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A Study on the Types of Coulombs in GC and HPLC

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Abstract:

This study is looking for two types of advanced and accurate analytical devices in analytical chemistry, both of which depend on the phases that the material passes through and on its concentration; They are sensitive to the lowest concentrations up to 0.001 ppm. as well as the search for the most important component parts of each device and for the materials that have a measure in these devices (gas chromatography and highperformance liquid chromatography) where the two are similar in terms of the presence of columns and detector. The most important applications for each device and the most important areas it serves were also identified.

General Introduction

The word chromatography is a separation method that was originally based on a series of experiments carried out by the Russian scientist Tweets in 1930. He was able to analyze chlorophyll and separate it into colored sections by filtering it through columns filled with a specific adsorbent and display it with a specific solvent [1].

Is used to separate and estimate microscopic amounts of compounds . In addition to qualitative and quantitative analysis, there are other uses, including preparatory, that is, preparing materials with high purity or to purify materials that contain unwanted impurities. It is also used to obtain information about some physicochemical properties and to study chemical reactions [2].

The International Organization defined chromatography for Applied and Pure Chemistry (IUPAC) as a method for separating a group of compounds in a form where the compounds are between two phases, one of which is fixed and the other is mobile. The stationary phase is either solid or liquid. The equilibrium can be described quantitatively between the stationary and mobile phases by a constant that depends on the temperature, which is the partition factor or so-called distribution coefficient, which can be calculated with the following equation [3].

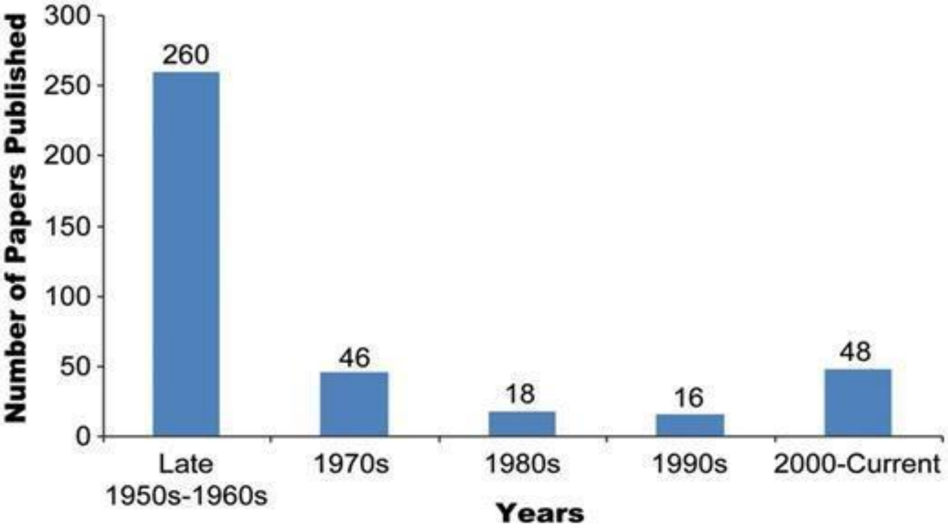
$$k = \frac{Cs}{Cm} \text{ ....(1)}$$

K : Distribution Coefficient

Cs : Concentration of solute in the stationary phase

C_m : Concentration of solute in the mobile phase

Gas chromatography (GC), invented by Martin and Synge in 1941 (1), was first applied for the separation of a series of fatty acids in 1951. After this, the GC technique developed rapidly and was the first analytical instrument to be controlled by a computer [1].



The term preparative GC (pGC) usually describes the use of large-diameter columns to purify relatively large quantities of materials by using a sample collection system at the column outlet . Currently, GC is one of the most important and widespread techniques in the field of analytical chemistry. However, the success of the GC technique is largely associated with chemical analysis instead of pGC .

Gas-liquid chromatography (GLC) is primarily used for the preparation of low- boiling halides or for the study of the structure or configuration of compounds.

The preparation quantities of GLC may go from Nano gram to microgram Although gas-solid chromatography (GSC) allows the harvest of much larger quantities of the target compounds, it has primarily been applied for more types of different chemical compounds or for individual pharmacology/toxicology research. Capillary columns, belonging to GLC, have predominantly been used in GC (including pGC) Capillary columns, belonging to GLC, have predominantly been used in GC (including pGC)[2].

However, the yielded compounds may be contaminated by the vaporized stationary phase when a high separation temperature is applied . Therefore, the stationary phase used in a pGC column must be physically and thermally stable, and chemically inert, to avoid taking part in chemical reaction during the preparation process . On the other hand, for infrared (IR) or nuclear magnetic resonance (NMR) analyses, the response of the stationary phase may mix with the compounds of interest. Therefore, the IR and NMR absorptions of 17 liquid stationary phases (including carboxylic esters, ethers and silicones) commonly used in pGC have been reported [4] .

It is still the most widely used automated analysis technique today, and this type of chromatographic. Process is called elution. The different chromatographic processes are named for the physical state of the mobile phase. Thus, in GC the mobile phase is a gas, and in LC the mobile phase is a liquid. Figure 1.1 shows a flow chart of the various common gas, liquid and liquid chromatographic techniques [5].

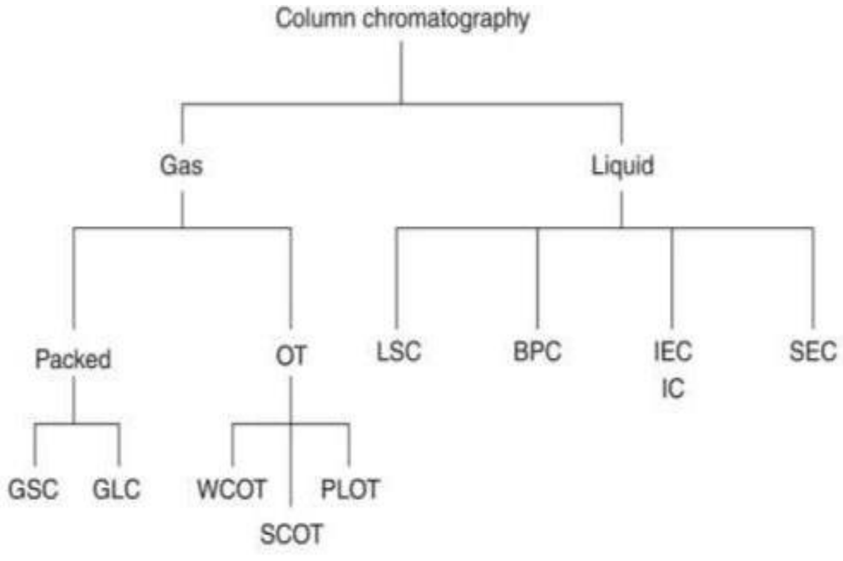


Figure 1.1 Classification of Chromatographic methods [5] .

In GC, the sample is evaporated and carried by the mobile gaseous phase (carrier gas) through the column. In most analyses, samples split (equilibrate) in and out of the stationary liquid phase. Depending on their solubility in the stationary phase at a given temperature, the components of the sample (called substances) separate. Solutes or analytes) from each other based on their relative vapor pressures and their connections to the stationary phase[6].

Within GS, a sub-classification is made according to the state of the stationary phase. If the stationary phase is a solid, then this technique is called gas-solid chromatography (GSC). If it is a liquid, this technique is called gas-liquid chromatography (GLC)).Some capillary columns represent GLC, while others represent GSC. Of the two main types, GLC is the most widely used.[7]

Gas chromatography is a method for separating components of mixture of volatile compounds. In most application the separation are made to identify and determine the quantity of each component of a sample of the mixture ,and analytical gas chromatographic apparatus include additional devices for this purpose .as with all chromatographic techniques the theory depends on the partitioning of the analyze between a stationary and mobile phase .the mobile phase is a gas [5].

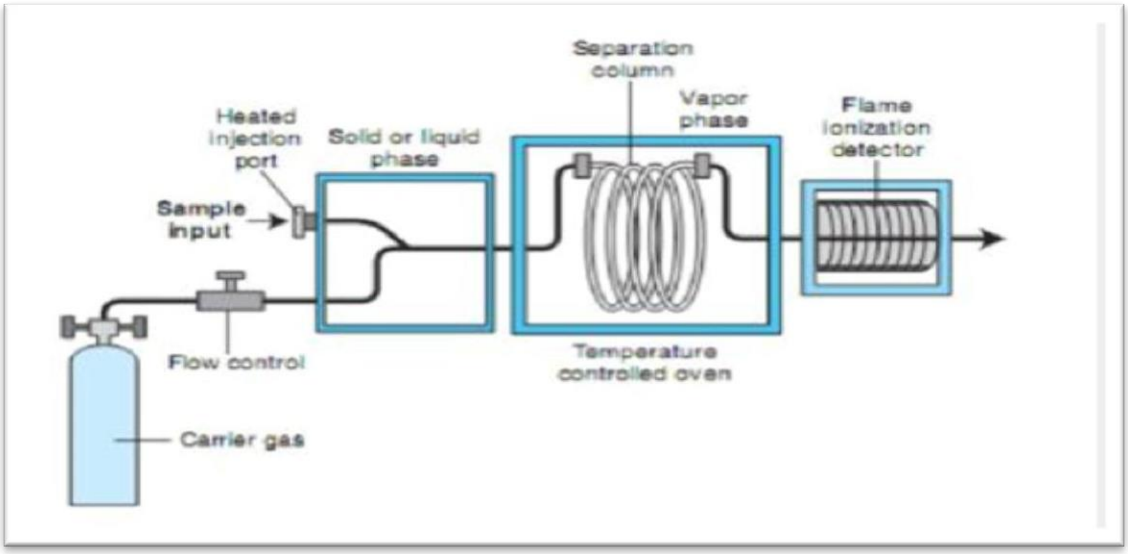


Fig 1-2Illustration of the device GC

which is means that the analyte must be volatile to move through the column and majority analytics have little interaction with mobile phase:

1. Gas-solide chromatography: the stationary phase is in solid at this technique .this functions on the absorbent principle.
2. Gas-liquid chromatograph: the stationary phase is in a liquid at this technique this function on the principle of partition and absorption

GC has replaced distillation as the preferred method for separating volatiles. In both techniques, temperature is a major variable. But gas chromatographic separations also depend on the chemical (polar) nature of the stationary phase. This additional variable makes GC more powerful [8].

The temperature for most gas chromatography operations is about 380 degrees Celsius, so samples need to have an appreciable vapor pressure (60 torr) and at that temperature the solutes usually do not exceed their boiling point of 500 degree Celsius and their weights are 1000 Da [8].

PRINCIPLE

Supply of carrier gas with attached pressure regulators and flow meters in a high-pressure cylinder .carrier gas: He, N, H2 and argon methane (it is favored because of high thermal conductivity .N2is preferred when signification carrier gas consumption is used). Pressure regulators: tank carrier gas moves through a toggle value, a flow meter (11000ml\min) capillary restrictors, and a pressure gage (14atm).the flow rate is balanced using a needle value mounted at the flow meter base and operated by capillary restrictors [6].

In gas chromatography, the elution order of analytic is governed by several factors such as latent vapor pressure Solubility's in stationary phase and propensities for molecular interaction in stationary phase all of these factor change with temperature and their concerted effect ultimately determine the equilibrium distribution of solute molecules between mobile phase and stationary phase.

Chromatography is the analytical techniques use for separation, purification, identification Of constituents from the mixture. This works on the principle of differential interaction of solute with two different phases (stationary phase and mobile phase). In this technique number of compound can be detected [8].

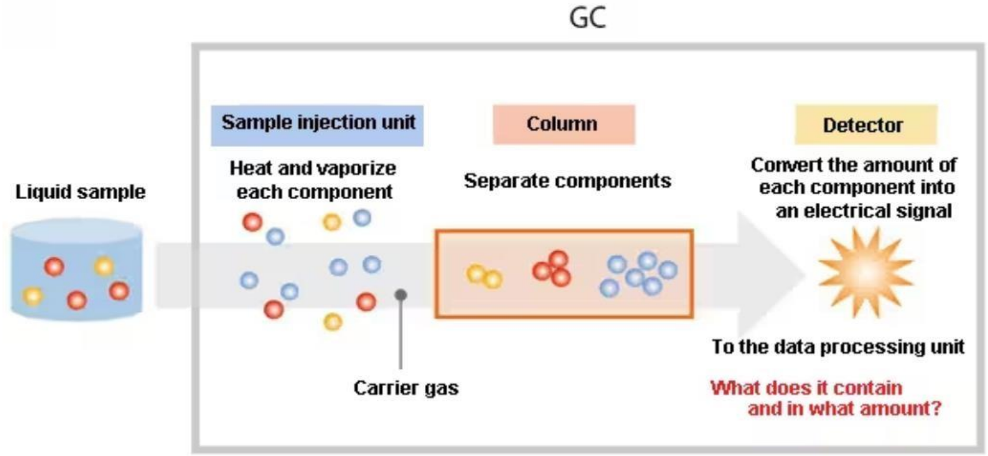


Fig 1-3 the principle of operation of the gas chromatography device

Compounds Suitable for GC Analysis

Components that can be analyzed with GC have the following three main features.

1. Compounds with a boiling point up to 400 °C
2. Compounds that are not decomposed at their vaporization temperature
3. Compounds that decompose at their vaporization temperature, but always by the Same amount. This is called pyrolysis GC [8].

1-3 **COLUMN:**

5-50 m coiled stainless steel |glass Teflon Types of columns depending on its use:

1- Analytical column 1-1.5 meters length ,4-6 mm diameter

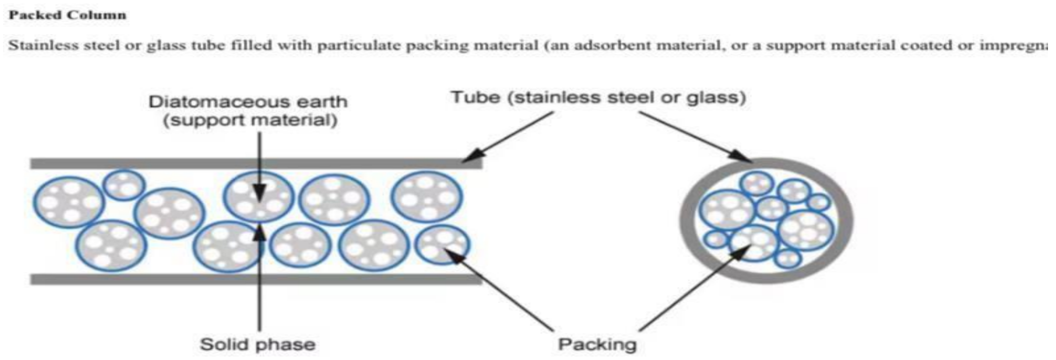
2- Preparative column 3-6meter length , 5-9mm dimeter[2]

Column types depending on its nature:

- packed column-partition and absorption
- Open tubular or capillary column
- WCOT-partition
- SCOT-partition
- PCOT-partition
- Recorder
- Separate thermostat compartment for detector and columns [10]

1-4 Structure of Packed Columns:

Packed columns, also known as packed beds, are made up of a long, narrow tube that is filled with a solid, inert support material that has been coated with a stationary phase .The stationary phase is responsible for the separation of analytes while the support material provides structural stability and ensures uniform gas flow through the column .Common support materials include diatomaceous earth, alumina, and silica, while stationary phases can range from non-polar to highly polar coatings[11].



Packed Column

Stainless steel or glass tube filled with particulate packing material (an adsorbent material, or a support material coated or impregn

1-5 Advantages of Packed Columns:

1. Simplicity: Packed columns are simple to utilize, making them appropriate for everyday analyses. Their ease of use is beneficial for operators with limited chromatographic knowledge.
2. Robustness: When opposed to capillary columns, which can be delicate and quickly contaminated packed columns are more durable and less prone to damage from sample matrices.
3. Versatility: Packed columns may tolerate a wide range of stationary phases, enabling for non-polar and polar compound separations. Because of their adaptability, they are appropriate for a wide range of applications [9].

1-6 Specialty Gas Chromatography Columns:

In addition to the standard non-polar and polar columns, there are specialty columns designed for specific applications:

1. Chiral Columns: Chiral columns are used to separate enantiomers, which are molecular mirror-image isomers. These columns, which contain a chiral stationary phase, are extremely useful in the pharmaceutical and agricultural sectors, where compound stereochemistry is crucial.
2. PLOT Columns: PLOT (porous-layer open tubular) columns are used to analyze gases and light volatile chemicals. They have a porous layer within the capillary column that allows for effective gas separation.
3. Packed Micro columns: Packed micro columns are miniature versions of packed columns used in situations where sample size is limited, such as environmental analysis or when working with valuable samples [12].

1-6 Advantages of Capillary Columns

1. High Resolution: Capillary columns have high resolution and are therefore useful for sorting complicated mixtures and closely eluting substances. Their small diameter reduces peak widening, resulting in crisp, well-defined peaks [5].
2. Sensitivity: Capillary columns are highly sensitive and may identify minute levels of chemicals in samples. This is critical in applications where low-level quantification is required.
3. Versatility: Capillary columns are suitable for separating non-polar, polar, and specialized chemicals because they support a wide range of stationary phases. Analysts can select the stationary phase that best meets their analytical needs
4. Reduced Sample Size: Capillary columns' smaller interior diameter necessitates smaller sample volumes, which is useful when working with restricted or valuable

1-7 Applications of Capillary Columns

1. Environmental Analysis: Capillary columns are widely used in environmental analysis to detect and quantify pollutants, such as volatile organic compounds (VOCs), in air, water, and soil samples.
2. Food and Beverage Analysis: Capillary columns are employed for analyzing flavor compounds, pesticides, and additives in food and beverages, ensuring product safety and quality.
3. Pharmaceuticals: In pharmaceutical laboratories, capillary columns are indispensable for testing the purity and potency of drugs and pharmaceutical raw materials[15].
4. Chiral Separations: Capillary columns with chiral stationary phases are used for separating enantiomers, which are essential in pharmaceuticals and the flavor and fragrance industry.
5. Petrochemicals: Capillary columns are crucial in the petrochemical industry for characterizing hydrocarbons and other complex mixtures.

1-10 Applications:

Gas chromatography (GC) is used widely in applications involving food analysis. Typical applications pertain to the quantitative and/or qualitative analysis of food composition, natural products, food additives, flavor and aroma components, a variety of transformation products, and contaminants, such as pesticides, fumigants, environmental pollutants, natural toxins, veterinary drugs, and packaging materials. The aim of this article is to give a brief overview of the many uses of GC in food analysis in comparison to high-performance liquid chromatography (HPLC) and to mention state-of-the-art GC techniques used in the major applications [1].

Past and current trends are assessed, and anticipated future trends in GC for food applications are predicted. Among the several new techniques being developed, the authors believe that, in food analysis applications, fast-GC/mass spectrometry (MS) will have the most impact in the next decade. Three approaches to fast- GC/MS include low-pressure GC/MS, GC/time-of-flight (TOF)-MS and GC/supersonic molecular beam (SMB)-MS, which are briefly discussed, and their features are compared When a mixed solution sample is injected into the GC system, the compounds contained in the sample, including the solvent components, are heated and vaporized unit[18].

With GC system, the mobile phase, referred to as the carrier gas, always flows in sequence from the sample injection unit to the column, and then to the detector. The were vaporized in the sample injection unit are transported by the carrier gas to the column. Once in the column, the mixture of compounds is separated into the vario amount of each compound is then measured by the detector [18].

2-1 HIGH performance liquid chromatography HPLC:

High-Performance Liquid Chromatography (HPLC) was developed in the late 1960s and early 1970s. Today it is widely applied for separations and purifications in a variety of are including pharmaceuticals, biotechnology, environmental, polymer and food industries. HPLC has over the past decade become the method of choice for the analysis of a wide variety of compounds. the flowing figure shows picture of the HPLC device[21].

Fig 2-1 the HPLC device

Liquid chromatography is a form of chromatography and is an analytical method used to separate a mixture of a solution into its individual components. Since 1969, it has rapidly developed into an ideal method for separation due to the simplicity and low cost of basic equipment.

The maximum separation efficiency in liquid chromatography can be obtained by reducing the flow rate of the mobile phase, and this results from the slow diffusion characteristic of the liquid phases. The only way to increase the speed of diffusion is by raising the temperature, but the gain in this case is very small in the available temperature range, and the alternative to that is the distance through which the molecules must spread[22].

As a solution to this problem, smaller particles were used to fill the column, and on this basis there was development in liquid chromatography to a more advanced formula, which is high-performance liquid chromatography (HPLC). In this case, the packed column needs a high inlet pressure to achieve a moderate flow speedfi.

(Bischoff HPLC column)

Fig 2-2 The Column

High-performance liquid chromatography (HPLC) is an advanced form of liquid chromatography in which the liquid mobile phase is pumped under mechanical pressure through a column containing the stationary phase.

High-performance chromatography is classified into several categories depending on the type of separation and the type of column, which are:

1. High-performance reversed-phase chromatography.
2. High performance chromatography
3. Ion exchange chromatograph [6].

Its main advantage over GC is that the analyses do not have to be volatile, so macromolecules are suitable for HPLC analysis. HPLC is accomplished by injection of a small amount of liquid sample into a moving stream of liquid (called the mobile phase) that passes through a column packed with particles of the stationary phase. The separation)of a mixture into its components depends on different degrees of retention of each component in the column[23].

The extent to which a component is retained in the column is determined by its partitioning between the liquid mobile phase and the stationary phase. In HPLC this partitioning is affected by the relative solute/stationary phase and solute/mobile phase interactions. Thus, unlike GC, changes in mobile phase composition can have an enormous impact on your separation.

Since the compounds have different mobility's, they exit the column at different times; i.e., they have different retention times, t ,R. The retention time is the time between injection and detection. Thus, HPLC is most often used when one is performing a target compound analysis, where one has a good idea of the compounds present in a mixture so reference standards can be used for determining retention times.

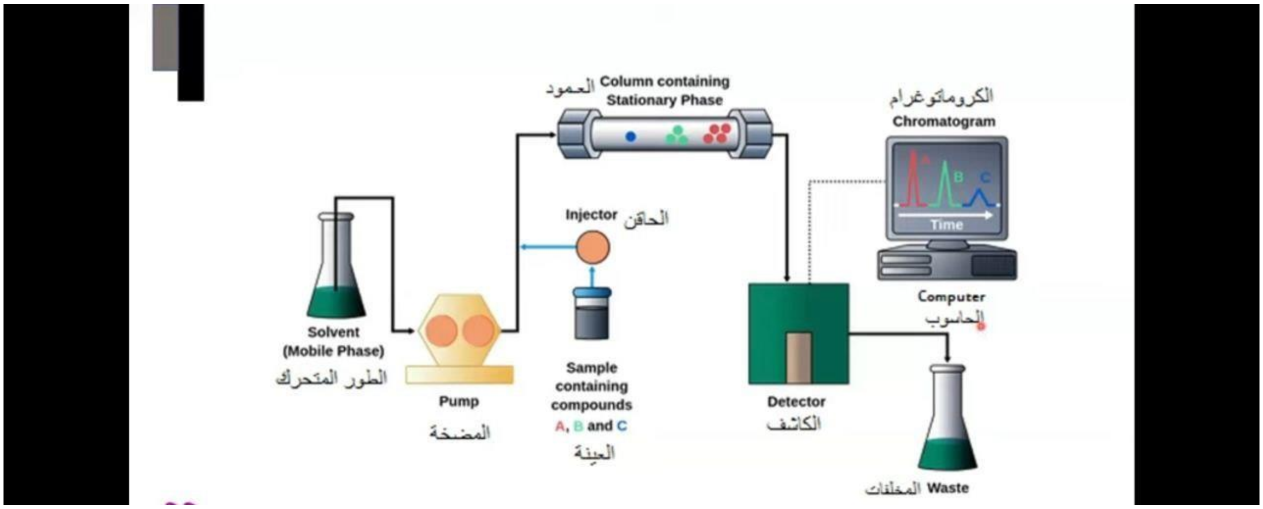
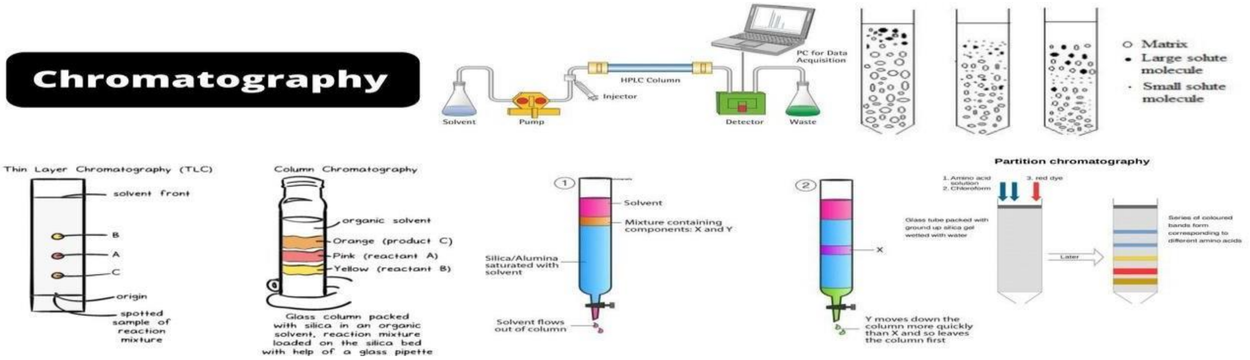


Fig 2-3 Principle of HPLC

2-2 Principle of HPLC

HPLC is a separation technique that involves: The injection of a small volume of liquid sample into a tube packed with tiny particles (3 to 5 micron (µm) in diameter called the stationary phase) where individual components of the sample are moved down the packed tube (column) with a liquid (mobile phase) forced through the column by high pressure delivered by a pump.

These components are separated from one another by the column packing that involves various chemical and/or physical interactions between their molecules and the packing particles. These separated components are detected at the exit of this tube (column) by a flow-through device (detector) that it is not necessary that the degassers and filters be integral parts of the HPLC system. Elution with a single solvent or solvent e factors which influence [21].



2-3 factors effect on separation efficiency:

1- Capacity

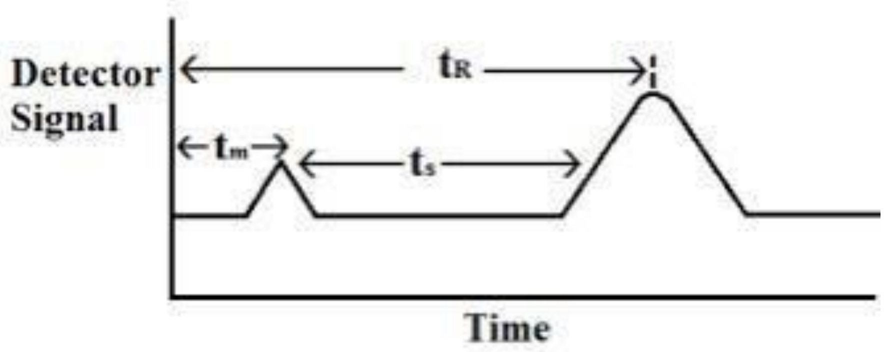
In order to obtain an effective separation in a high-performance chromatography, it is necessary that the column has the ability to hold the model to enable it to separate the components of the model .The capacity coefficient K is a measure of how the column is filled. The effect of the model with the filling material is expressed by the following equation [8]:

$$K=\frac{t1-t0}{t0} \text{ ....(2)}$$

K= capacity coefficient

T1= Rotation time

T0= dead time



The capacity factor depends on the type of solvent and its strength, and the high value of the capacity boring indicates the high ability of the column to retain the analyzed material, so the use of columns with a high amplitude factor is indifferent in improving the separation process, and the value of K ranges from 1-5 and gives this value a good balance between separation and analysis time[6].

2- Selectivity:

Selectivity for any chromatography system is a measure of the difference in detention times between two values and shows how the chromatographic system can separate two compounds and selectivity is symbolized by the symbol **α**

$$\alpha=\frac{t2-t0}{t1-t0}=\frac{k2}{k1} \text{ ....(3)}$$

α = Selectivity coefficient

t0= Dead Time

k2= Capacity coefficient of the second material t1

t2= The time of detention of the second solution.

K1= Capacity coefficient of the first material

2-4 The Factor Which Influence The HPLC Performance:

1. **Internal diameter:** The internal diameter (ID) of an HPLC column is a critical aspect that determines the quantity of analyte that can be loaded onto the column and also influences

sensitivity. Larger columns are usually seen in industrial applications such as the purification of a drug product for later use. Low ID columns have improved sensitivity and lower solvent consumption at the expense of loading capacity[24].

2. **Particle size:** Most traditional HPLC is performed with the stationary phase attached to the outside of small spherical silica particles (very small beads). Smaller particles generally provide more surface area and better separations, but the pressure required for optimum linear velocity increases by the inverse of the particle diameter squared.
3. **Pore size:** Many stationary phases are porous to provide greater surface area. Small pores provide greater surface area while larger pore size has better kinetics, especially for larger analytes. Pore size defines the ability of the analyte molecules to penetrate inside the particle and interact with it is inner surface. This is especially important because the ratio of the outer particle surface to its inner one is about 1:1000. The surface molecular interaction mainly occurs on the inner particle surface.
4. **Pump pressure:** Pumps vary in pressure capacity, but their performance is measured by their ability to yield a consistent and reproducible βlow rate. Modern HPLC systems have been improved to work at much higher pressures, and therefore be able to use much smaller particle sizes in the column
5. **Temperature:** For proper function of the HPLC the temperature has its own in influence. Mostly HPLC columns can work at room temperature or around (25 35 ◦c) are good. But there is also an unexceptional case that requires a higher mixture of constant composition is termed an isocratic elution. In gradient elution, two (and sometimes more) solvent systems that differ significantly in polarity are used and varied in composition during the separation [25].

SN	Device components	Function	Types/notes
1	Mobile phase Reservoir	Preserving the mobile phase ,which in turns carries the materials to be analyzed through the device	The mobile phase must be free of impurities and gases.The mobile phase flow process is either constant components (Isocratic) orIngredients gradually (Gradient).
2	Pump	Pumping the mobile phase andensuring its flow continuous high pressure and constant flow	The most famous types are :1.Syringe Pump **2**.Reciprocating Pump
3	Injector	Inject a specified amount ofsample at the beginning of a column chapter	There are two types 1.Manual injector 2.Automatic injector
4	Column	During which the sample components are separated fromeach other	It represents the heart of the Device and contain(stationary phase)
5	Detector	It detects the components of the	There are a large number of

		sample emerging from the separation column and converts the amount of each into an electrical signal	different types , the most famous of which is a detector UV-VIS absorbance detector
6	Data Processing Unit (computer)	Processing the signal coming from the detector , displaying the chromatogram , and then determining the identity of the sample components and estimating their quantities[26]	A qualitative and quantitative analysis of the data is conducted (Qualitative & Quantitative)

Table 2-1 shows the parts of the device with an explanation of each part.

2-6 HPLC Columns

High-Performance Liquid Chromatography (HPLC) is a common analytical technique used in chemistry, biochemistry, and other sciences to separate, identify, and quantify compounds. The use of columns is crucial in any HPLC laboratory as they serve as the separation mechanism in the technique. Columns are available in different types depending on the separation mechanism and the nature of the sample to be analyzed [21].

Their use is critical to obtaining accurate and reliable analytical results in HPLC laboratories. Without an appropriate column, it would be impossible to separate the components of the sample, and the analysis would be inaccurate and incomplete. Therefore, the use of columns in HPLC labs is necessary and essential for effective and accurate analysis of samples.

In this blog post, we will be discussing the different types of columns used in High- Performance Liquid Chromatography (HPLC). We will explain what they are, how they work, and what their main benefits and drawbacks are. By the end of this blog post, you will have a good understanding of the different types of columns available in the market and be able to make an informed decision when selecting an HPLC column that suits your requirements the best [26].

Fig 2-3 auto sampler

2-5 Types of HPLC columns

1. Reverse-Phase HPLC Columns

The most widely used type of column in HPLC is the reverse-phase column. These columns use a non-polar stationary phase and a polar mobile phase. This causes the polar molecules to elute faster from the column, while non-polar molecules elute slower. Reverse-phase columns are effective at separating non-polar compounds, such as lipids and sterols. At HPLCs, we offer a wide range of reverse-phase HPLC columns that are optimized for different types of samples.

2. Ion-Exchange HPLC Columns

Ion-exchange columns separate analytes based on their charge. The stationary phase in these columns contains charged groups that attract analytes with opposite charges. The mobile phase contains a salt that competes with the analytes for binding to the column. Ion exchange columns are used to separate charged molecules, such as amino acids and proteins. Our ion-exchange HPLC columns are designed for high-resolution separations of complex mixtures.

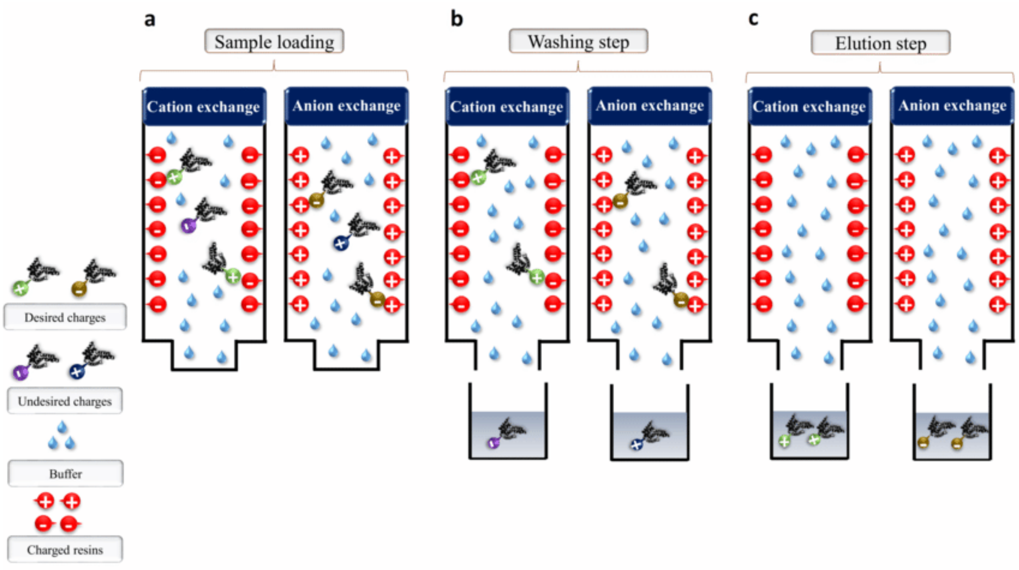


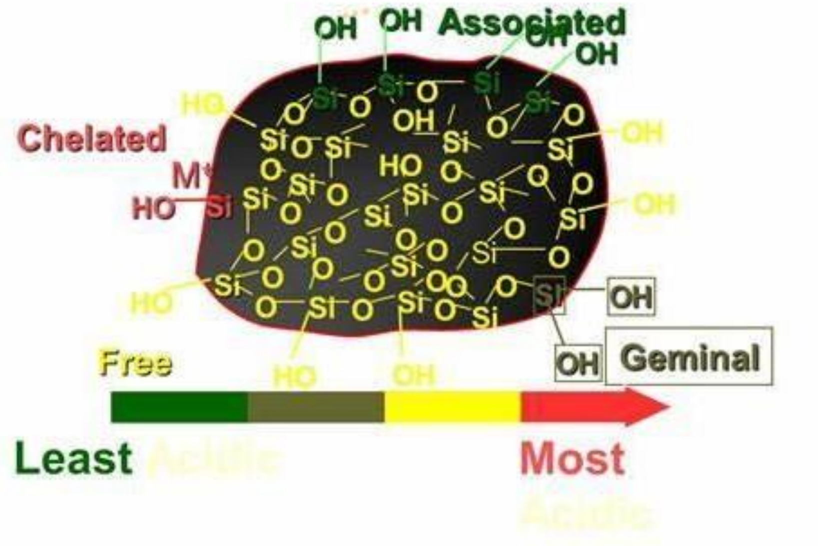
Fig 2-4 Ion-Exchange HPLC Columns

3. Size-Exclusion HPLC Columns

Size-exclusion columns use a porous stationary phase that separates analytic based on their size. Small molecules are trapped inside the pores of the column, while larger molecules pass through the column faster. Size-exclusion columns are used to separate molecules based on their molecular weight. Our size-exclusion HPLC columns are designed for high-resolution separations of polymers and proteins.

4. Normal-Phase HPLC Columns

Normal-phase HPLC columns have a polar stationary phase and a non-polar mobile phase. They are useful for separating polar compounds such as carbohydrates, amino acids, and peptides.



5. Chiral HPLC Columns

Chiral HPLC columns have a stationary phase with chiral selectors that separate enantiomers. They can be used with a range of detectors and are sensitive and accurate.

Fig 2-6 Chiral HPLC Columns

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