav = average peak width

Numerical Problems

Your colleague has collected data from injecting a mixture of compounds into a chromatography column and has asked for your help with the analysis. The column length is 25.7 cm.

A chromatogram from a mixture of A, B, C, and D produced the following data:

	Retention Time (min)	Width of peak base
Nonretained	3.1	-
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16

a. Please calculate the number of theoretical plates for each peak.
b. Use the average number of theoretical plates to calculate the plate height, H, for the column.
c. Please calculate the retention factor for each peak.
d. Calculate the resolution between species B and C.
e. Calculate the selectivity factor for species B and C.
f. Calculate the length of column necessary to give a resolution of 1.5.

Numerical Problems

23-50. Calculate the number of theoretical plates needed to achieve a resolution of 2.0 if:

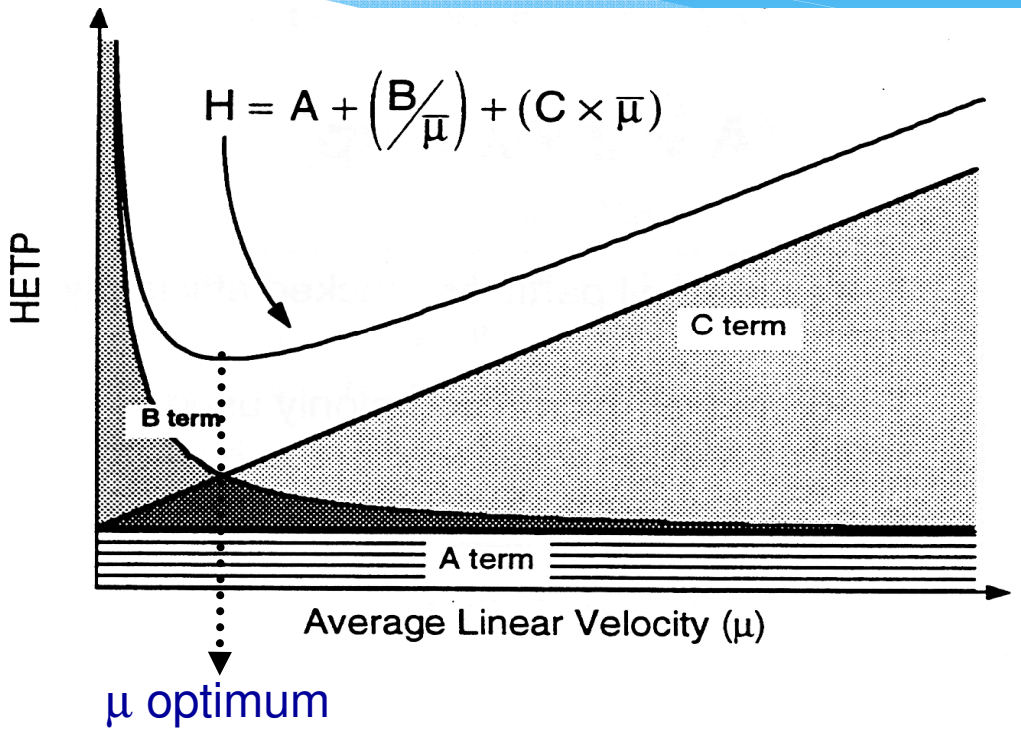
(a) $\alpha = 1.05$ and $k_2 = 5.00$.

(b) $\alpha = 1.10$ and $k_2 = 5.00$.

(c) $\alpha = 1.05$ and $k_2 = 10.00$.

(d) How can you increase N, α, and k_2 in a chromatography method? In this problem, which has a larger effect on resolution, α or k_2?

Plot of van Deemter equation shows how H changes with the linear velocity (flow-rate) of the mobile phase



Optimum linear velocity (μ_{opt}) - where H has a minimum value and the point of maximum column efficiency:

$$\mu_{opt} = \sqrt{B/C}$$

μ_{opt} is easy to achieve for gas chromatography, but is usually too small for liquid chromatography requiring flow-rates higher than optimal to separate compounds

Example 12: Given the following data and a 24.7 cm column

Compound	Retention Time (min)	Peak Width(W_B, 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 the resolution between species C and D. What column length is required for a resolution of 1.5?

TYPES OF COLUMN

* Packed Column
* Open Tubular column

PACKED COLUMN

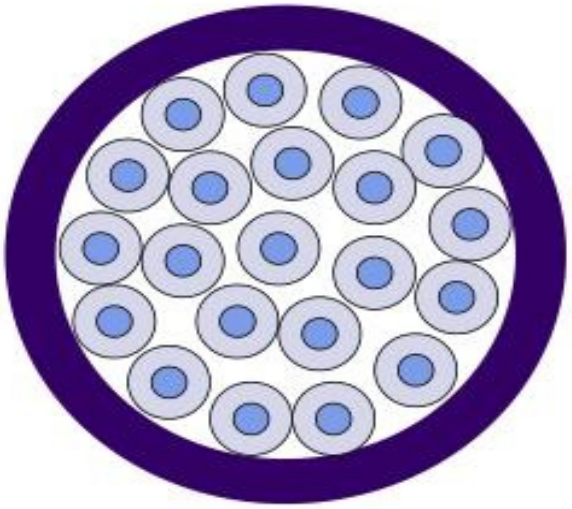
* It is completely packed or filled with stationary phase

Packed Column

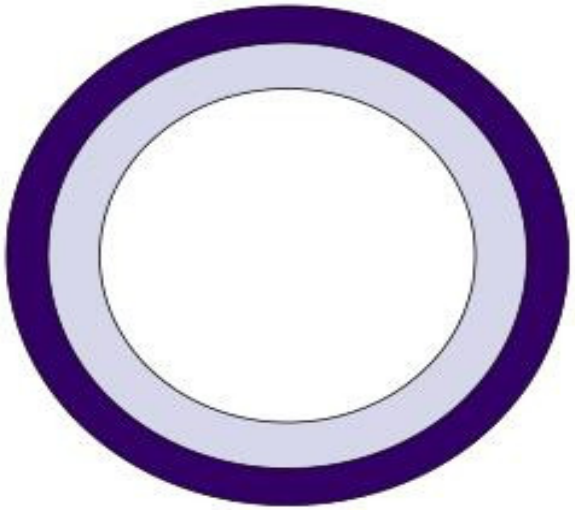
- Glass or metals
- 2-3 m long, 2-4 mm i.d.
- Densely packed with packing materials or solid support coated with thin layer of stationary liquid phase
- Diatomaceous earth
- Size: 60-80 mesh (250-170 μm) or 80-100 mesh (170-149 μm)

Packed vs Capillary

- Length: 2-50 m or more
- Stainless steel, glass, fused silica, or Teflon



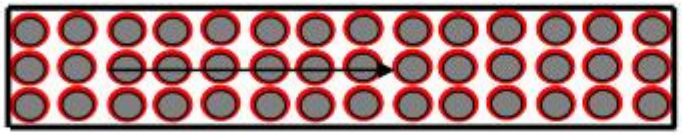
Packed Column



Open-tubular Column (capillary column)

A. Types of Columns in GC

1. Packed-bed column (d > 2 mm, packing particle from 100 to 250 micron



2. Micro-packed column (d < 1 mm, d_p/d_c less than 0.3)

3. Packed capillary column (d < 0.6 mm, packing particle 5-20 micron)

4. Wall coated open tubular columns (WCOT)

Thin layer of stationary phase coated directly on the wall of the tube

5. Support coated open tubular (SCOT)
   Liquid phase + glass powder or particle support

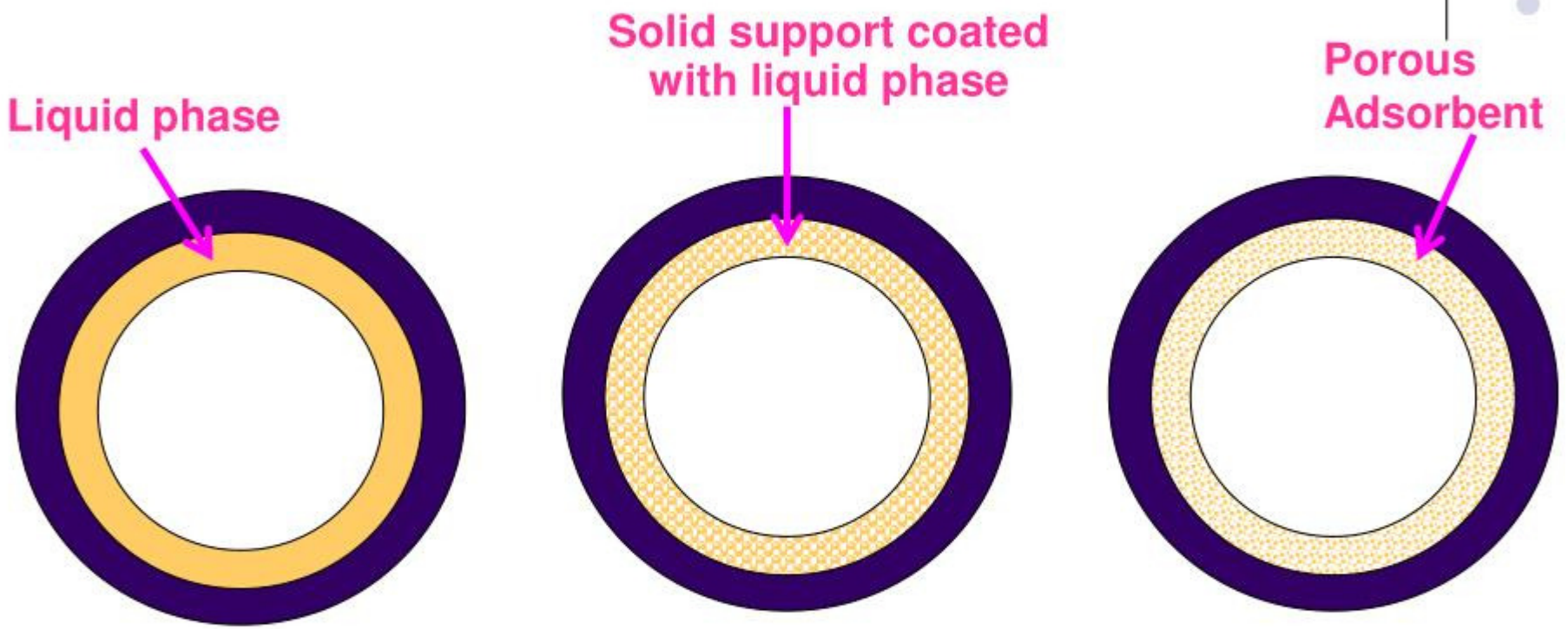
6. Porous layer open tubular column (PLOT)
   Particle support

Open tubular columns

Compared with packed columns, open tubular columns offer：

1. higher resolution
2. shorter analysis time
3. greater sensitivity
4. lower sample capacity

Types of Open Tubular Column

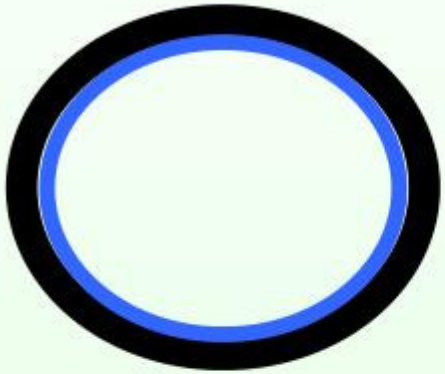


Wall-coated Open Tubular (WCOT)

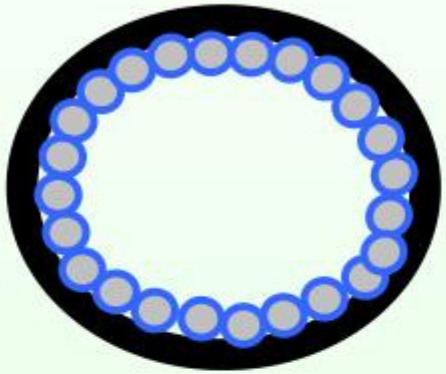
Support-coated Open Tubular (SCOT)

Porous Layer Open Tubu (PLOT)

FSOT: Fused-silica open tubular column



wall-coated
open tubular column
(WCOT)
FSOT: fused-silica, OTC



support-coated
open tubular column
(SCOT)

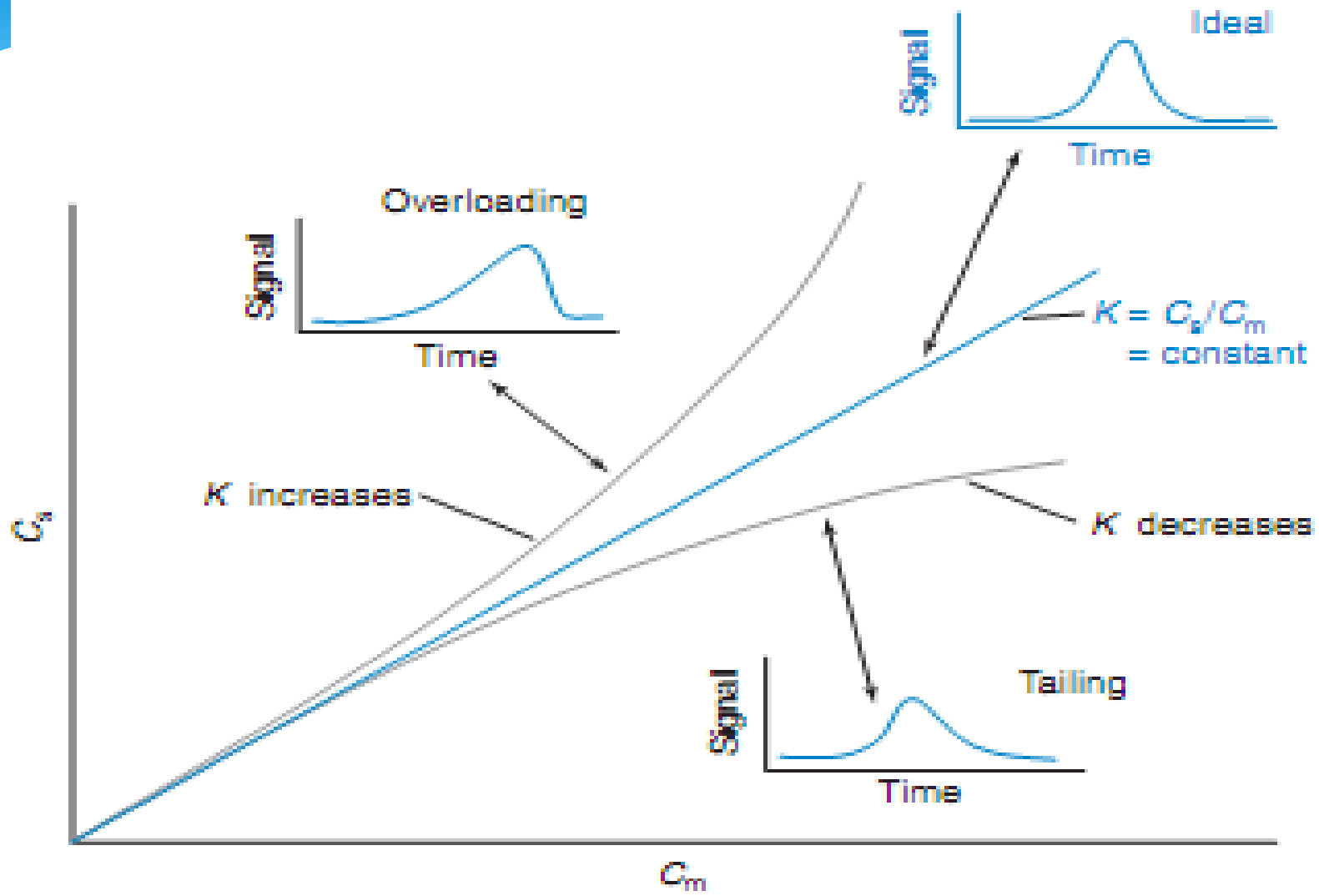
porous-layer
open tubular column
(PLOT)

Decreasing thickness of stationary phase leads to

1. decreased plate height
2. decreased retention time
3. decreased capacity for analyte

ASYMMETRICAL BAND SHAPES

* Gaussian Shape Band (Ideal Shape)
* Overloading
* Tailing



Common isotherms and their resulting chromatographic bandshapes.

Overloading

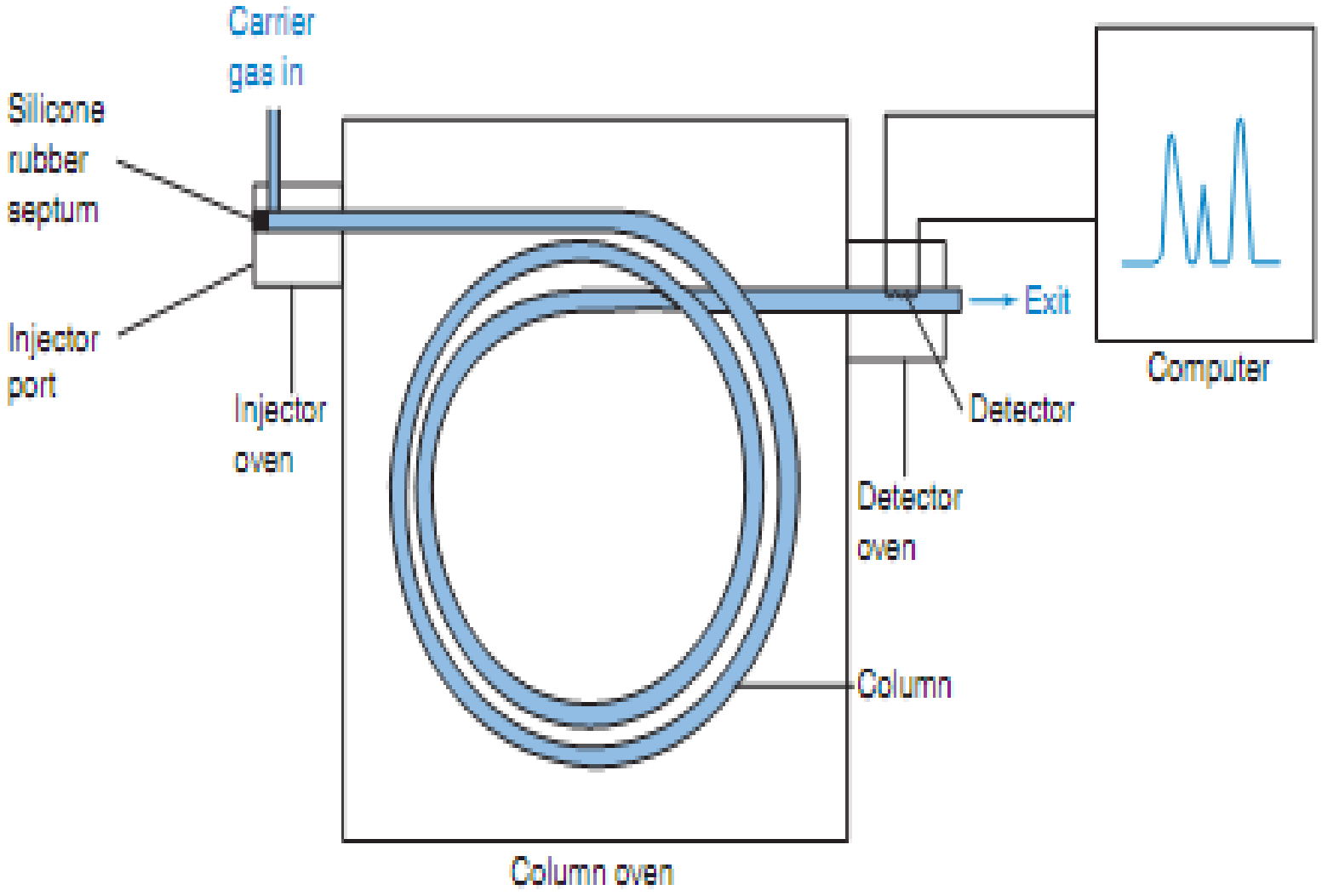
* Overloading occur when we apply large mount of sample.
* Under action of gravity, pressure of sample push it out of the column.
* It can be controlled by applying small sample amount.

Tailing

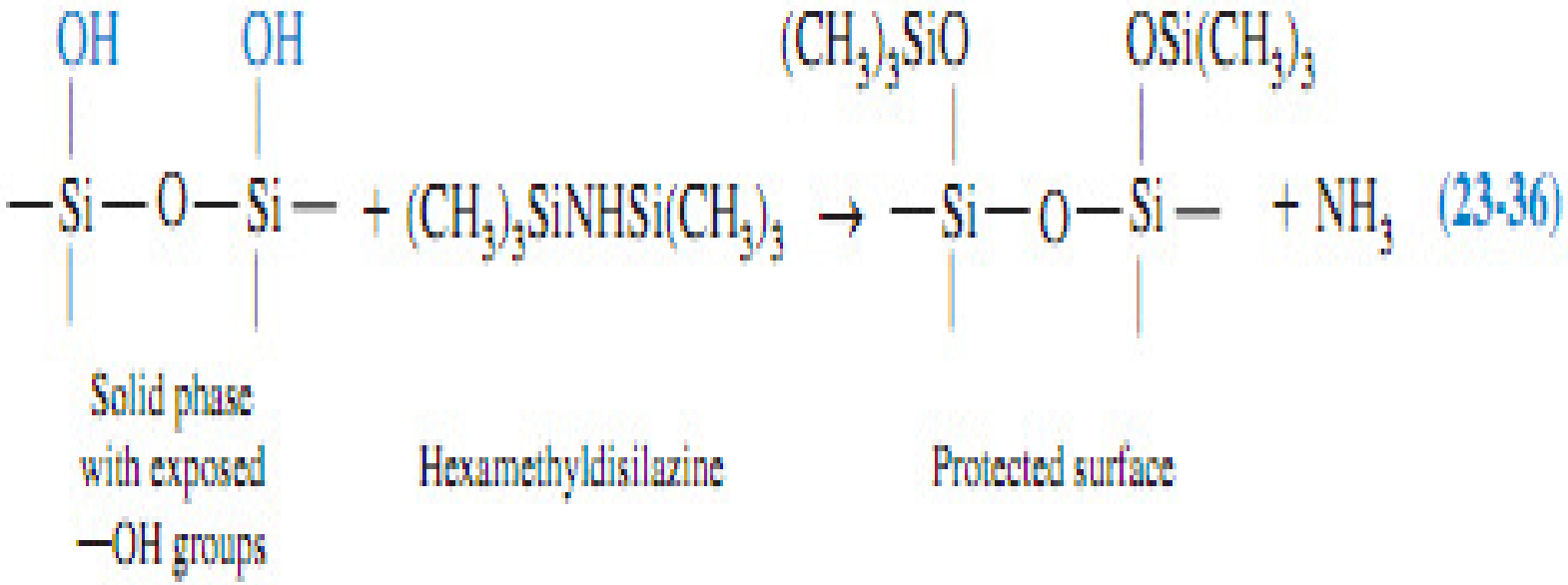
* Tailing occur when sample strongly bonded with stationary phase.
* Mostly –OH group of silica strongly attach sample with it.

GAS CHROMATOGRAPHY

* Gas chromatography (GC) is a common type of chromatography used in analytical chemistry for separating and analyzing compounds that can be vaporized without decomposition.


Carrier
gas in
Silicone
rubber
septum
Injector
port
Injector
oven
Exit
Detector
Computer
Detector
oven
Column
Column oven

Sites that bind solute strongly cause tailing. Silica surfaces of columns and stationary phase particles have hydroxyl groups that form hydrogen bonds with polar solutes, thereby leading to serious tailing. **Silanization** reduces tailing by blocking the hydroxyl groups with nonpolar trimethylsilyl groups:



GAS CHROMATOGRAPHY

Gas chromatography:

mobile phase: gas

stationary phase: usually a nonvolatile liquid, but sometimes a solid

analyte: gas or volatile liquid

PRACTICAL REQUIREMENTS

- Carrier gas
- Flow regulators & Flow meters
- Injection devices
- Columns
- Temperature control devices
- Detectors
- Recorders & Integrators

CARRIER GAS

» Hydrogen

better thermal conductivity

disadvantage: it reacts with unsaturated compounds & inflammable

» Helium

excellent thermal conductivity

it is expensive

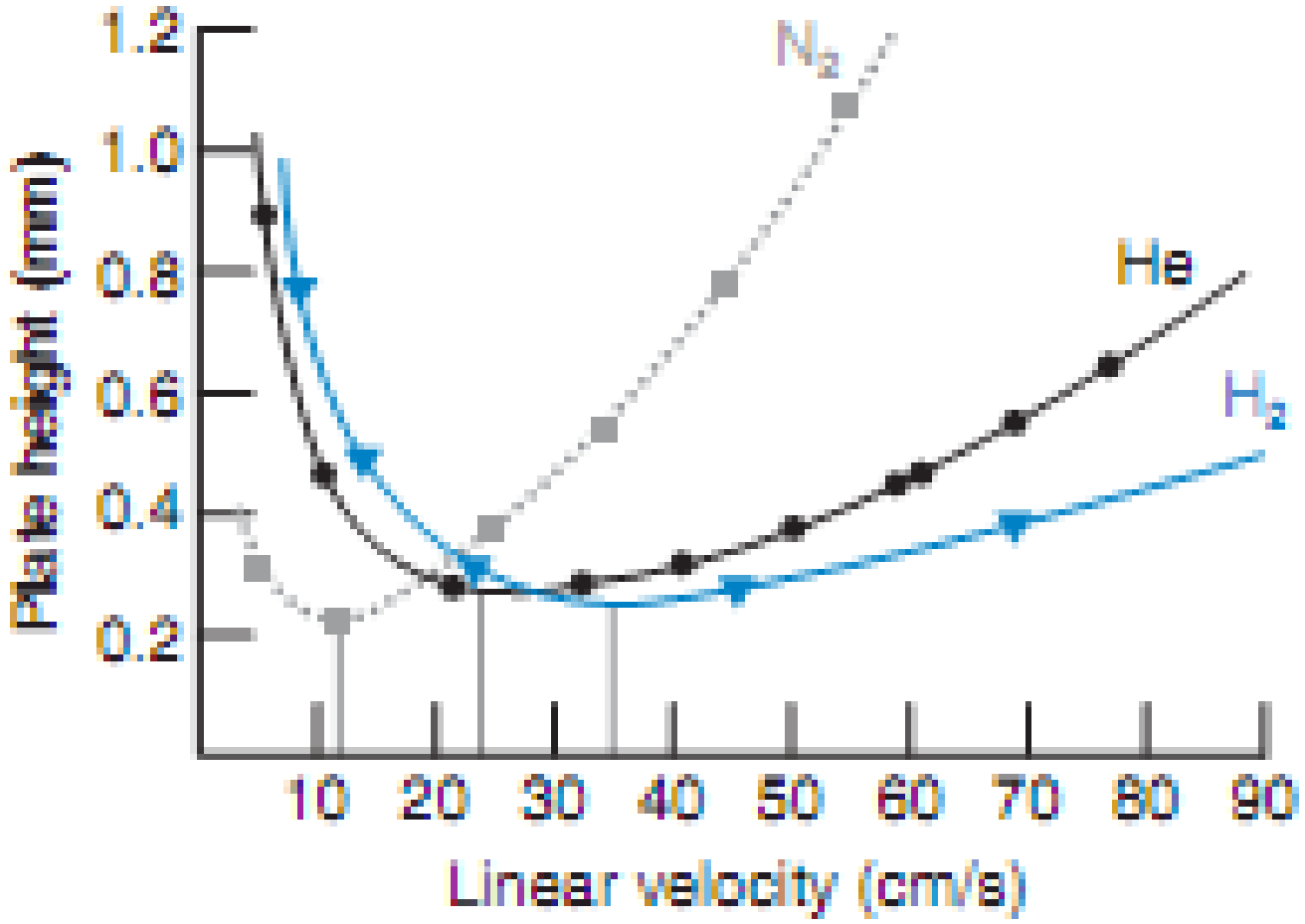
» Nitrogen

reduced sensitivity

it is inexpensive

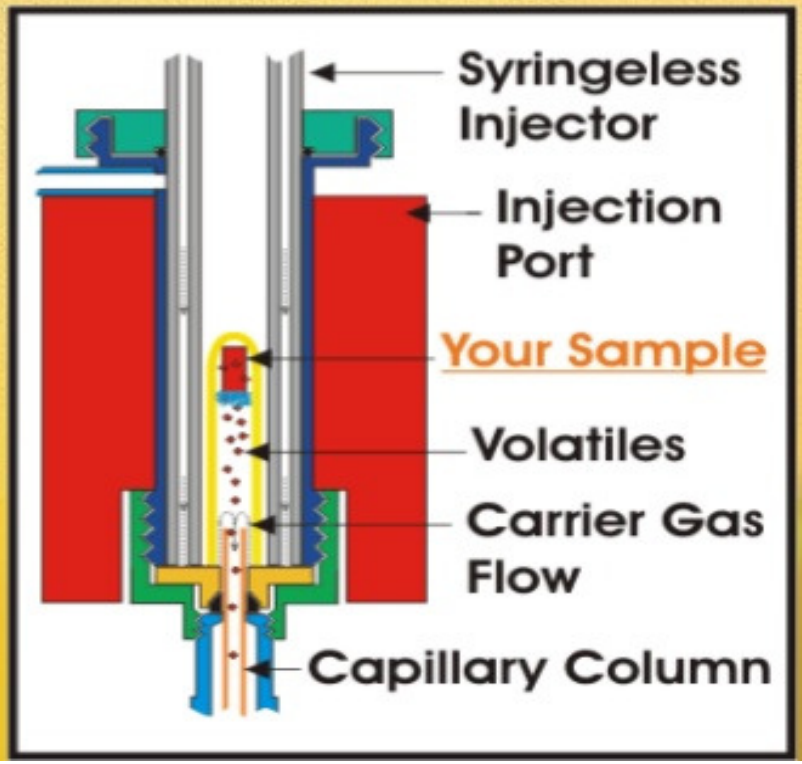
Requirements of a carrier gas

- ✓ Inertness
- ✓ Suitable for the detector
- ✓ High purity
- ✓ Easily available
- ✓ Cheap
- ✓ Should not cause the risk of fire
- ✓ Should give best column performance


1.2
1.0
0.8
0.6
0.4
0.2
Plate height (mm)
N_2
He
H_2
10
20
30
40
50
60
70
80
90
Linear velocity (cm/s)

Injection Devices

- Gases can be introduced into the column by valve devices
- liquids can be injected through loop or septum devices

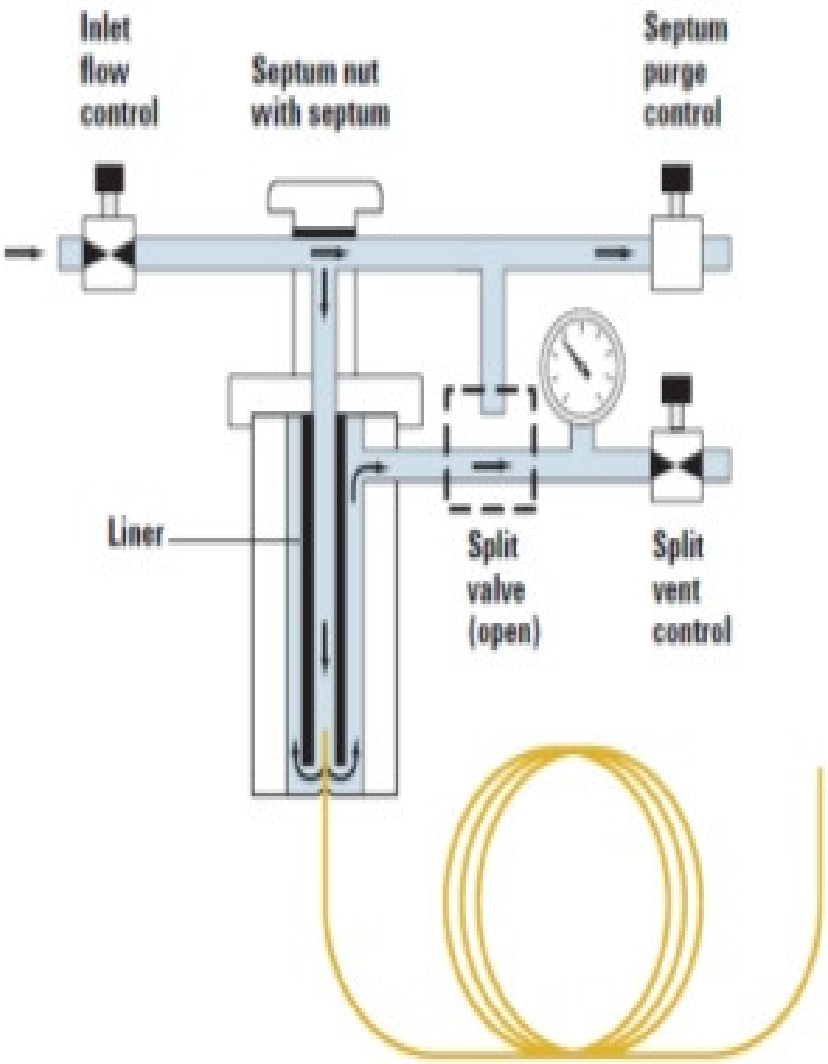


Mode of Injections

Split injection:	routine method
	0.1-1 % sample to column
	remainder to waste
Splitless injection:	all sample to column
	best for quantitative analysis
	only for trace analysis, low [sample]
On-column injection:	for samples that decompose above boiling point - no heated injection port
	column at low temperature to condense sample in narrow band
	heating of column starts chromatography

Split injector

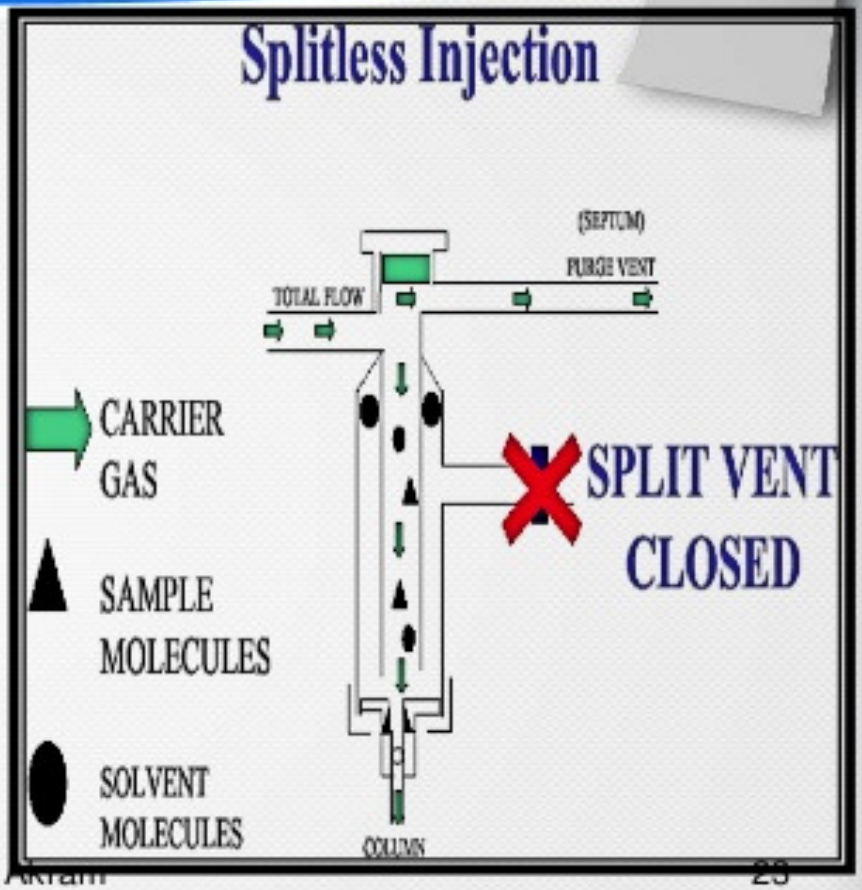
- In the split injection mode, sample enters the hot liner and volatilized rapidly.
- Vaporized sample is mixed with a carrier gas (diluted).
- Finally, a large part of the diluted vaporized sample is split away from the Colum, while a small part will enter the column.
- This mode of injection is used for analysis of samples of high analyte concentrations.



Agilent, fundamental gas chromatography, G1176-90000

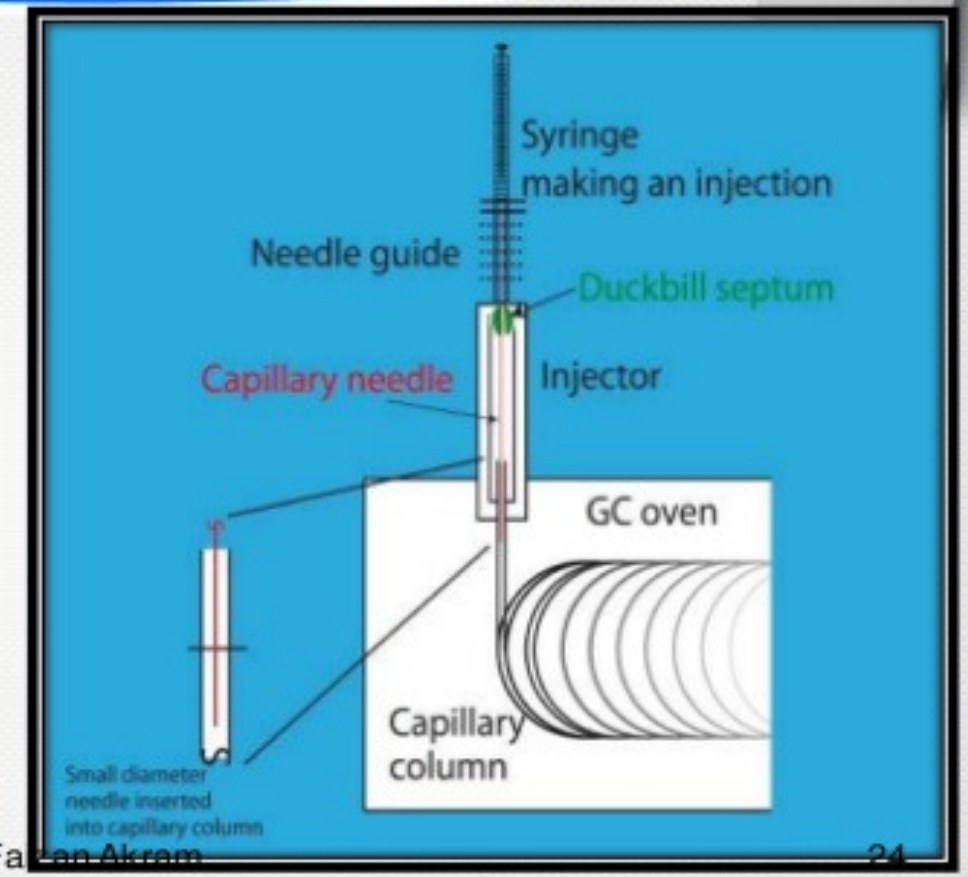
➢ ***Split less injection:***

- all sample to column
- best for quantitative analysis
- only for trace analysis, low [sample]
- Injector temperature for splitless injection is lower (220) than that for split injection, because the sample spends more time in the port and we do not want it to decompose.



➢ ***On-Column Injection***

- On-column injection is used for samples that decompose above their boiling points and is preferred for quantitative analysis.
- Solution is injected directly into the column, without going through a hot injector



COLUMNS

- Important part of GC
- Made up of glass or stainless steel
- Glass column- inert , highly fragile

COLUMNS can be classified

❖ Depending on its use

1. Analytical column

   1-1.5 meters length & 3-6 mm d.m

2. Preparative column

   3-6 meters length, 6-9mm d.m

❖Depending on its nature

1.Packed column: columns are available in a packed manner

S.P for GLC: polyethylene glycol, esters, amides, hydrocarbons, polysiloxanes…

2.Open tubular or Capillary column or Golay column

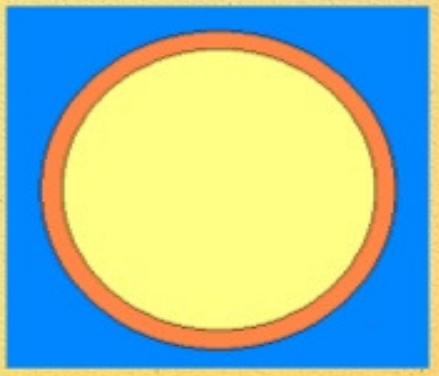
- Long capillary tubing 30-90 M in length
- Uniform & narrow d.m of 0.025 - 0.075 cm
- Made up of stainless steel & form of a coil
- Disadvantage: more sample cannot loaded

3.SCOT columns (Support coated open tubular column

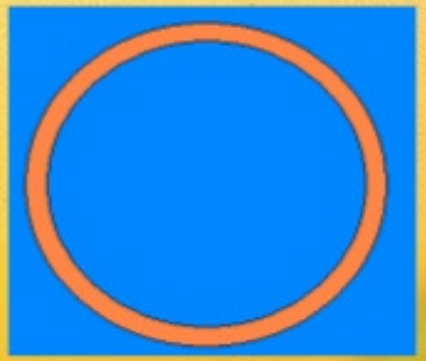
- Improved version of Golay / Capillary columns, have small sample capacity
- Made by depositing a micron size porous layer of supporting material on the inner wall of the capillary column
- Then coated with a thin film of liquid phase

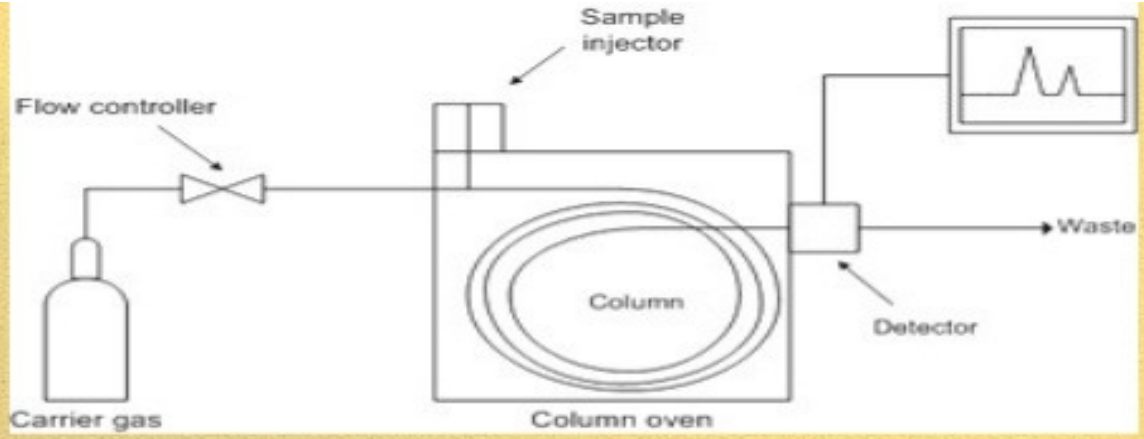
Columns

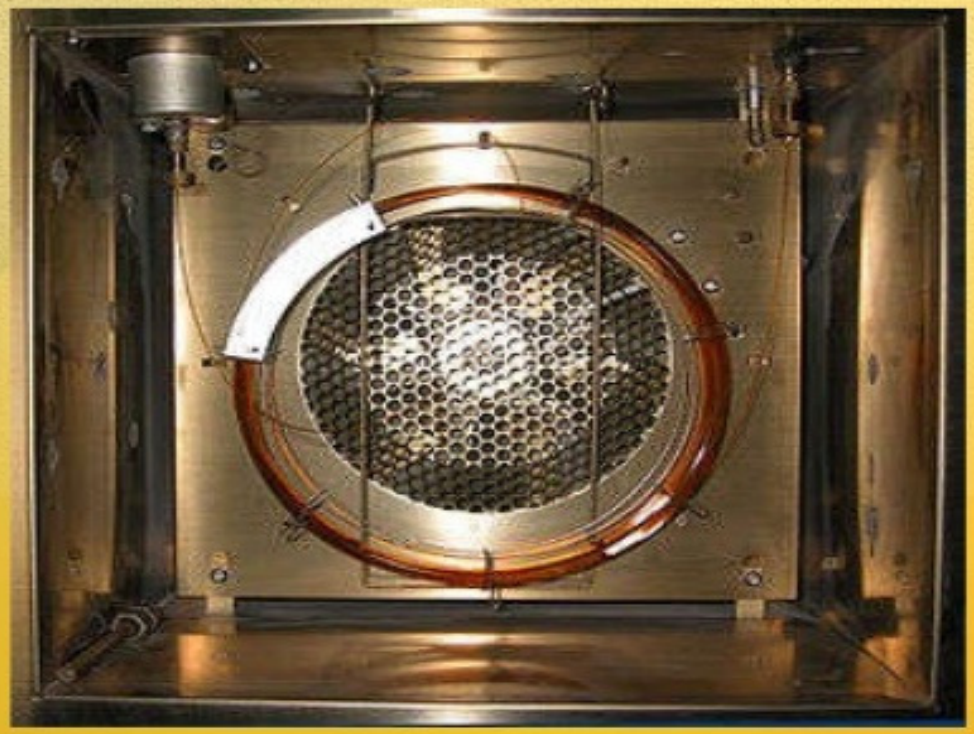
- Packed



- Capillary




Sample injector
Flow controller
Waste
Column
Detector
Carrier gas
Column oven



Equilibration of the column

- Before introduction of the sample
- Column is attached to instrument & desired flow rate by flow regulators
- Set desired temp.

❖ Conditioning is achieved by passing carrier gas for 24 hours

Temperature Control Devices

- **Preheaters**: convert sample into its vapour form, present along with injecting devices
- **Thermostatically controlled oven**:

  temperature maintenance in a column is highly essential for efficient separation.

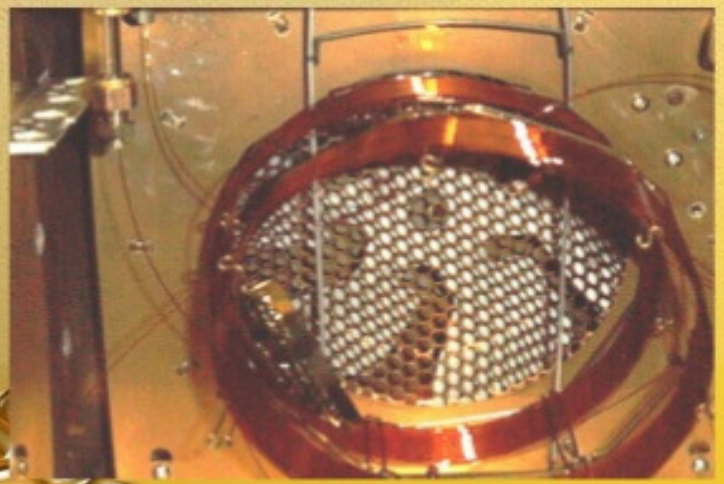
Two types of operations

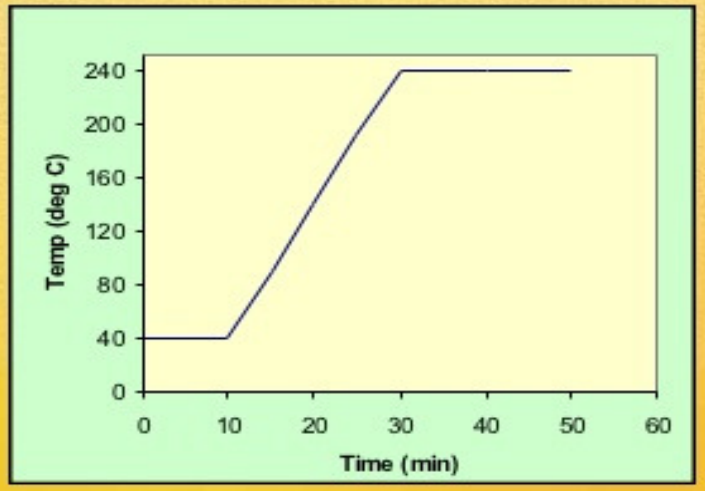
- Isothermal programming:-
- Linear programming:- this method is efficient for separation of complex mixtures

Instrumentation - Oven

Temperature Control

- Isothermal
- Gradient





DETECTORS

- Heart of the apparatus

The requirements of an ideal detector are-

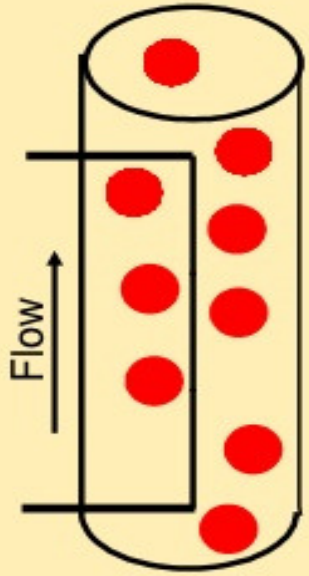
- Applicability to wide range of samples
- Rapidity
- High sensitivity
- Linearity
- Response should be unaffected by temperature, flow rate…
- Non destructive
- Simple & inexpensive

1.Thermal Conductivity Detector (Katharometer, Hot Wire Detector)

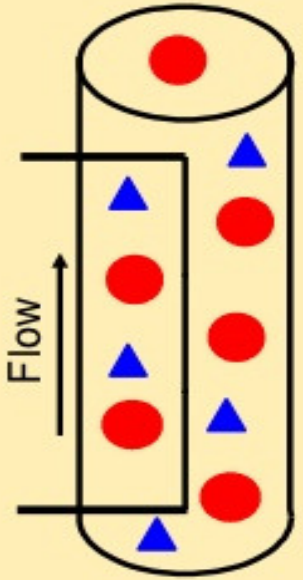
Measures the changes of thermal conductivity due to the sample (μg). Sample can be recovered.

Thermal Conductivity Basics

The TCD is a nondestructive, concentration sensing detector. A heated filament is cooled by the flow of carrier gas.



When the carrier gas is contaminated by sample ▲, the cooling effect of the gas changes. The difference in cooling is used to generate the detector signal.



Thermal Conductivity Detector

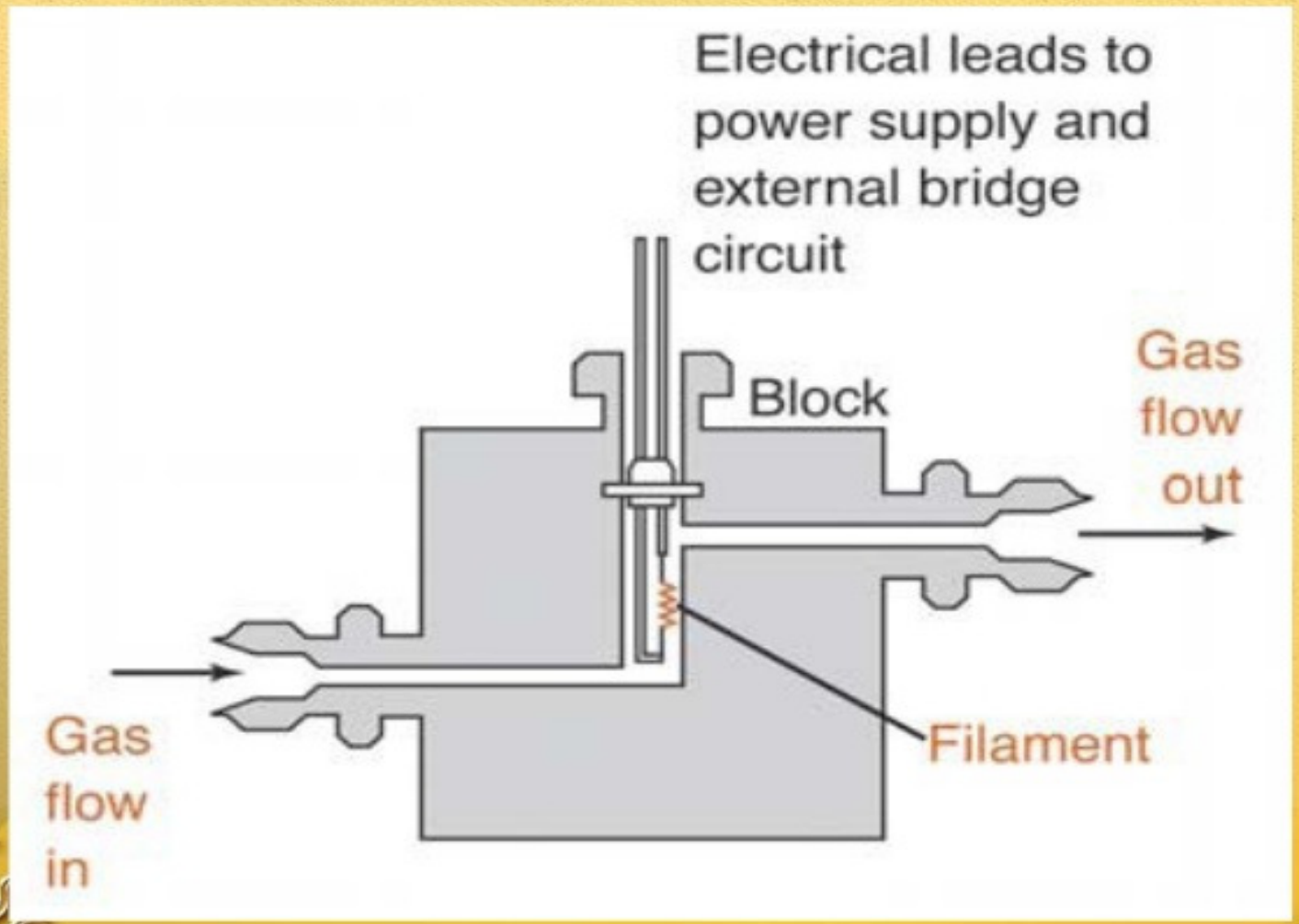
When a separated compound elutes from the column , the thermal conductivity of the mixture of carrier gas and compound gas is lowered. The filament in the sample column becomes hotter than the control column.

The imbalance between control and sample filament temperature is measured by a simple gadget and a signal is recorded

Measures heat loss from a hot filament –

e filament heated to const T

- when only carrier gas flows heat loss to metal block is constant, filament T remains constant.
- when an analyte species flows past the filament generally thermal conductivity goes down, T of filament will rise. (resistance of the filament will rise).


Electrical leads to power supply and external bridge circuit
Block
Gas flow out
Gas flow in
Filament

Advantages of Katharometer

- ❖ Linearity is good
- ❖ Applicable to most compounds
- ❖ Non destructive
- ❖ Simple & inexpensive

Disadvantages

- ✓ Low sensitivity
- ✓ Affected by fluctuations in temperature and flow rate
- ✓ Biological samples cannot be analyzed

Flame Ionization Detector

- **Destructive detector**
- The effluent from the column is mixed with H & air, and ignited.
- Organic compounds burning in the flame produce ions and electrons, which can conduct electricity through the flame.
- A large electrical potential is applied at the burner tip
- The ions collected on collector or electrode and were recorded on recorder due to electric current.

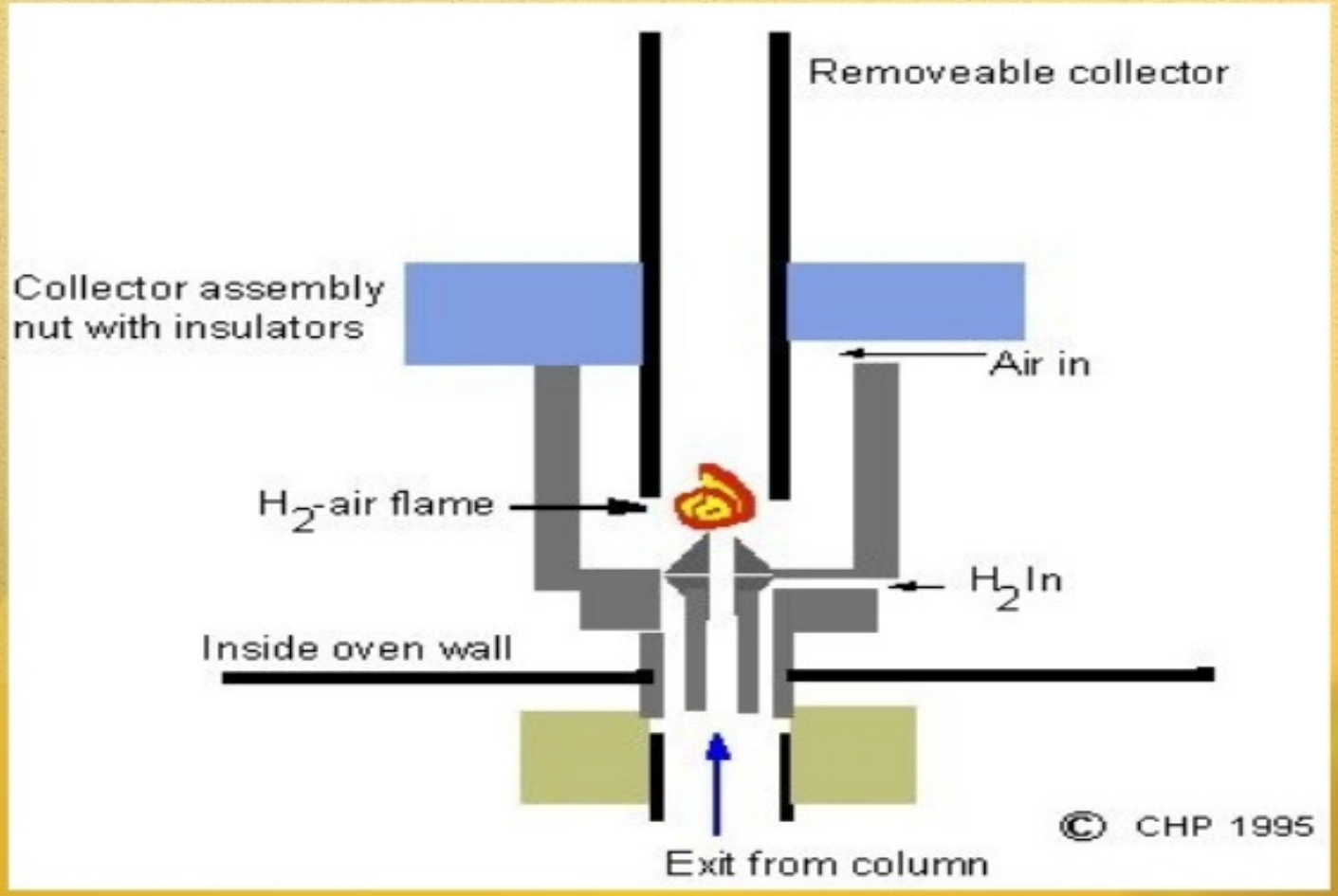
FIDs are mass sensitive rather than conc. sensitive

ADVANTAGES:

- µg quantities of the solute can be detected
- Stable
- Responds to most of the organic compounds
- Linearity is excellent

- DA: destroy the sample

FID


Removeable collector
Collector assembly
nut with insulators
Air in
H_2-air flame
H_2 In
Inside oven wall
Exit from column
© CHP 1995

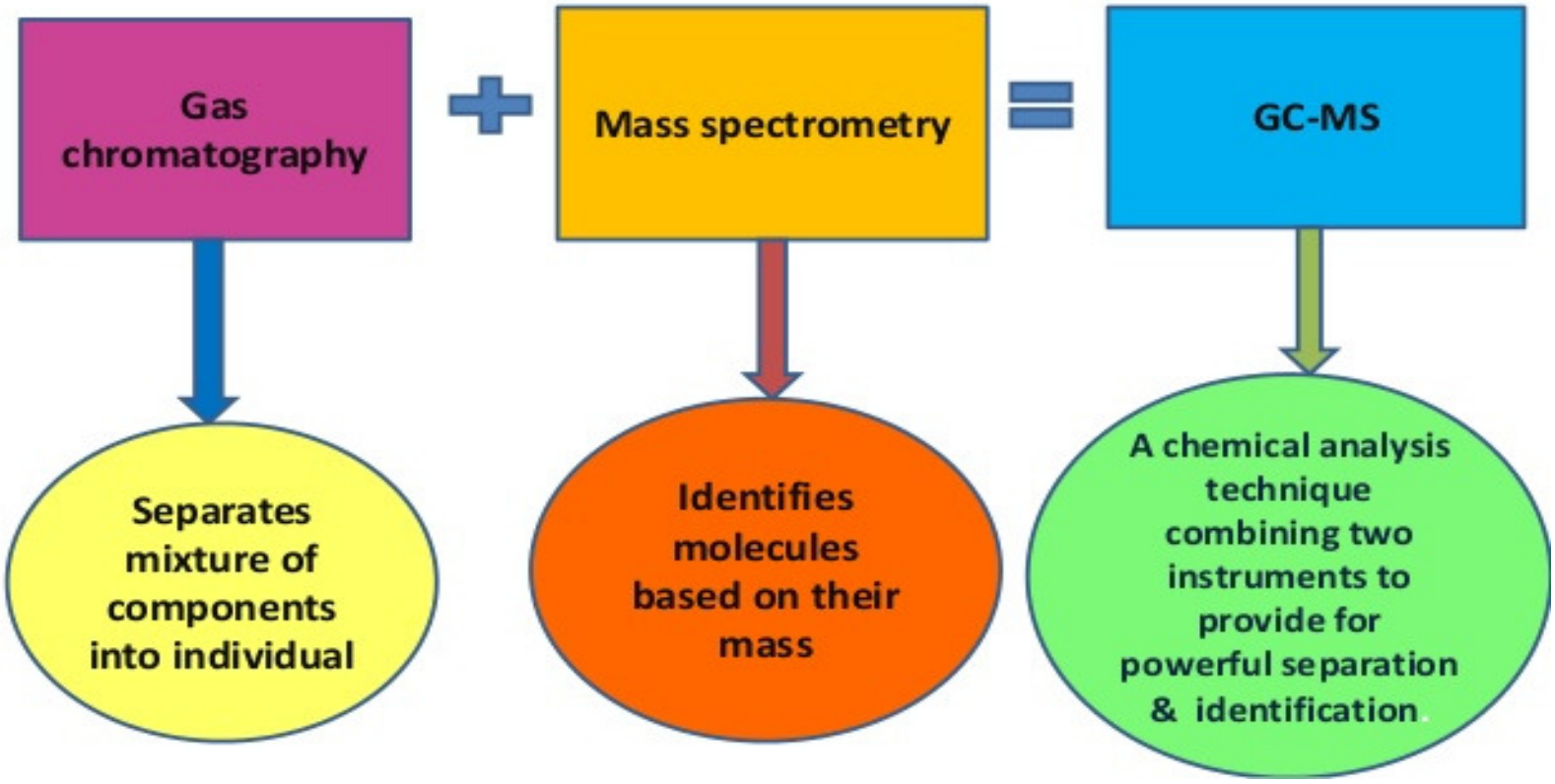
GC – Mass Spectrometry (GC-MS)

GC-MS is a sophisticated instrumental technique that produces, separates, and detects ion in the gas phase.

Today, relatively inexpensive compact benchtop system are available and widely used in laboratories.

GC-MS – A BRIEF

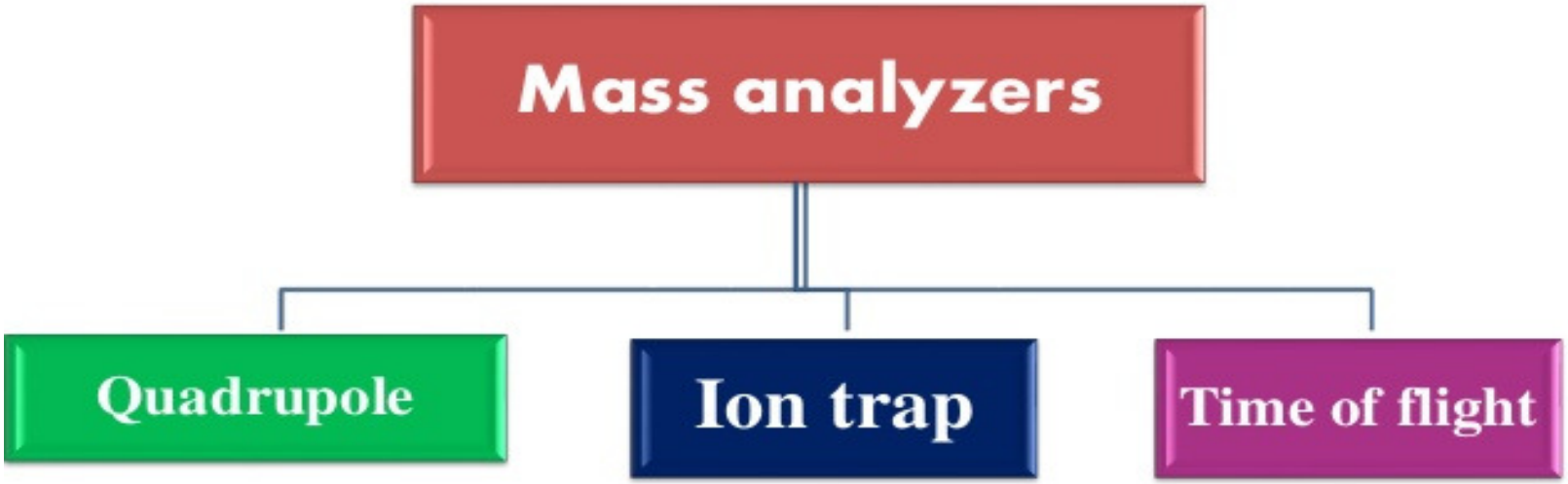
- It's a Hyphenated Technique
- Invented By James & Martin in 1952

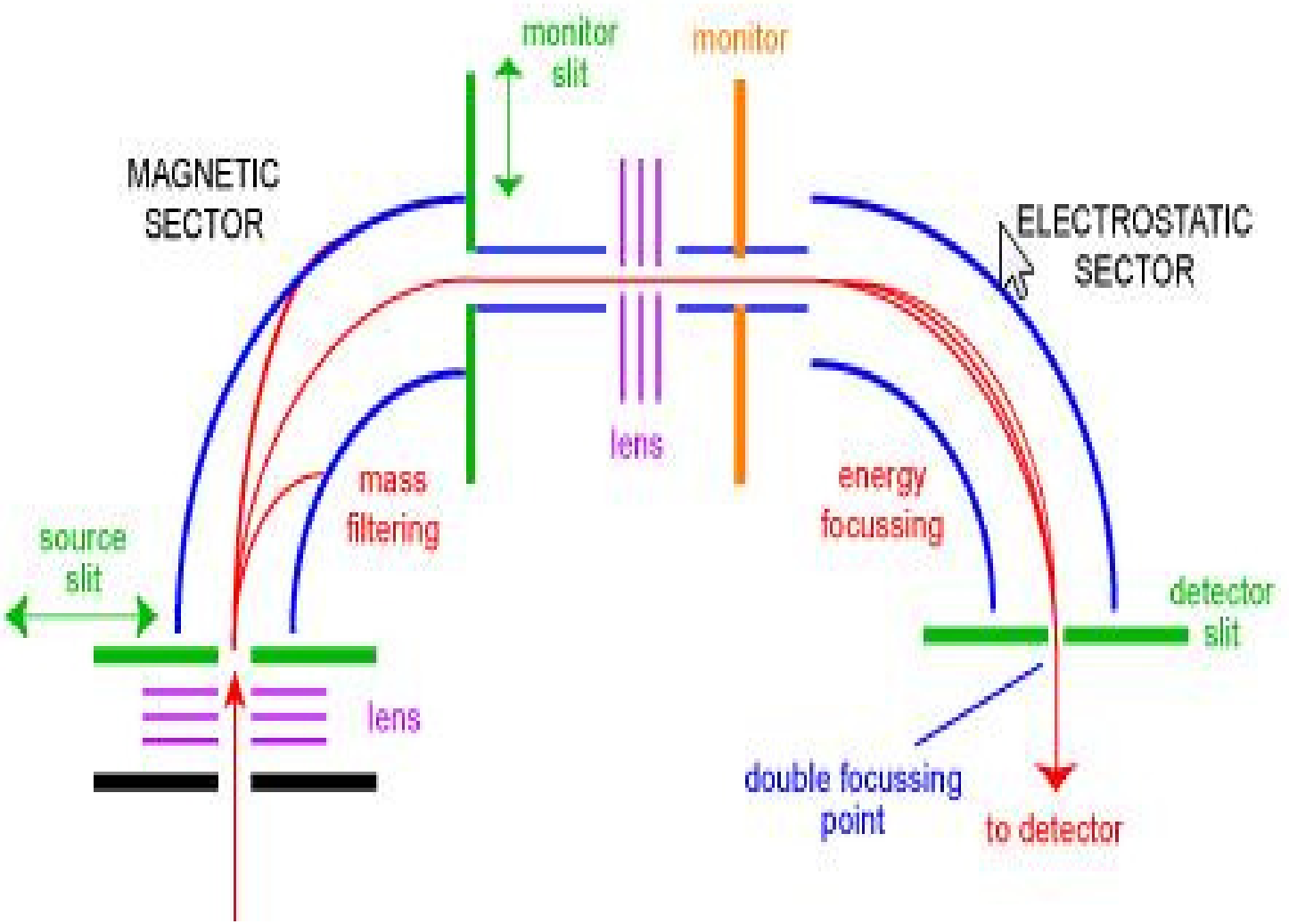


Mass Analyzers

They deflects ions down a curved tubes in a magnetic fields based on their kinetic energy determined by the mass, charge and velocity.

The magnetic field is scanned to measure different ions.




monitor slit
monitor
MAGNETIC SECTOR
ELECTROSTATIC SECTOR
lens
mass filtering
energy focussing
source slit
detector slit
lens
double focussing point
to detector