# Separation Methods

Dr. Fida Am Ali

# What is the separation

- ***Intruduction:***
- A sample that requires analysis is often a mixture of many components in a complex matrix.
- For samples containing unknown compounds, The components must be separated from each other so that each individual component can be identified by other analytical methods.
- The separation properties of the components in a mixture are constant under constant conditions, and therefore once determined they can be used to identify and quantify each of the components. Such procedures are typical in chromatographic and electrophoretic analytical separation.

# Types of separation methods

There are a many methods of separation such as:

- filtration
- Centrifugen
- Crystallization
- Distillation
- Extraction
- Ion exchange
- Chromatography
- Electrophoresis

But the main four methods of separation are:

1- *The extraction and 2- ion exchange*: in which isolating an analyte from interferences.

3- *chromatographic separation:* in which the separation, identification, and determination of the chemical components in complex mixtures are achieved.

4- Electrophoretic separation: is a technique by which the ionic molecules can be separated based on the mobility of ions in an electric field. In this method the separation, Identification and may be determination of chemical components are achieved

# important concepts

- *The sample matrix:* is the medium containing an analyte of interest.

- *An interferent:* is a chemical species that causes a systematic error in an analysis by enhancing the analytical signal

- A masking agent is a reagent that chemically binds an interferent and prevents it from causing errors in an analysis

# Analytical Separations by Extraction

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# What is the extraction

Extraction use two immiscible phases to separate a solute from one phase to the other.

The distribution of a solute between two phases is an equilibrium condition described by partition theory

# Partition Theory, Distribution Coefficient

- A solute **S** will distribute itself between two phases (after shaking and allowing the phases to separate) and, within limits, the ratio of the concentrations of the solute in the two phases will be a constant:

$$K_D = \frac{[S]_1}{[S]_2}$$

- Where $K_D$ is the **distribution coefficient, distribution constant or partition coefficient** and the subscripts represents solvent 1 (e.g., an organic solvent) and solvent 2 (e.g., water).

If the distribution coefficient is large, the solute will tend toward quantitative distribution in solvent 1.

# Distribution Ratio

- The **distribution ratio** is the ratio of the concentrations of **all** the species of the solute in each phase. Consider, for example, the extraction of benzoic acid from aqueous solution. Benzoic acid (HBz) is a weak acid in water. The distribution coefficient is given by:

$$K_D = \frac{[HBz]_e}{[HBz]_a} \quad 1$$

Where ***e*** represents the ether solvent and ***a*** represents the aqueous solvent.

- In this example the distribution ratio is given by:

$$D = \frac{[HBz]_e}{[HBz]_a + [Bz^-]_a} \qquad (2)$$

- We can derive the relation between D and $K_D$ from the equilibria involved. The acidity constant $K_a$ for the ionization of the acid in aqueous phase is given by:

$$K_a = \frac{[H^+]_a [Bz^-]_a}{[HBz]_a} \qquad 3$$

Hence,

$$[Bz^-]_a = \frac{K_a [HBz]_a}{[H^+]_a} \qquad (4)$$

From equation 1: $[HBz]_e = K_D [HBz]_a$ (5)

Substitutions of equations 4 and 5 into equation 2:

$$D = \frac{K_D}{1 + K_a/[H^+]_a} \qquad (6)$$

- This equation predicts that when $[H^+]_a > K_a$, D is nearly equal to $K_D$, and if $K_D$ is large, the benzoic acid will be extracted into the ether layer, D is maximum under these conditions.

- On the other hand, if $[H^+]<K_a$, then D will be small, and the benzoic acid will remain in the aqueous layer. That is, in alkaline solution, the benzoic acid is ionized and cannot be extracted, while in acid solution, it is largely undissociated.

# Percent Extracted (Recovery)

- The fraction of solute extracted is equal to millimoles of solute in the organic layer divided by the total number of millimoles of solute. The millimoles are given by the molarity times the millilitres. Thus, the percent extracted is given by:

$$P\% = \mathbf{E\%} = \frac{\mathbf{[S]_0\ V_0}}{\mathbf{[S]_0\ V_0 + S_a V_a}} \times \mathbf{1oo} \qquad \mathbf{(7)}$$

Where $V_0$ and $V_a$ are the volumes of the organic and aqueous phases.

# Types of Extraction Methods

- There are two main methods of extraction, solvent extraction and solid-phase extraction.
- The techniques of solvent extraction and solid phase extraction and related techniques are very useful for isolating analytes from complex sample matrix prior chromatographic analysis.
- Solvent extraction involves the distribution of a solute between two immiscible liquid phases.
- This technique is extremely useful for very rapid separations of both organic and inorganic substances.
- Solid phase extraction is a technique in which hydrophobic functional groups are bonded to solid particle surfaces and act as the extracting phase.

- The apparatus used for solvent extraction is the **separatory funnel** illustrated in the next figure:

Separatory funnel.

- Most often, a solute is extracted from an aqueous solution into an immiscible organic solvent. After the mixture is shaken for about a minute, the phases are allowed to separate and the bottom layer (the denser solvent) is drawn off in a completion of the separation.
- Many substances are partially ionized in the aqueous layer as weak acids. This introduces a pH effect on the extraction.

# Example 1

Twenty millilitres of aqueous solution of 0.1N benzoic acid is shaken with 10 ml ether.

After the layers are separated, it is determined by titration that 0.5 millimole benzoic acid remains in the aqueous layer. What is the distribution coefficient ?

And what is the percent extracted ( The recovery)?

# Solvent extraction of metals

- Solvent extraction has one of its most important applications in the separation of metal cations.
- In this technique, the metal ion, through appropriate chemistry, distributes from an aqueous phase into a water-immiscible organic phase. This technique depends on adding a chelating agent to the aqueous solution in order to eliminate the charge of metal ion and constitute an insoluble complx in water. Then the organic solvent is added to extract the complex , and the solvent, that is used for extraction the metal complex should be dichloromethane or chloroform or $CCl_4$

# Ion pairs Extraction

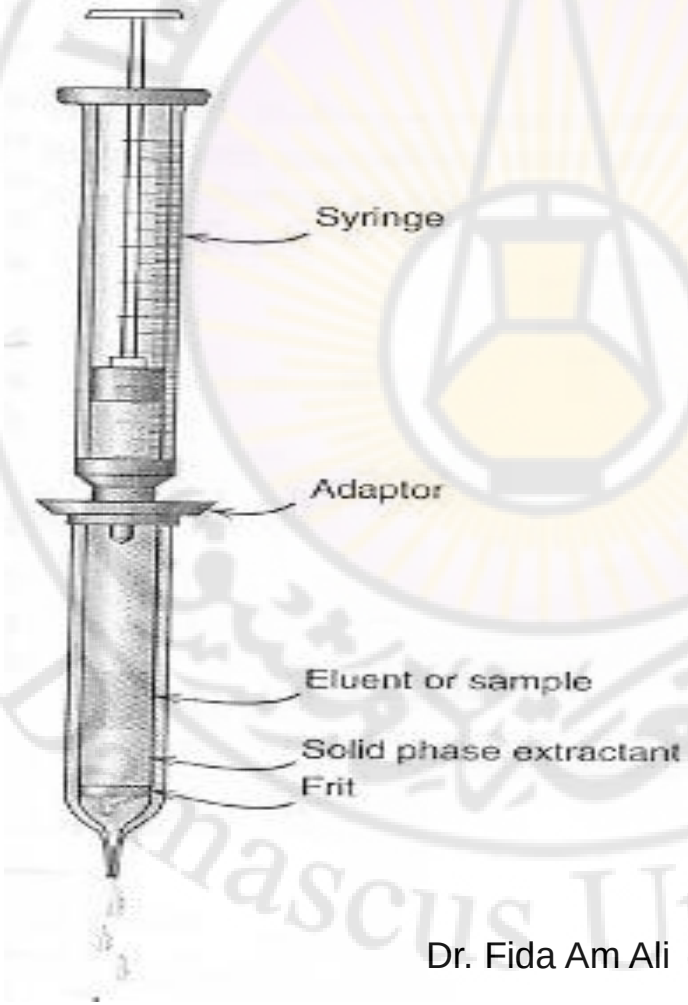
This technique is useful to extract the ionic organic compound from an aqueous solution to an organic phase.

This technique depends on adding an ionic surfactant (such as SLS, D.O.S.S) to the aqueous solution and the charge of the surfactant should be opposite to the charge of ionic organic compound, so an ion pair molecule (uncharged molecule) will be constituted and this constituted molecule will be insoluble in water but soluble in organic solvents. Thus an organic solvent will be added to extract the uncharged molecule

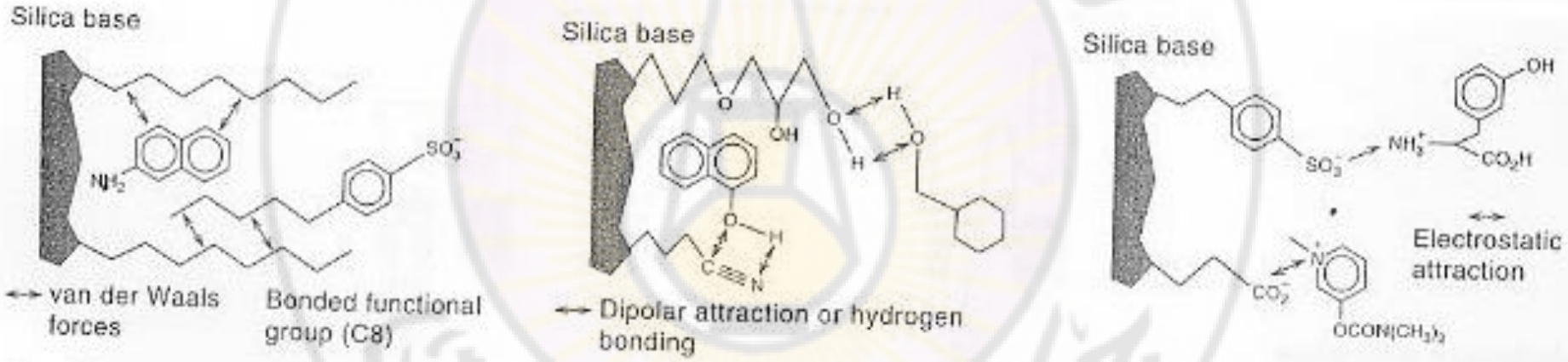
# Solid- phase Extraction

- **The solid –phase extraction (SPE)** has become a widely used technique for sample clean up and concentration prior to chromatographic analysis in particular.
- In this technique, hydrophobic organic functional groups are chemically bonded to a solid surface, for example, powdered silica.
- A common example is the bonding of $C_{18}$ or $C_8$ chains on silica, with particle sizes on the order of 40 µm. These groups will interact with hydrophobic organic compounds by **Van der waals** forces or by **Hydrogen bonding** and extract them from an aqueous sample in contact with solid surface.

- The powdered phase is generally placed in a small cartridge, similar to plastic syringe. Sample is placed in the cartridge and forced through by means of a plunger or a vacuum ( see the next figure).



- Trace organic molecules are extracted, preconcentrated on the column, and separated a way from the sample matrix. Then they can be eluted with a solvent such as methanol and then analyzed, for example, by chromatography.
- The nature of the extracting phase can be varied to allow extraction of different classes of compounds.
- The next figure illustrates bonded phases based on Van der waals forces, hydrogen bonding and electrostatic attraction.




Solid-phase extractants utilizing nonpolar, polar, and electrostatic interactions.

# Principles of Chromatography

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# General Description of Chromatography

- In chromatographic methods the solutes are separated according to their distribution of the solute between a stationary phase and a mobile phase.
- While the mechanisms of retention for various types of chromatography differ, they are all based on establishment of an equilibrium for the distribution of solute between the two phases.
- The more a solute interacts with the stationary phase, the slower it is moved along a column.

# Classification of Chromatographic Techniques

- Chromatographic processes can be classified according to the type of equilibration process involved, which is governed by the type of stationary phase. Various bases of equilibration are: 1)- adsorption, 2)- partition, 3)- ion exchange, and 4)- pore penetrations or size exclusion chromatography.

# Adsorption Chromatography

- The stationary phase is a solid on which the sample components are adsorbed.
- The mobile phase may be a liquid ( liquid- solid chromatography) or a gas (gas- solid chromatography), the components distribute between the tow phases through a combination of adsorption and desorption processes. Thin layer chromatography (TLC) is a special example of adsorption chromatography in which the stationary phase is plane, in the form of a solid supported on inert plate, and the mobile phase is a liquid

# Partition Chromatography

- The stationary phase of partition chromatography is a liquid supported on an inert solid, the mobile phase may be a liquid (liquid- liquid chromatography) or gas (gas- liquid chromatography).
- Paper chromatography is an example of liquid-liquid chromatography, in which the stationary phase is supported on an inert paper and the mobile phase is a liquid
- In the normal mode of operations of liquid-liquid partition, a polar stationary phase (e.g., methanol on silica) is used with a nonpolar mobile phase (e.g., hexane) this is called **Normal phase chromatography (NPC).**
- This favors retention of polar compounds and elution of nonpolar compounds and is called **normal- phase chromatography**

- If a nonpolar stationary phase is used, with a polar mobile phase, then nonpolar solutes are retained more and polar solutes more readily eluted.This is called **reversed-phase chromatography**

# Ion Exchange and size exclusion chromatography

- Ion exchange chromatography uses an ion exchange resin as the stationary phase. Cation exchanger or anion exchanger resins may be used as a stationary phase. This depends on the nature of the sample components. The mechanism of separation is based on ion exchange equillibria.

If cations are exchanged , A cation exchange resin such as sulphonic cation resin should be used as a stationary phase and , If anions are exchanged , an anion exchange resin should be used such as quaternary anion resin

$$x RN(CH_3)_3^+ OH^- + A^{-x} \rightleftharpoons [RN(CH_3)_3^+]_x A^{-x} + x\, OH^-$$

$$\underset{\text{solid}}{x RSO_3^- H^+} + \underset{\text{solu}}{M^{+x}} \rightleftharpoons \underset{\text{solid}}{(RSO_3^-)_x\ M^{+x}} + \underset{\text{solu}}{x\, H^+}$$

- Size exclusion chromatography is a type of chromatography, in which the stationary phase is a molecular sieve.
- These sieves are polymeric carbohydrates and acrylamides that have open network formed by the cross linking of the polymeric chains. They may be hydrophilic and capable of absorbing water, whereupon swelling causes an opening of this structure. The degree of cross linking will determine the size of holes.
- Solvated molecules larger than the largest pores of the swollen gel cannot penetrate the gel particles and will pass straight through the column through the spaces between the individual particles.

Smaller molecules, will penetrate the open network in the particles to a varying degree, depending on their size and shape. They are therefore retarded to varying degrees and will be eluted in order of decreasing molecular size.

- Gels with a high degree of swelling are used to fractionate large molecules, whereas the denser (lower swelling) gels are used for separation a low- molecular-weight compounds.

Gel filtration is a type of size-exclusion chromatography in which the stationary phase is hydrophilic porous polymer and the mobile phase is a buffer solution. It is used for separating polar species.

Gel permeation is a type of size exclusion chromatography in which the stationary phase is hydrophobic porous polymer and the mobile phase is an organic solvent such as THF,. It is used to separate nonpolar species.

- Size exclusion chromatography can be used for separation proteines, enzymes, piptides, and hormons.

- The exclusion limit is a molecular weight of the molecule that will just permeate the gel and be retarded

# Column Chromatography

-By the separation of components on a glass chromatographic column, the columns that have 20 to 100 cm lengths and10 to 50 mm diameter were packed with 50-200 µm solid particles that formed the stationary phase.

a small volume of sample is placed at the top of column, which is filled with the stationary phase .

- Mobile phase solvent is added to the column and is allowed to slowly emerge from the bottom of the column.
- The individual components interact with the stationary phase to different degree.
- The distribution equilibrium is described by the distribution constant:

$$\mathbf{K_c} = \frac{[\mathbf{X}]_\mathbf{s}}{[\mathbf{X}]_\mathbf{m}} = \frac{C_s}{C_m} \qquad \mathbf{(1)}$$

- Where $[X]_s$ is the molar concentration of component X in the stationary phase at equilibrium and $[X]_m$ its molar concentration in the mobile phase.
- This equilibrium constant is governed by the temperature, the type of compound, and the stationary and mobile phases.
- It is also called the distribution coefficient or the partition coefficient in partition chromatography.
- Solutes with large $K_c$ value will be retained more strongly by stationary phase than those with a small value.

# Theory of Column Efficiency in Chromatography

## Theoretical Plates:

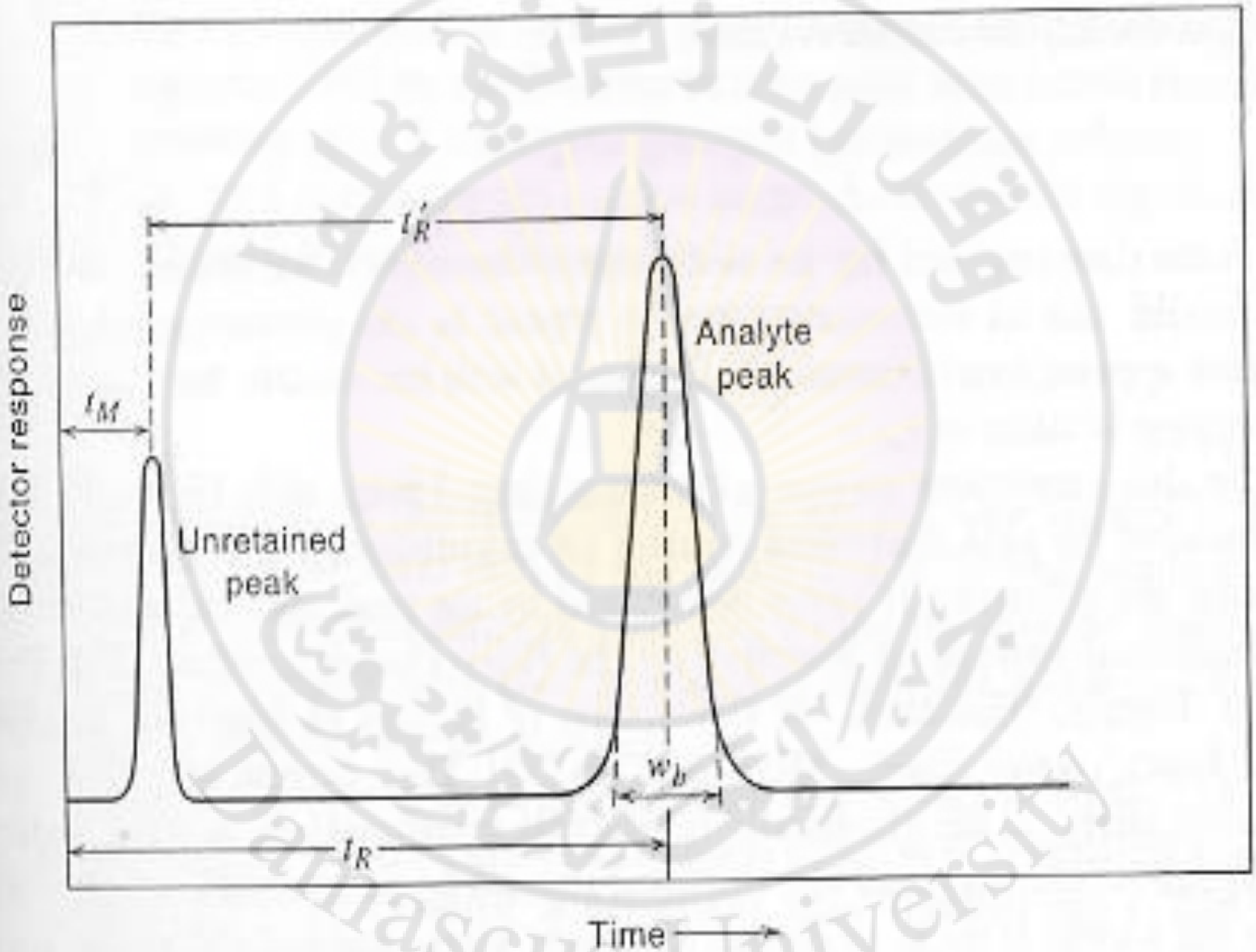
- The separation efficiency of a column can be expressed in term of the number of theoretical plates in the column.
- A Theoretical plate is a plate of stationary phase in chromatography, which can be thought of as representing a single equilibrium step. In reality, they are a measure of the efficiency of a column.
- For high efficiency, a large number of plates is necessary.
- The **Height Equivalent to a Theoretical Plate HETP,** is the length of the column divided by the number of Theoretical Plates.

$$HETP = \frac{L}{N}$$

- To avoid a long column, then HETP should be as short as possible.
- The number of plates or efficiency can be obtained from a chromatogram from the expression

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

- Where N is the number of plates of a column toward a particular compound, $t_R$ is the retention time and w is the peak width. These are illustrated in the next figure.


Detector response
$t'_R$
Analyte peak
$t_M$
Unretained peak
$w_b$
$t_R$
Time

- It should be noted that w is not the base width of the peak but the width obtained from the intersection of the baseline with tangents drawn through the inflection points at each side of the peak.

# Example 1

Calculate the number of plates in the column resulting in the chromatographic peak in the last figure. (W=9 $t_R$= 52)

# Retention Time

- The chromatogram is the diagram of separation which consists of a number of peaks, their positions are determined by a retention times and their peak areas are related proportional to the concentrations of separated components.

The retention time $t_R$ is the time between injection of a sample in a column or in a chromatograph and the appearance of a solute peak at the detector of a chromatograph in its highest concentration.

- In the last figure, the small peak on the left is a species that is not retained by the stationary phase. The time $t_M$ after sample injection for this peak to appear is sometimes called the dead time.

Often the sample or the mobile phase will contain an unretained species. When they do not, such species may be added to aid in peak Identification.
- The larger peak on the right in the last figure is that of an analyte species. The average rate of solute migration, v, is

$$V = \frac{L}{t_R} \qquad (3)$$

Where L is the length of the column. Similarly, the average velocity, u, of the molecules of the mobile phase is

$$u = \frac{L}{t_m} \qquad (4)$$

# The Relationship between Migration Rate and Partition constant

- In order to relate the rate of migration of a solute to its partition constant, we express the rate as a fraction of the velocity of the mobile phase

$$V = u \times \ fraction of\ time\ solute\ spends\ in\ mobile\ phase$$

This fraction, however, equals the average number of moles of solute in the mobile phase at any instant divided by the total number of moles of solute in the column:

$$V = u \times \frac{moles of\ \ solute in\ mobile\ \ phase}{total\ moles\ of\ \ solute}$$

$$V = u \times \frac{C_m V_m}{C_m V_m + C_s V_s} = u \times \frac{1}{1 + C_s V_s / C_m V_m}$$

$C_m$: molar concentration of solute in the mobile phase.

$V_m$: The volume of the mobile phase.

$C_s$: molar concentration of solute in the stationary phase.

$V_s$: The volume of the stationary phase.

Substitution of equation(1) into this equation gives an expression of the rate of solute migration as a function of volumes of the stationary and mobile phases:

$$V = u \times \frac{1}{1 + KV_s / V_m} \qquad (5)$$

# The Capacity Factor

- The *capacity factor* is an important experimental parameter that is widely used to describe the migration rates of solutes on columns. For a solute A, the capacity factor $K`_A$ is defined as

$$K'_A = \frac{K_A V_s}{V_m} \qquad (6)$$

- Where $K_A$ is the partition constant for the species A.
- Substitution of equation (6) into (5) yields:

$$V = u \times \frac{1}{1 + K'_A} \qquad (7)$$

- In order to show how K`$_A$ can be derived from a chromatogram, we substitute Equation 3 and 4 into Equation 7:

$$\frac{L}{t_R} = \frac{L}{t_m} \times \frac{1}{1 + K'_A} \qquad (8)$$

This equation rearranges to

$$K'_A = \frac{t_R - t_M}{t_M} \qquad (9)$$

- When the capacity factor for a solute is much less than unity, elution occurs so rapidly that accurate determination of the retention times is difficult.
- When the capacity factor is larger than 20 to 30, elution times become inordinately long.

-Ideally, separations are performed under conditions in which the capacity factors for the solutes in a mixture are in the range between 1 and 5

# Relative Migration Rates: The Selectivity Factor

-The *selectivity factor α* of a column for the two species A and B is defined as

$$\alpha = \frac{K_B}{K_A} \qquad (10)$$

- Where $K_B$ is the partition constant for more strongly retained species B and $K_A$ is the constant for the less strongly held or more rapidly eluted species A.
- According to this definition, α is always greater than unity.
- Substitution of Equation 6 and analogous equation for solute B into 10 provides after rearrangement a relationship between the selectivity factor for two solutes and their capacity factor:

$$\alpha = \frac{K'_B}{K'_A} \qquad (11)$$

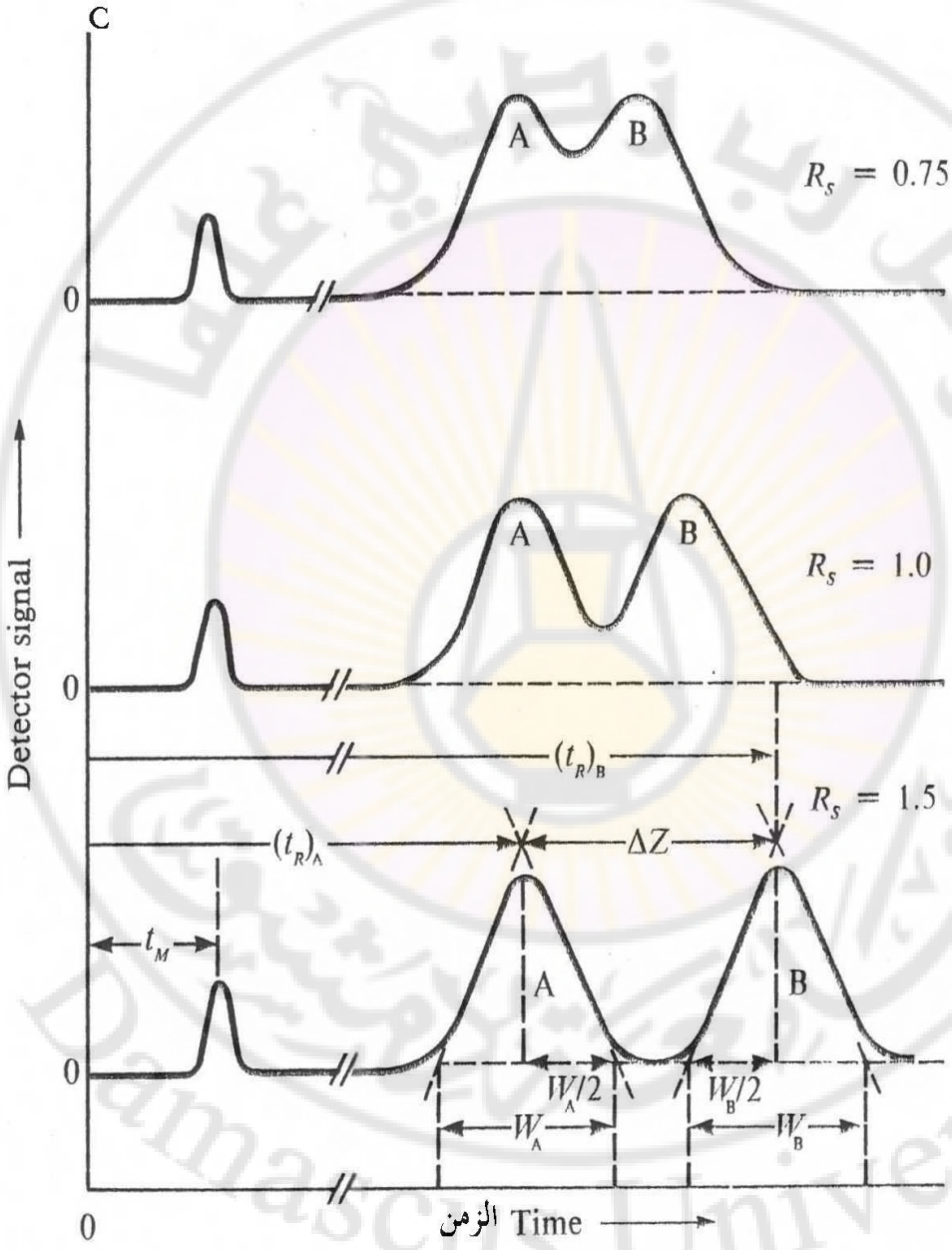
- Substitution of Equation9 for the two solutes into Equation 11 gives an expression that permits the determination of α from an experimental chromatogram

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

# Column Resolution

- The resolution of a column provides a quantitative measure of its ability to separate two analytes. For separation two solutes A and B on a column the resolution is defined as:

$$R_s = \frac{2\Delta t_R}{W_A + W_B}$$



# Example 2

Substances A and B have retention times of 16.40 min and 17.63 min, respectively, on a 30.0 cm column. An unretained species passes through the column in 1.30 min. The peak width for A and B are 1.11 and 1.21 , respectively. Calculate a)- column resolution b)- average number of plates in column, c)- plate height d)- the selectivity factor α

# Applications of chromatography

1- Qualitative analysis

2- Quantitative analysis

# Qualitative analysis

- Chromatography is widely used for recognizing the presence or absence of components in mixtures that contain a limited number of species whose identities are known. For example, 30 or more amino acids in protein hydrolysation can be detected by means of a chromatogram.
- On the other hand, because a chromatogram provide but a single piece of information about each species in a mixture, the application of technique to the qualitative analysis of complex samples of unknown composition is limited.
- Its important to note that while a chromatogram may not lead to positive identification of the species in a sample, it often provide sure evidence of the absence of species.

# Quantitative Analysis

- Chromatography owes its enormous use to its speed, simplicity , relatively low cost, and wide applicability as a tool for separations. It can also provide quantitative information about separated species.
- Quantitative chromatography is based on a comparison of either the height or area of analyte peak with that of one or more standards.

# Analysis Based on Peak Area

- Peak area independent of broadening effect caused by column temperature, eluent flow rate, and the great ability of substance to retain by stationary phase.
- From this standpoint, therefore, area is more satisfactory analytical parameter than peak height.
- On the other hand, peak heights are more easily measured and, for narrow peak, more accurately determined.
- Most modern chromatographic instruments are equipped with electronic integrators that provide precise measurements of relative peak areas.

# Gas Chromatography (GC)

# Description of GC

- In gas chromatography (GC) the components of a vaporized sample are distributed between a mobile gaseous phase and a liquid or solid stationary phase.
- There are two types of gas chromatographic separation depending on the type of used stationary phase. They are:

  1- Gas liquid chromatography (GLC), in which the stationary phase is a liquid supported on an inert solid and it's the most widely used in chromatographic separation.

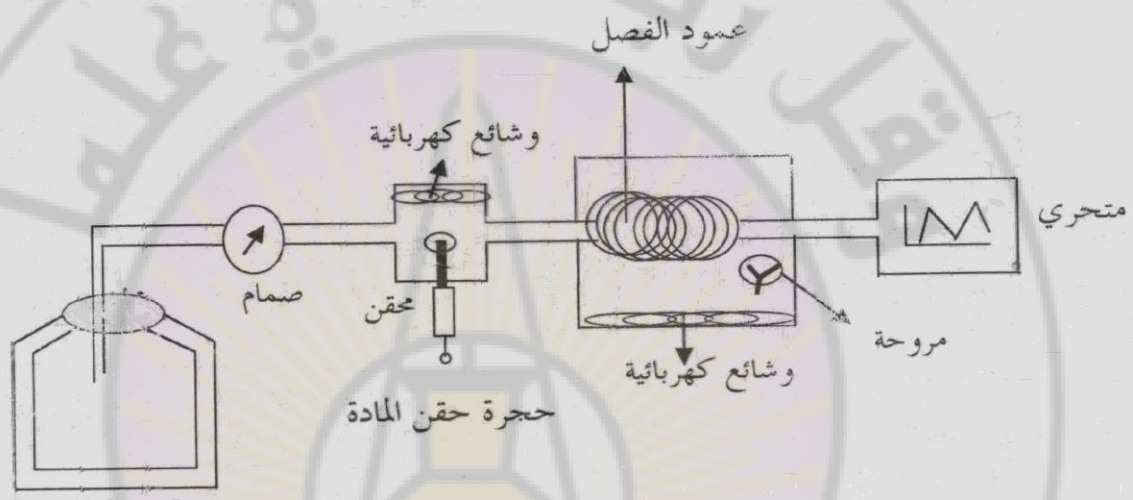
  2- Gas solid chromatography (GSC), in which the stationary phase is an adsorbent.

# Apparatus (Performing GC separation)

- In gas chromatography, the sample is converted to vapor state ( if it is not already a gas) by injection into heated port, and the eluent is a gas (carrier gas).
- The stationary phase is generally a nonvolatile liquid supported on a capillary wall or on inert solid particles. It may also be an solid substance, which can be adsorbent.
- There are a large number of liquid phases available, and it is by changing the liquid phase rather than the mobile phase, that different separation accomplished. The most important factor in gas chromatography is the selection of proper column (stationary phase) for the particular

**Separation to be attempted.**

- **The nature of the liquid or solid phase will determine the exchange equilibrium with the sample components, and this will depend on the solubility or adsorbability of the sample, the polarity of the stationary phase and sample molecules. The mobile phase has no interaction with the sample components.**
- **The basic component of gas chromatographic apparatus are shown in the next figure:**


عمود الفصل
وشائع كهربائية
متحري
صمام
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حجرة حقن المادة
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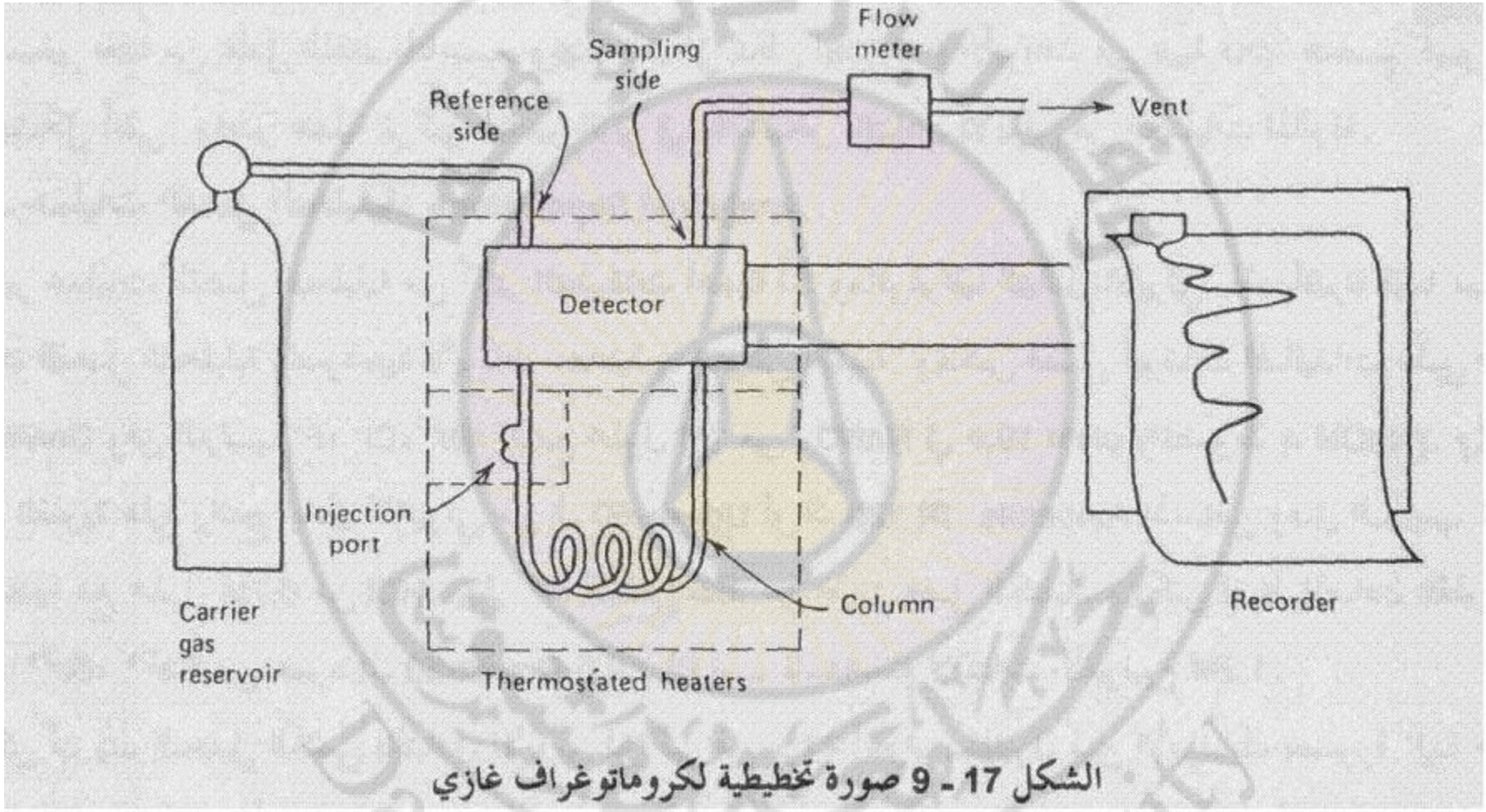
Gas Chromatography (IQOG-CSIC).mp4

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## 1- Carrier gas supply:

**The gaseous mobile phase must be chemically inert. Helium is the most common mobile phase, although argon, nitrogen. These gases are available in pressurized tanks. Pressure regulator and flow meter are required to control the flow rate of the gas.**

## 2- Sample injection system:

**The sample is rapidly injected by means of hypodermic microsyringe through silicon rubber septum into the column. The sample inject port is heated to temperatures at which the usually about 50˚C above the boiling point of the highest boiling solute**

## 3- Gas Chromatography Columns:

**The two types of columns used in GC are packed columns and capillary columns.**

**Packed columns were the first type and were used for many years. Capillary columns are more commonly used today, but packed columns are still used for many applications.**

**3-1- Packed Columns: Columns can be in any shape that will fill heating oven. Columns forms include coiled tubes, U-shaped tubes, and W-shaped tubes, but coils are most commonly used. Typical packed columns are 1 to 10 m long and 0.2 to 0.6 cm in diameter.**

**Short columns may be made of glass, but longer column may be made of stainless steel. But for inertness glass is preferred for shorter columns.**

**The column is packed with small particles that may themselves serve as the stationary phase (GSC) or more commonly are coated with a nonvolatile liquid phase of varying polarity (GLC).**

**3-2- Capillary columns: can provide very high resolution, compared with packed columns. They increase number of plates with the stationary phase supported on the inner wall.**

**Capillaries are made of stainless steel and they are 0.1 to 0.5 mm in diameter, with lengths of 15 to 100 m and can have hundred thousand plates.**

**Capillary columns offer advantages of high resolution with narrow peaks, short analysis time, and high sensitivity.**

# 4- GC. Detectors:

**Since the initial experiments with GC were begun, over 40 detectors have been developed. Some are designed to respond to most compounds in general, while others are designed to be selective for particular types of substances.**

**Characteristics of ideal Detector used in GC:**

- **High sensitivity**
- **Good stability and reproducibility**
- **Short response time**
- **Nondestructive of sample as possible.**
- **The response that is measured by detector, must be related with concentrations or quantities of separated components of sample.**

**We must say that no detector exhibits all these characteristics but most of them.**

**We describe some of the more widely used detectors.**

# 4-1- Thermal conductivity detector (TCD):

The original GC detector was the **TCD.** As a gas is passed over a heated filament wire, the temperature and the resistance of wire will vary according to the thermal conductivity of the gas.

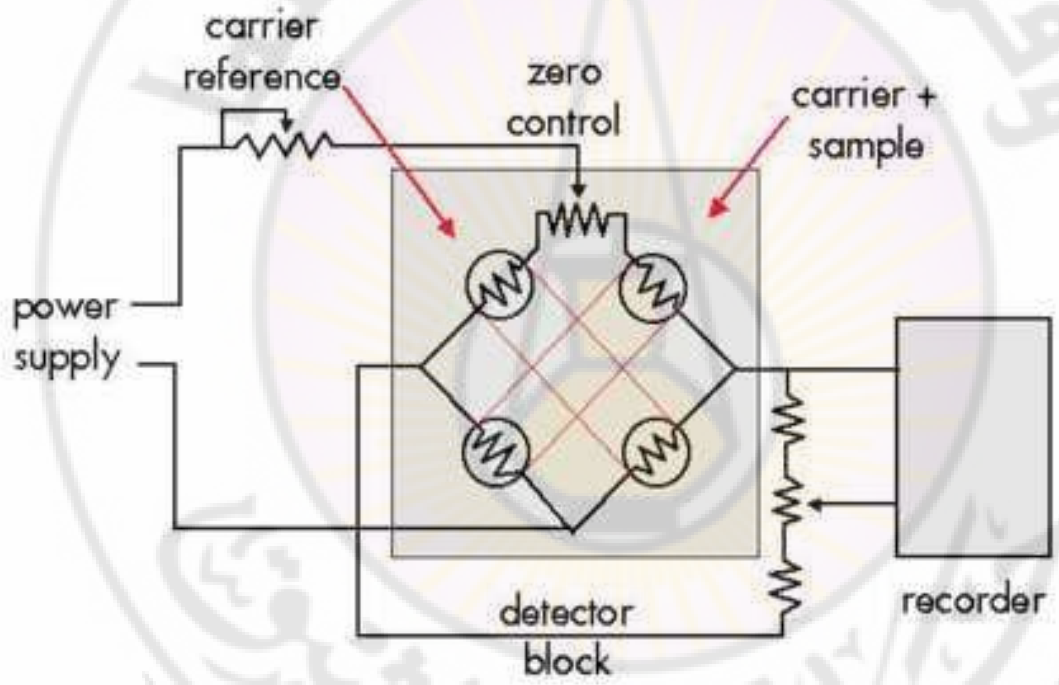
The pure carrier gas is passed over one filament, and the effluent gas containing the sample constituents is passed over another.

These filaments are in opposite arms of a Wheatstone bridge circuit that measures the difference in their resistance. So long as there is no sample gas in the effluent, the resistance of the wires will be the same.

But whenever a sample component is eluted with the carrier gas, a small resistance change will occurs in the effluent arm. The change in the resistance is proportional to the thermal conductivity of the separated component, which is related to the concentration of the sample component in the carrier gas, is registered on the recorder.

-The characteristics of TCD are their simplicity and approximately equal response for most substances . They are also not very sensitive (0.1 µg).

# Thermal conductivity detector



**- The TCD**

1- is a very sensitive detector for organic compounds

2- is selective for organic compounds

3- consist of an electrical cell, its cathode coated with $Ni^{63}$

4- measures a response which is related to the quantity of separated component

a b c d

# 4-2- Flame Ionization Detector (FID)

- **Most organic compounds form ions in a flame. This forms the basis of flame ionization detector (FID). The ions are measured (collected) by a pair of oppositely charged electrodes.**
- **This detector gives excellent sensitivity. Thus is about 1000 times more sensitive than the thermal conductivity detector (0.1ng)**

# Flame ionization detector

Sample components enter at the base of the detector. They mix with hydrogen and enter the flame.

Ions are produced that can be measured.

# 4-3- The electron capture detector (ECD)

- The **ECD** is very sensitive for compounds that contain electronegative atoms and is selective for these.

- The detector cathode consists of a metal foil coated with a β- emitting element, usually nickel-63.
- The cell is normally polarized with an applied potential, and electrons emitted from the source at the cathode strike gas molecules, causing electrons to be released.

- The resulting cascade of thermal electrons is attached to the anode, and establishes a standing current.

When a compound possessing electron affinity is introduced into the cell, it captures electrons to create a large negative ion.

- The negative ion has a mobility in an electric field about 100.000 less than electrons, and so a decrease in current results.

- Relatively few compounds show significant electronegativities, and electron capture is quite selective, allowing the determination of trace constituents in the presence of noncapturing substances. High- electron- affinity atoms or groups include halogens, carbonyls, nitro groups.

- The ECD is widely used for pesticides.

- Compounds with law electron affinities may be determined by preparing appropriate derivatives. Most important biological compounds possess law electron affinities. Steroids such as cholestrol can be determined by preparing their chloracetates derivatives.


Electron capture

β⁻ are emitted by an $^{63}Ni$ source.

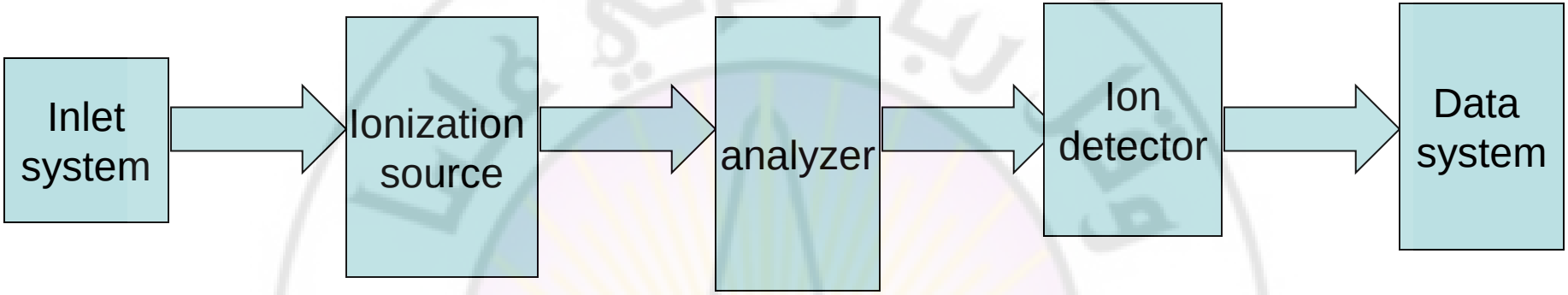
Electrophores will absorb β⁻, reducing the current.

This is the basis for the response

# GC-MS

- One of the most powerful detector for gas chromatography is the mass spectrometer MS. The combination of gas chromatography and mass spectrometry is known as GC/MS.
- mass spectrometer measures the mass- to- charge ratio (m/z) of ions that have been produced from the sample.
- A diagram of a typical molecular- mass spectrometer is shown in the next Figure.

- mass spectrometer




-Sample molecules enter the mass spectrometer through an inlet system.

- Sample molecules are converted to ions and are often fragmented in the ionization source.
- The ions then pass into an analyzer, were they are separated according to their mass- to- charge ratio.
- The separated ions strike an ion detector, where they produce an electrical signal that is recorded and plotted by the data system.

There are other types of detectors, that may used in GC in some cases:

**Flame thermoionic detector**: IS essentially a two-stage flame ionization detector designed to give an increased specific response for nitrogen and phosphorous containing substances. A second flame ionization detector is mounted above the first, with flame gases from the first passing into the second flame. The two stages are divided by a wire mesh screen coated with an alkali salt or base such as sodium hydroxide. The presence of sodium increase the sensitivity 1000 time (0.1PG)

**Electrolytic conductivity detector:** compounds containing halogens, nitrogen, or sulfur are mixed with a gas in a tub. The products are next dissolved in a liquid, which produces a coductive solution. The change in coductivity a result of the presence of the active compound is then measured

**Coulometric detector:** measures the amount of electricity that is needed to perform electrochemical reaction for the separated components. This detector is selective for substances that have electochemical activities

# Gas-Solid Chromatography( stationary phase)

- Gas-solid chromatography is based on adsorption of gaseous substances on solid surface.
- Gas- solid chromatography is performed with both packed and capillary columns, where a thin layer of the adsorbent is affixed to the inner walls of capillary.
- Gas solid chromatography is widely used to separate a mixture of gases (e.g. Methane, Carbon dioxide, Ethylene and Ethane).
- There are many solid stationary phases that are used in GSC such as: Alumina- Chromosorb and celite (diatomic soil)

- **A separation of a mixture of species A and B is achieved by GSC on a column of cellite,**

**A = ester, B = benzoic acid. What are the results of separation?**

1- A is affixed on the stationary phase more than the other

2- A is adsorbed on the stationary phase by hydrogen bonds

3- B has the minimum interaction with the stationary phase.

4- B distributes between carrier gas and solid phase by partition mechanism which is related to the polarity of the stationary phase and the polarity of compound

a b c d e

**- A separation of a mixture of species A, B and C is achieved by GSC on a column of alumina,**

**A = keton, B = salicylic acid, C= Amine . What are the results of separation?**

1- B is the last component that leave the column

2- B has the longest retention time

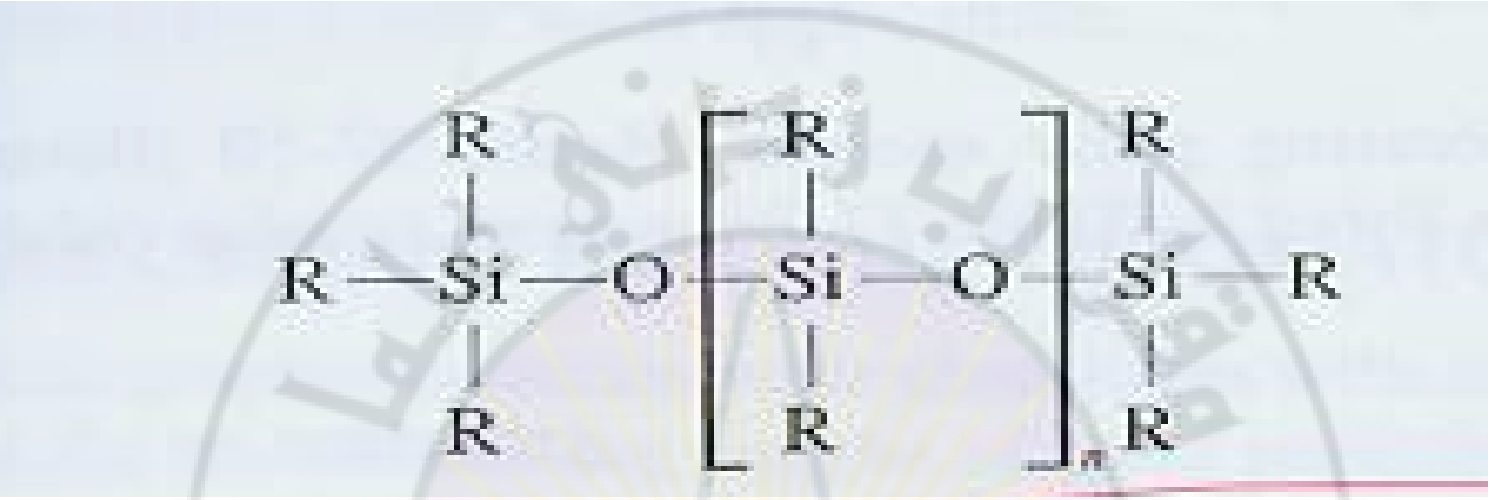
3- C will be the first component that leaves the column

4- A will be adsorbed on the stationary phase more than C and less than A

a b c d e

# Liquid phases for GLC

- **Desirable properties for stationary phase in GLC include: 1)- low volatility 2)- thermal stability 3) high purity.**
- **The most widely used stationary phases for both packed and capillary columns in gas liquid chromatography in order of increasing polarity are illustrated in the next table. These six liquids can probably provide satisfactory separations for 90% or more of the samples encountered by scientist.**
- **Five of the liquids listed in the next table are polydimethyl siloxanes (PDS) derivatives that have the general structure**



- **In the first of these, polydimethyl siloxane, the –R groups are all – $CH_3$, giving a liquid that is relatively nonpolar. In the other polysiloxanes in the table, a fraction of the methyl groups are replaced by functional groups such as phenyl**
- **(-$C_6H_5$), cyanopropyl (-$C_3H_6CN$), and trifluoropropyl (- $C_3H_6CF_3$).**

| L.S.P | Commercial column | Temp | application |
|---|---|---|---|
| polydimethyl siloxane P.D.S | OV1 | 350˚ C | Hydrocarbons- non-polar compounds |
| 5% phenyl P.D.S | OV3 | 350˚ C | Fatty acids methyl esters - Alkaloids |
| 50% phenyl P.D.S | OV17 | 250˚C | Pesticides- Sugars |
| 50%trifluoro-propyl P.D.S | OV210 | 200 ˚C | Chlorinated aromatic-nitro aromatic |
| Polyethylene glycol | Carbowax | 250˚C | Free acids- essential oils |
| 50%cyano-propyl P.D.S | OV275 | 240˚C | Free acids - Alcohols |

**7- A separation of a mixture of species A, B and C is achieved by GLC on the column OV3, where they have approximate boiling points,**
**A = olefin, B = ester, and C= amine. The results of separation are as follows**:

1- C affixed on the stationary phase more than the other
2- B is adsorbed on the stationary phase by hydrogen bonds
3- A has the less interaction with the stationary phase so it migrates the first.
4- C has a narrow peak with long retention time

a b c d e

# Temperature Selection

- The proper temperature selection in GC is a compromise between several factors. The **injection temperature** should be relatively high, consistent with thermal stability of the sample, to give fastest rate of vaporization to get the sample into the column in a small volume decreased spreading and increased resolution result.
- The **column temperature** is a compromise between speed, sensitivity and resolution. At high column temperature, the sample components spend most of their times in the gas phase and so they are eluted quickly, but resolution is poor. At law temperature they spend more time in the stationary phase and eluted slowly, resolution is increased but sensitivity is decreased due to increased spreading of the peaks.

- The **detector temperature** must be high enough to prevent condensation of the sample components.

- Separation can be facilitated by temperature programming, and most gas chromatographs have temperature programming capabilities. The temperature is automatically increased at a preselected rate during the running of the chromatogram. In this way the compound eluted with more difficulty can be eluted in a reasonable time without forcing the others from the column too quickly.

# Factors that affect on gas separation

There are several factors affect on gas separation, the most important of them are:

1- The polarity:

The successful gas separation depends on the similarity of polarity between the sample components and the stationary phase.

- When the polarities of the analyte and the stationary phase are different, the analyte will leave the column at the first, and its peak will be narrow with short retention time, while the analyte has relative long retention time and its peak will be sometimes broad, when its polarity and stationary phase polarity are the same.

## 2- The temperature:

When the temperature increases, the bonds between the stationary phase and the sample components will be weaker, so the fixing of the analyte on the stationary phase will reduce, and the rate of the mobile phase will increase. As a result the peak of the analyte will be narrow with short retention time, and vice versa.

## 3- The mobile phase rate:

If the rate of the mobile phase increases, the analyte will remain short time on the stationary phase and leave the column quickly. Then its peak will be narrow with short retention time, and vice versa.

We must say, that there are certain conditions to separate sample components successfully, according to the temperature, mobile phase rate, and polarity.

# Quantitative Measurements using GC

- The concentrations of eluted solutes are proportional to the areas under the recorded peaks. Electronic integrations in GC instruments print out the areas of peaks, and the retention times of peaks are also generally printed.
- The method of standard additions is a useful technique for calibrating, especially for occasional samples. One or more aliquots of the sample are spiked with a known concentration of standard, and the increase in peak area is proportional to the added standard. This method has the advantage of verifying that the retention time of the unknown analyte is the same as that of the standard.

# Application of gas chromatography

Gas chromatography is widely used in the following analyses:

1- Using gas separation to detect traces of anaesthetic drugs in the blood.

2- Using gas separation to analyze food components and food additives or compounds that permeate to the food.

3- Using gas separation for making quality control on medicaments

4- Using gas separation in environmental analyses such as the determination of pollutants in ground water

- When silica particles are bonded with hydrophobic phase, they become “waterproof” and must be conditioned in order to interact with aqueous samples.
- This is accomplished by passing methanol or similar solvent through the sorbent bed. This penetrates into the bonded layer and permits water molecules and analyte to diffuse into the bonded phase.

After conditioning water is passed to remove the excess solvent prior to adding the sample.

- In this technique the analyte and other sample constituents are extracted on the sorbant extraction bed. A rinsing step removes some of the undesired analyte, while elution removes desired analyte.

# Gas Chromatography (GC)

# Description of GC

- In gas chromatography (GC) the components of a vaporized sample are distributed between a mobile gaseous phase and a liquid or solid stationary phase.
- There are two types of gas chromatographic separation depending on the type of used stationary phase. They are:

  1- Gas liquid chromatography (GLC), in which the stationary phase is a liquid supported on an inert solid and it's the most widely used in chromatographic separation.

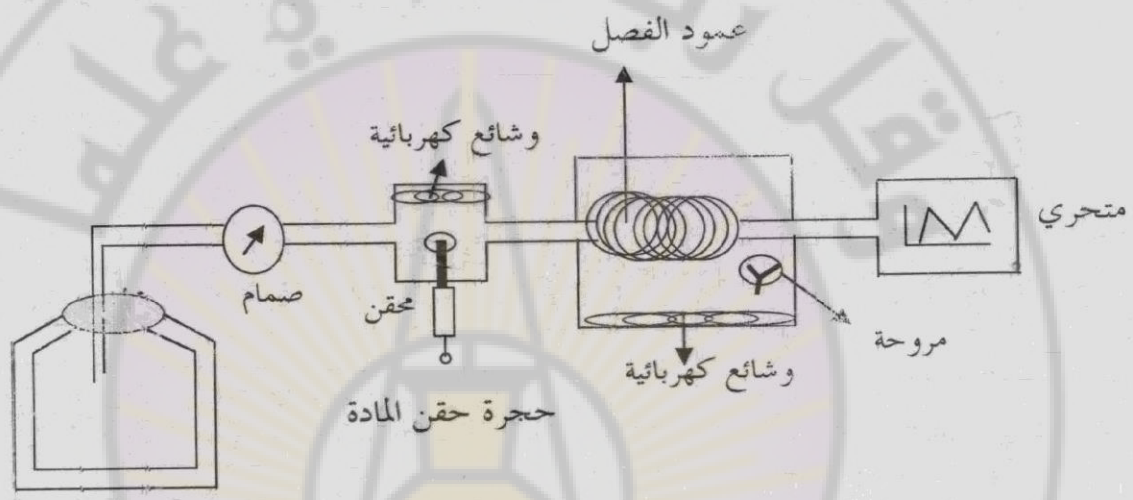
  2- Gas solid chromatography (GSC), in which the stationary phase is an adsorbent.

# Apparatus (Performing GC separation)

- In gas chromatography, the sample is converted to vapor state ( if it is not already a gas) by injection into heated port, and the eluent is a gas (carrier gas).
- The stationary phase is generally a nonvolatile liquid supported on a capillary wall or on inert solid particles. It may also be an solid substance, which can be adsorbent.
- There are a large number of liquid phases available, and it is by changing the liquid phase rather than the mobile phase, that different separation accomplished. The most important factor in gas chromatography is the selection of proper column (stationary phase) for the particular

**Separation to be attempted.**

- **The nature of the liquid or solid phase will determine the exchange equilibrium with the sample components, and this will depend on the solubility or adsorbability of the sample, the polarity of the stationary phase and sample molecules. The mobile phase has no interaction with the sample components.**
- **The basic component of gas chromatographic apparatus are shown in the next figure:**


عمود الفصل
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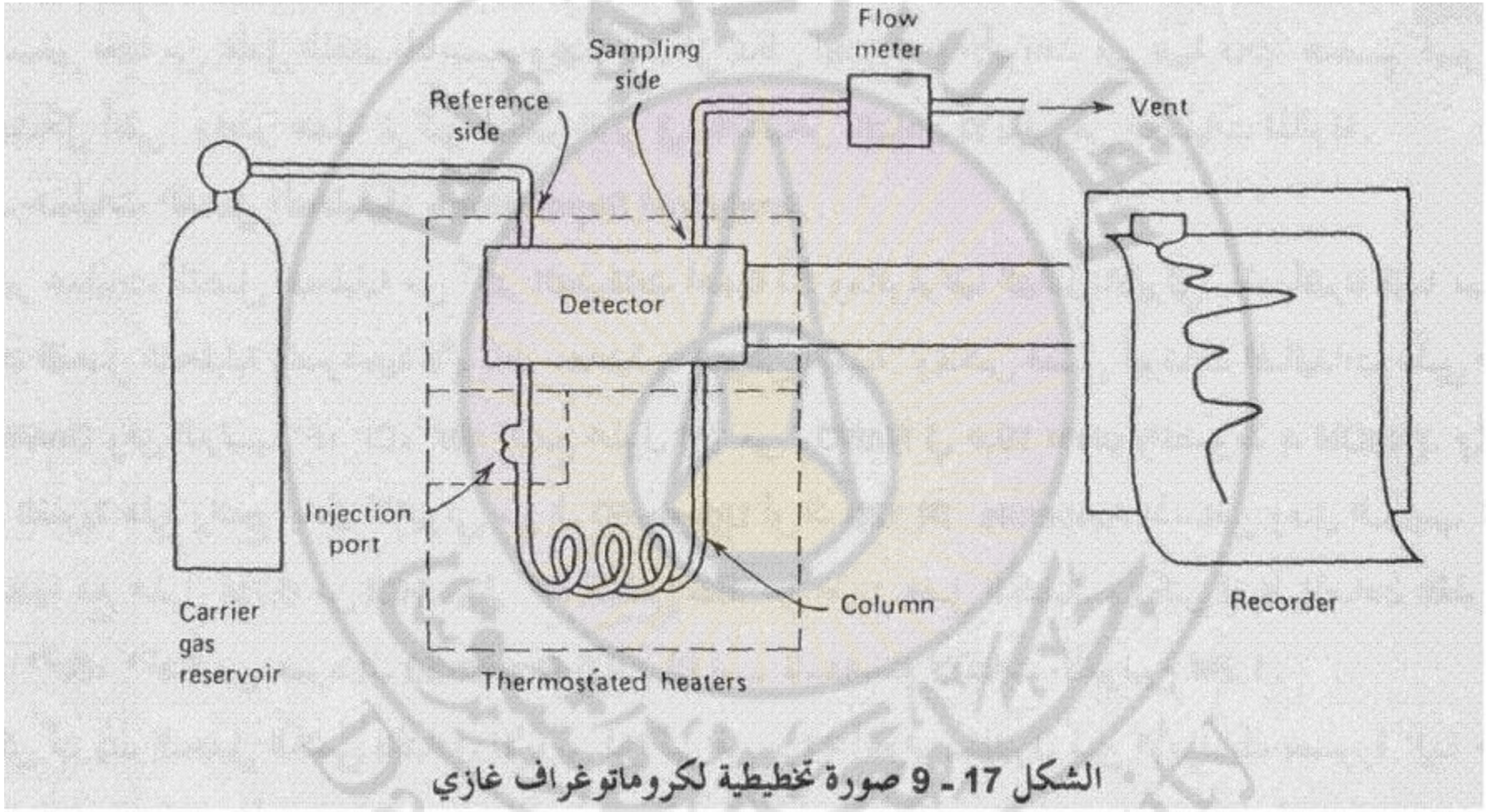
Gas Chromatography (IQOG-CSIC).mp4




الشكل 17 - 9 صورة تخطيطية لكروماتوغراف غازي

## 1- Carrier gas supply:

**The gaseous mobile phase must be chemically inert. Helium is the most common mobile phase, although argon, nitrogen. These gases are available in pressurized tanks. Pressure regulator and flow meter are required to control the flow rate of the gas.**

## 2- Sample injection system:

**The sample is rapidly injected by means of hypodermic microsyringe through silicon rubber septum into the column. The sample inject port is heated to temperatures at which the usually about 50˚C above the boiling point of the highest boiling solute**

## 3- Gas Chromatography Columns:

**The two types of columns used in GC are packed columns and capillary columns.**

**Packed columns were the first type and were used for many years. Capillary columns are more commonly used today, but packed columns are still used for many applications.**

**3-1- Packed Columns: Columns can be in any shape that will fill heating oven. Columns forms include coiled tubes, U-shaped tubes, and W-shaped tubes, but coils are most commonly used. Typical packed columns are 1 to 10 m long and 0.2 to 0.6 cm in diameter.**

**Short columns may be made of glass, but longer column may be made of stainless steel. But for inertness glass is preferred for shorter columns.**

**The column is packed with small particles that may themselves serve as the stationary phase (GSC) or more commonly are coated with a nonvolatile liquid phase of varying polarity (GLC).**

**3-2- Capillary columns: can provide very high resolution, compared with packed columns. They increase number of plates with the stationary phase supported on the inner wall.**

**Capillaries are made of stainless steel and they are 0.1 to 0.5 mm in diameter, with lengths of 15 to 100 m and can have hundred thousand plates.**

**Capillary columns offer advantages of high resolution with narrow peaks, short analysis time, and high sensitivity.**

# 4- GC. Detectors:

**Since the initial experiments with GC were begun, over 40 detectors have been developed. Some are designed to respond to most compounds in general, while others are designed to be selective for particular types of substances.**

**Characteristics of ideal Detector used in GC:**

- **High sensitivity**
- **Good stability and reproducibility**
- **Short response time**
- **Nondestructive of sample as possible.**
- **The response that is measured by detector, must be related with concentrations or quantities of separated components of sample.**

**We must say that no detector exhibits all these characteristics but most of them.**

**We describe some of the more widely used detectors.**

# 4-1- Thermal conductivity detector (TCD):

The original GC detector was the **TCD.** As a gas is passed over a heated filament wire, the temperature and the resistance of wire will vary according to the thermal conductivity of the gas.

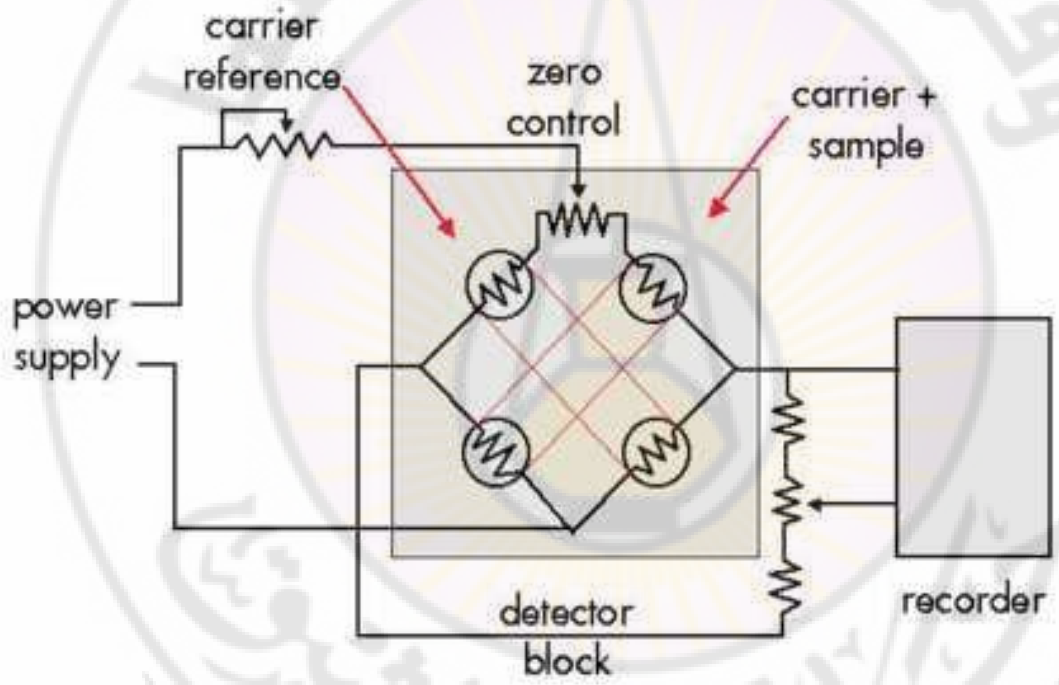
The pure carrier gas is passed over one filament, and the effluent gas containing the sample constituents is passed over another.

These filaments are in opposite arms of a Wheatstone bridge circuit that measures the difference in their resistance. So long as there is no sample gas in the effluent, the resistance of the wires will be the same.

But whenever a sample component is eluted with the carrier gas, a small resistance change will occurs in the effluent arm. The change in the resistance is proportional to the thermal conductivity of the separated component, which is related to the concentration of the sample component in the carrier gas, is registered on the recorder.

-The characteristics of TCD are their simplicity and approximately equal response for most substances . They are also not very sensitive (0.1 µg).

# Thermal conductivity detector



**- The TCD**

1- is a very sensitive detector for organic compounds

2- is selective for organic compounds

3- consist of an electrical cell, its cathode coated with $Ni^{63}$

4- measures a response which is related to the quantity of separated component

a b c d

# 4-2- Flame Ionization Detector (FID)

- **Most organic compounds form ions in a flame. This forms the basis of flame ionization detector (FID). The ions are measured (collected) by a pair of oppositely charged electrodes.**
- **This detector gives excellent sensitivity. Thus is about 1000 times more sensitive than the thermal conductivity detector (0.1ng)**

# Flame ionization detector

Sample components enter at the base of the detector. They mix with hydrogen and enter the flame.

Ions are produced that can be measured.

# 4-3- The electron capture detector (ECD)

- The **ECD** is very sensitive for compounds that contain electronegative atoms and is selective for these.

- The detector cathode consists of a metal foil coated with a β- emitting element, usually nickel-63.
- The cell is normally polarized with an applied potential, and electrons emitted from the source at the cathode strike gas molecules, causing electrons to be released.

- The resulting cascade of thermal electrons is attached to the anode, and establishes a standing current.

When a compound possessing electron affinity is introduced into the cell, it captures electrons to create a large negative ion.

- The negative ion has a mobility in an electric field about 100.000 less than electrons, and so a decrease in current results.

- Relatively few compounds show significant electronegativities, and electron capture is quite selective, allowing the determination of trace constituents in the presence of noncapturing substances. High- electron- affinity atoms or groups include halogens, carbonyls, nitro groups.

- The ECD is widely used for pesticides.

- Compounds with law electron affinities may be determined by preparing appropriate derivatives. Most important biological compounds possess law electron affinities. Steroids such as cholestrol can be determined by preparing their chloracetates derivatives.


Electron capture

β⁻ are emitted by an $^{63}Ni$ source.

Electrophores will absorb β⁻, reducing the current.

This is the basis for the response

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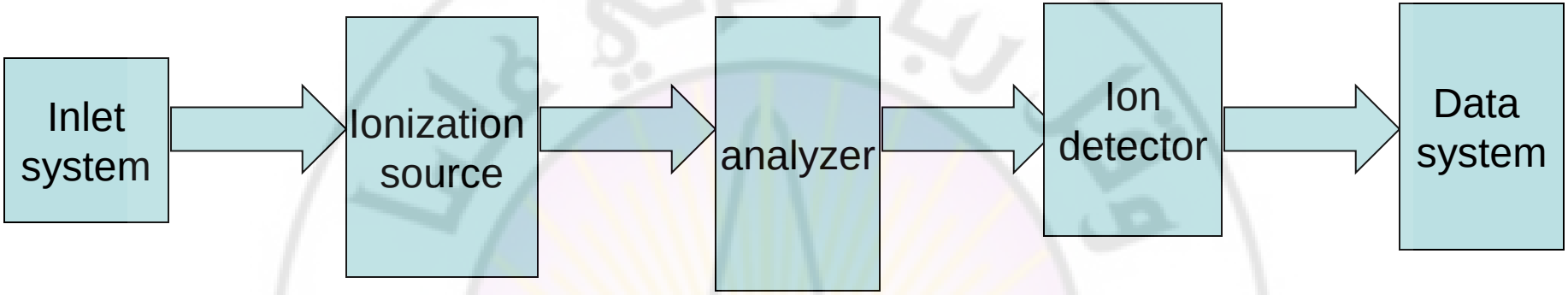


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- **Desirable properties for stationary phase in GLC include: 1)- low volatility 2)- thermal stability 3) high purity.**
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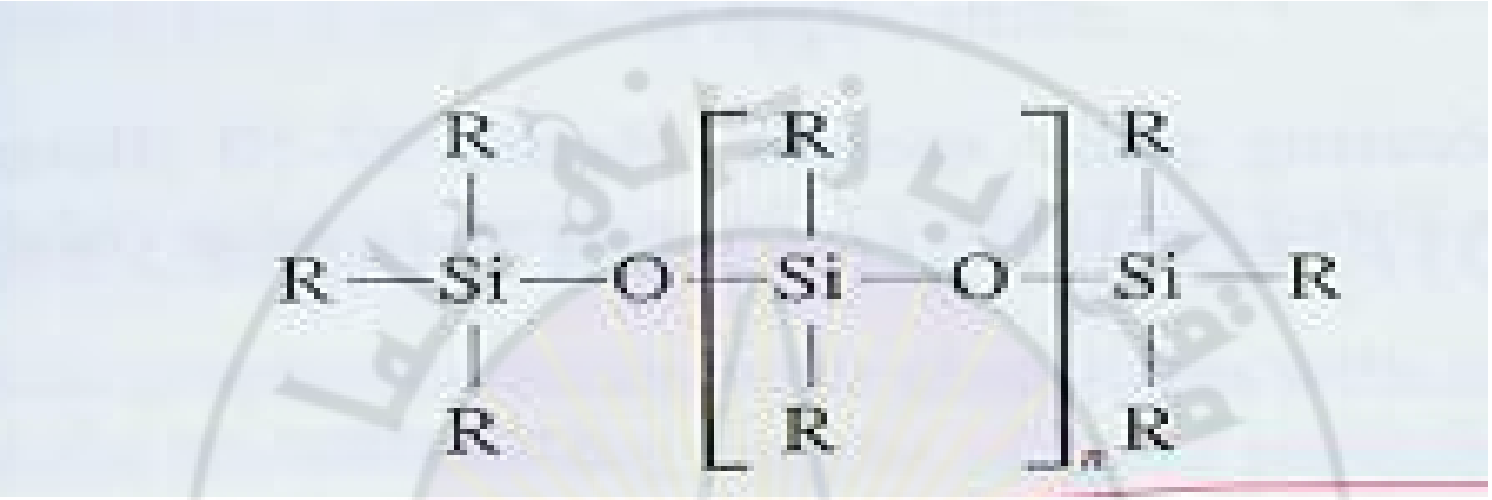

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# Ion Exchange Separation

Dr. Fida Am Ali

# Definition of Ion Exchange Separation

- Ion Exchange separation depends on the exchange of retained ions on porous, essentially insoluble solid phase with the ions in aqueous solution that is brought in contact with the solid phase.

- So There are two phases : Solid phase and aqueous phase
- The most widely use solid phase is synthetic resins , thus the solid phase is ion exchange resin

# Conditions for successful ion exchange separation

1- The exchange should be rapid

2- The contact surface between the tow phases should be large

3- The ion exchange resin should not give any ions to the aqueous phase except the ions that should be exchanged

4- The ion exchange resin should be porous, non soluble in water

5- The ion exchange resin should be resistant to acids and bases

# Ion Exchange Resins and their kinds

- The ion Exchange resins are highly molecular weight polymer (consist of polystyrene crosslinked with divinylbenzene) that contain a large number of ionic functional group per molecule .
- **Kinds of Ion Exchange Resins**

There are two types of Ion Exchange resins:

1- Cation exchange resins (acidic exchange resins)

2- Anion exchange resins ( basic exchange resins)

## Cation Exchange Resins:

These resins contain acidic cation exchangers and have four types

1- Sulfonic exchange resin R- $SO_3^-$ $H^+$

2- carboxylic exchange resin R – $COO^-$ $H^+$

3- Amino diacetic exchange resin R- N- $(CH_2COO^-)_2$ 2 $H^+$

4- phosphonic exchange resin R- $PO_3^{-2}$ $2H^+$

## Anion Exchange Resins:

These resins contain basic anion exchangers and have three types

1- Quaternary ammonium anion exchanger

$R\text{-}N^{+}\text{-}(CH_3)_3OH^{-}$

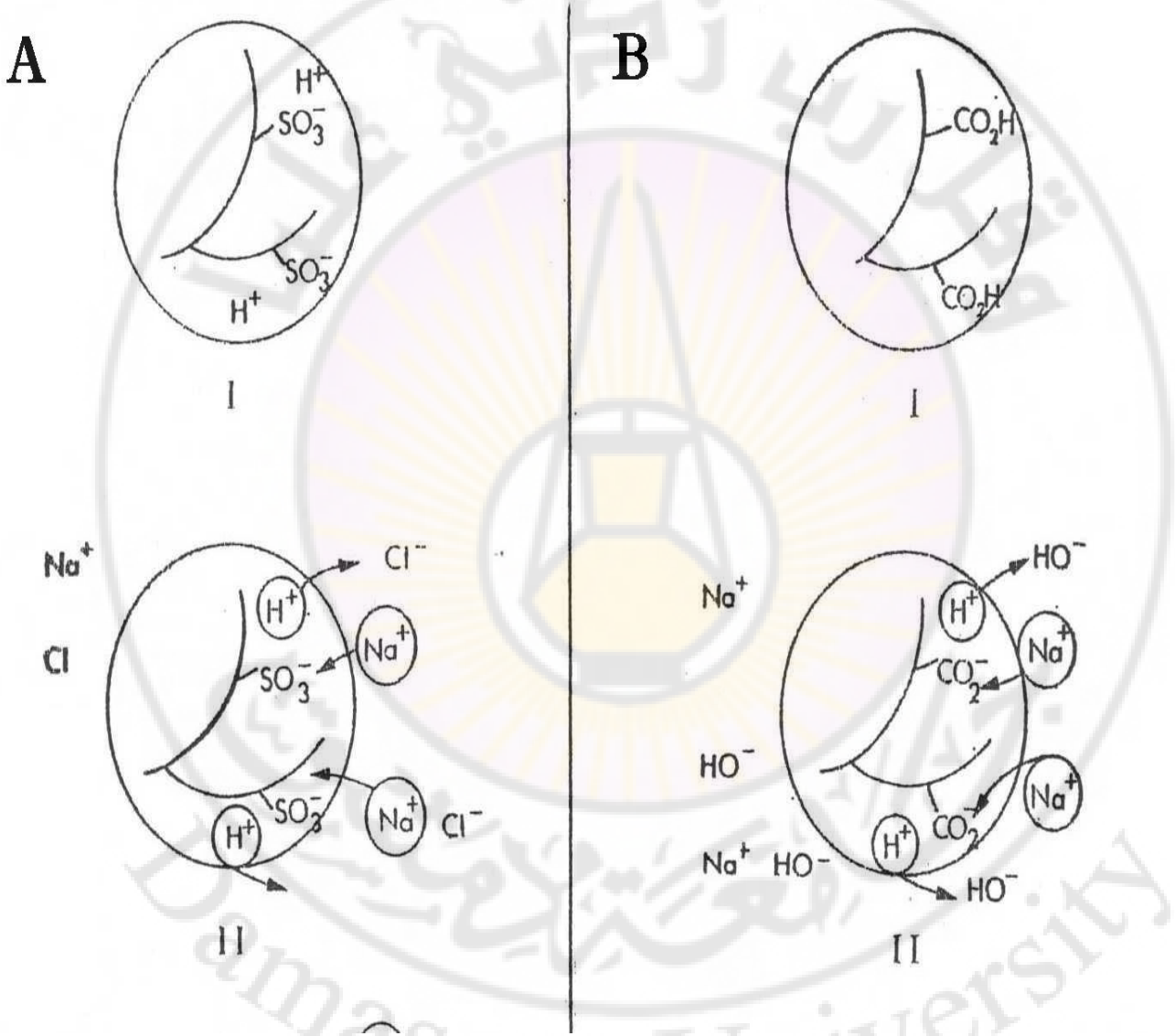
2- amino anion exchanger $R\text{-}NH^{+}\text{-}R'_2\ OH\text{-}$

3- Solfonium anion exchanger $R\text{-}S^{+}\text{-}R'_2\ OH^{-}$

# Mechanism of Ion Exchange

The exchange of ions occurs between ions retained on resin and ions exist in aqueous solution. So the exchanged ions should have the same charge but different nature.

The exchange occurs between charge units in aqueous to charge units in resin . So if $Na^{+}$ exist in aqueous solution it will exchange with one $H^{+}$in resin but if $Ca^{+2}$ exist in aqueous solution it will exchange with two $H^{+}$ in resin.



We can write an equilibrium reaction for each ion exchange process as the followings:

$$\underset{\text{solid}}{xRSO_3^- H^+} + \underset{\text{solu}}{M^{+x}} \rightleftharpoons \underset{\text{solid}}{(RSO_3^-)_x\ M^{+x}} + \underset{\text{solu}}{x\ H^+}$$

$$xRN(CH_3)_3^+\ OH^- + A^{-x} \rightleftharpoons [RN(CH_3)_3^+]_x\ A^{-x} + x\ OH^-$$

**The Exchange Capacity of Resin** : is the total number of equivalents of replaceable hydrogen or hydroxyl per unit volume or per unit weight of resin

# Ion Exchange Equillibria

Ion exchange equillibria can be treated by the law of mass action. For example when a dilute solution containing calcium ions passed through a column packed with sulfonic resin, the following equillibrium is established:

$$Ca^{+2}_{(aq)} + 2\ H^{+}_{(res)} \rightleftharpoons Ca^{+2}_{(res)} + 2\ H^{+}_{(aq)}$$

$$K = \frac{[Ca^{+2}_{(res)}][H^{+}_{(aq)}]^2}{[Ca^{+2}_{(aq)}][H^{+}_{(res)}]^2} \qquad (1)$$

$$\frac{[Ca^{+2}_{(res)}]}{[Ca^{+2}_{(aq)}]} = K\frac{[H^{+}_{(res)}]^2}{[H^{+}_{(aq)}]^2} = K_D \qquad (2)$$

$K_D$ represent the affinity of the resin for calcium ion. In general, where $K_D$ for an ion is large, a strong tendency for the resin to retain that ion exist, where $K_D$ is small, the opposite is true. Selection of a common reference ion ( such as $H^+$ ) permits a comparison of distribution coefficint for various ions on a given type of resin. Such experiments reveal that the poly charged ions are much more strongly retained than singly charged ions,differences that exist among values for $K_D$ appear to related to the size of the ion. Thus for the ions passed through sulfonic resin the $K_D$ for ions decrease in the order:

$AL^{+3}>Ba^{+2}>Pb^{+2}>Ca^{+2}>Ni^{+2}>Cd^{+2}>Cu^{+2}>Zn^{+2}>Mg^{+2}>Ag^{+}$

$>K^{+}>Na^{+}>H^{+}$

And for the ions passed through quaternary ammonium anion exchanger resin the $K_D$ for ions decrease in the order:

$SO_4^{2-} > I^- > HSO_4^- > NO_3^- > Br^- > Cl^- > HCO_3^- > CH_3COO^- > OH^-$

# Applications of Ion Exchange resins

1)- Eliminate the ions that interfere with an analysis

2)- preparing the deionized water

3)-Estimating the total salt content of sample

4)- water softning

# Example

100 ml of aqueous solution contain $SO_4^{-2}$ passed through a column packed with quaternary ammonium anion exchanger. After contact between the tow phases , the aqueous solution left the column and the concentration of hydroxyl ions in it was estimated to give the value of 20 mmmol/L

1)- Calculate the molarity of $SO_4^{-2}$ in the aqueous solution

2)- Calculate the number of millimoles of $SO_4^{-2}$ in the aqueous solution

3)- Calculate the concentration of $SO_4^{-2}$ in aqueous solution by ppm

# Application of gas chromatography

Gas chromatography is widely used in the following analyses:

1- Using gas separation to detect traces of anaesthetic drugs in the blood.

2- Using gas separation to analyze food components and food additives or compounds that permeate to the food.

3- Using gas separation for making quality control on medicaments

4- Using gas separation in environmental analyses such as the determination of pollutants in ground water

# Thin layer chromatography (TLC)

Dr. Fida Am Ali

# Thin- layer Chromatography (TLC)

- Thin layer chromatography (TLC) is a planar form of chromatography useful for wide-scale qualitative analysis and can also be used for quantitative analysis.

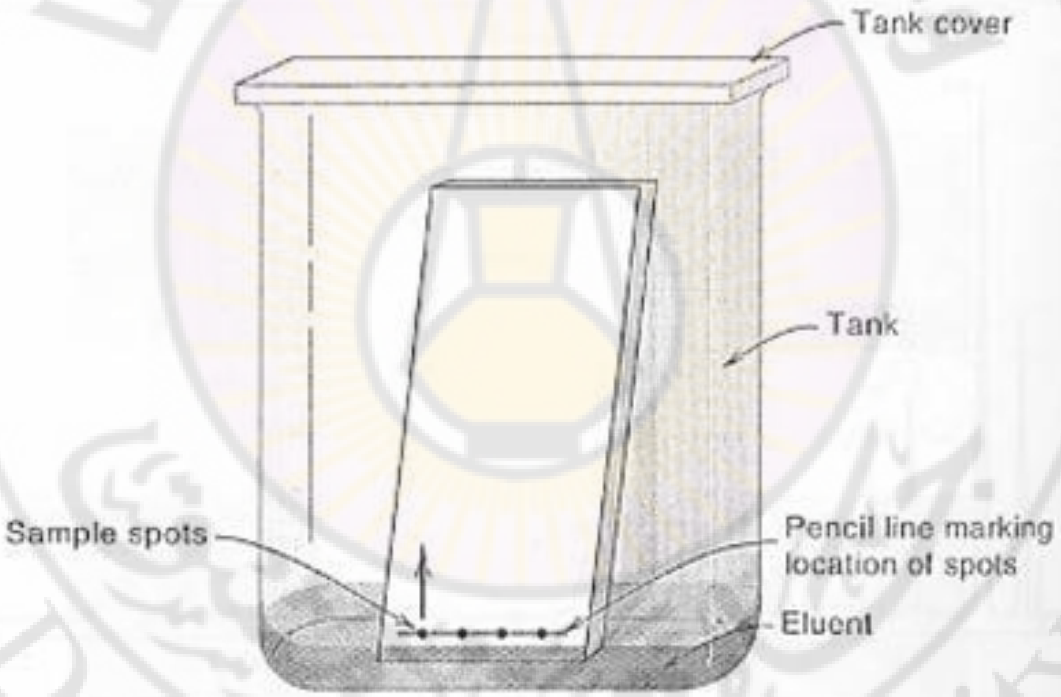
-The stationary phase is a thin layer of finely divided adsorbent supported on a glass or aluminum plate.

- A sample is spotted onto the plate with a micropipet, and the chromatogram is developed by placing the bottom of the plate in a suitable solvent (see the next figure). The solvent is drawn up the plate by capillary action, and the sample components move up the plate at different rates, depending on their solubility in the mobile phase and their ability of retention by the stationary phase.

- The individual solute spots are noted or are made visible by treatment with a reagent that forms a colored derivative.
- The spots will generally move at a certain fraction of the rate at which the solvent moves, and they are characterized by the $R_f$ value:

$$\mathbf{R_f} = \frac{\mathbf{distance\ solute\ moves}}{\mathbf{distance\ solvent\ front\ moves}}$$

- Where the distances are measured from the center of where the sample was spotted at the bottom of the plate. The solvent front will be line across the plate.
- The distance the solute move is measured at the center of the solute spot or at its maximum density, if tailing occurs.



# Developing the Chromatogram

- A typical set up for thin layer chromatography is shown in the last figure. A thin pencil line is drawn across the plate a few centimeters from the bottom, and the sample is spotted on this for future reference in $R_f$ measurements.
- The spot must be made as small as possible for maximum separation and minimum tailing. It is best done to evaporate the solvent after each drop.
- The plate is placed in a *chamber* with its end dipping in the elution solvent. A closed chamber must be used to saturate the atmosphere with the solvent and prevent it from evaporating from the surface of the plate as it moves up. The developing may take 10 min to 1 h, but it requires no operator time.
- Sample size range from 10 to 100 µg per spot.

# Detection of the spots

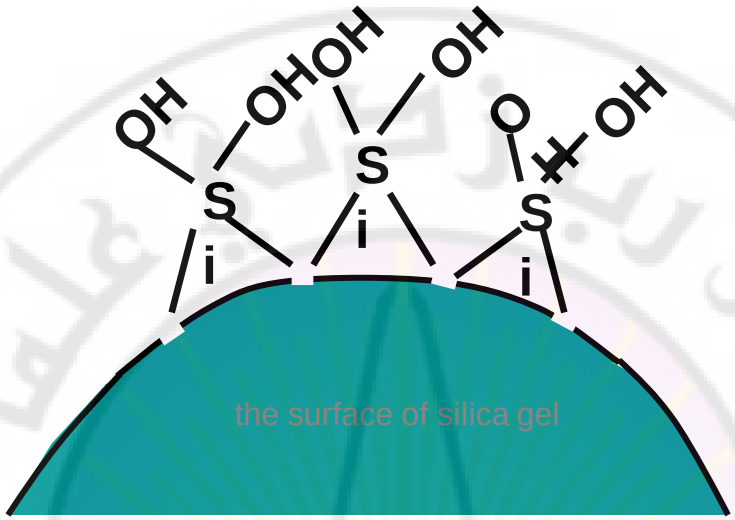
- If the solutes are fluorescent, they can be detected by shining an ultraviolet light on the plate.
- Color developing reagents are often used. For example, amino acids and amines are detected by spraying the plate with a solution of ninhydrin, which is converted to a blue or purple color.
- Colorless or nonfluorescent spots can be visualized by exposing the developed plate to iodine vapor .
- The iodine vapor interacts with the sample components, either chemically or by solubility, to produce a color.
- Thin layer plates are commercially available that incorporated a fluorescent dye in the powdered adsorbent.

- When held under ultraviolet light, dark spots appear where sample spots occur due to quenching of the plate fluorescence.

# Stationary phases for TLC

- The same stationary phases that are used in column chromatography can be used in TLC.
- The stationary phase consists of a finely divided powder (particle size 10 to 50 µm).
- The most commonly used stationary phases are adsorbents.
- Silica gel, alumina and powdered cellulose are the most popular.
- The most popular adsorbent is silica gel.

It is believed that silanol radicals ( -Si-OH ) on the surface of silica gel act as the active site that bind with the sample components by hydrogen bonds.




- Adsorbed water prevents other polar molecules from reaching the surface, so the gel is activated by heating to remove the adsorbed water.
- Alumina also contains oxygen atoms.
- Alumina is preferred for the separation of weekly polar compounds, but silica gel is preferred for separation of polar compounds such as amino acids and sugars.
- Magnesium silicate, calcium silicate, and activated charcoal may also be used as adsorbents.

Sucarose

cellulose

Starch

Talk

Calcium Carbonate

Calcium Sulfate

Calcium phosphte

Magnesium Carbonate (Floresil)

Calcium Oxide

Aluminum silicate ( Floridian)

Silica

Charcoal

Magnesium Oxide

Alumina

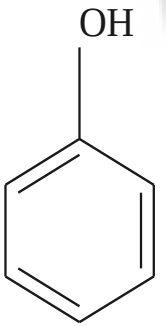
Increasing Polarity

# Mobile Phases for TLC

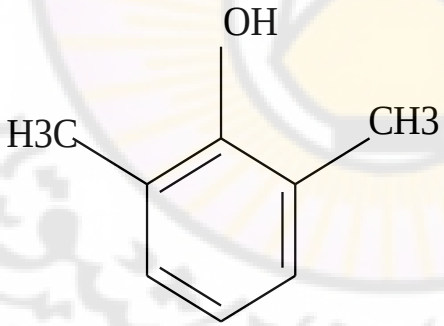
- In adsorption chromatography, the eluting power of solvent increases in order of their polarities (e.g, from hexane to acetone to alcohol to water).
- A single solvent, or at most two or three solvents, should be used whenever possible, because mixed solvents tend to chromaograph as they move up the thin layer, causing a continual change in the solvent composition with distance on the plate.

# Example 1

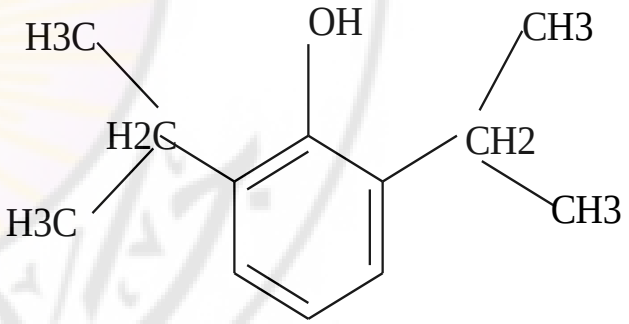
I, II and III three components in a mixture, which must be separated on a thin layer plate. The stationary phase is silica gel supported on an aluminum plate. The mobile phase is hexane. Which compound in the mixture will be separated at the first? and why?




I




II




III

## Example 2

-A separation of a mixture of species A, B and C is performed by TLC on a thin layer of silica and the toluen is used as the mobile phase, where A= amine B = ester C = carboxylic acid. What are the results of separation?