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GAS CHROMATOGRAPHY: A REVIEW

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ABSTRACT

In many academic and industrial research facilities, gas chromatography (GC or GLC) is a frequently used analytical technique for quality control, as well as for the identification and quantitation of mixture components. The paper's aim is to evaluate and describe the many phases of developing and validating a GC algorithm. For the examination of complicated mixtures, gas chromatography is a sensitive, accurate, repeatable, quantitative, and flexible method. This methodology is crucial for the examination of pharmaceuticals and medications. However, the application of GC is restricted to molecules that can undergo derivatization processes to produce volatile thermally stable chemicals or molecules. The discovery, development, and production of pharmaceuticals depend heavily on the development and validation of methods. Method development is the process of demonstrating that an analytical method is appropriate for use to measure the concentration of an API in a particular compound dosage form, allowing for the use of streamlined procedures to confirm that an analysis procedure will reliably deliver an accurate measurement of an active ingredient in the preparation of a compound. The technique validation is crucial for the development of analytical methods and is rigorously examined for robustness, system applicability, linearity, accuracy, precision, range, detection limit.

Keywords: Gas Chromatography, Carrier Gas, Injection Devices, Detectors.

I. INTRODUCTION

The technique of gas chromatography is distinctive and adaptable. Its examination of gases and vapors from extremely volatile components was used in its early stages of development[1]. An analytical tool called a gas chromatograph (GC) is used to determine the composition of distinct sample components[2-3].In analytical science, gas chromatography (GC) is a popular type of chromatography that is used to separate and study compounds that may be vaporized without disintegrating. Regular uses of GC include determining a substance's cleanliness or identifying the separate components of a blend[4-7].Gas-liquid chromatography (GLC) was made possible by the work of Martin and Synge, followed by James and Martin, whose contributions to the field revolutionized chemical separations and analyses[8].

Principle

In gas chromatography, the substance to be analyzed is partitioned between Mobile and stationary phase . During sepretion, the sample is vaporized and carried through column by the mobile gas phase. The different components get separated based on their vapour pressure and affinities for stationary phase . The affinity of components towards stationary phase is termed as distribution constant (Kc) , which is also known as **Partition Coefficient.**

Kc = [A]s / [A]m

Where,

[A]s = Concentration of component A in stationary phase

[A]m = Concentration of component A in mobile phase

The movement of different component through column is controlled by distribution constant (Kc), thus the chromatographic sepretion occurs based on difference in distribution constant. Fig 1 show a schematic representation of gas chromatography. The distribution constant depends on the temperature and the chemical nature of stationary phase. Thus temperature can be used for enhancing the components for sepretion of different components through the column or a different stationary phase.

For isolating compounds in a complicated sample mixture, a gas chromatograph is a chemical analytical tool. A gas chromatograph makes use of a column, a narrow tube through which various chemical components of a sample pass at varying speeds in a gas stream depending on their diverse chemical and physical properties and their interaction with a specific column filling, known as the stationary phase. Chemicals are electronically

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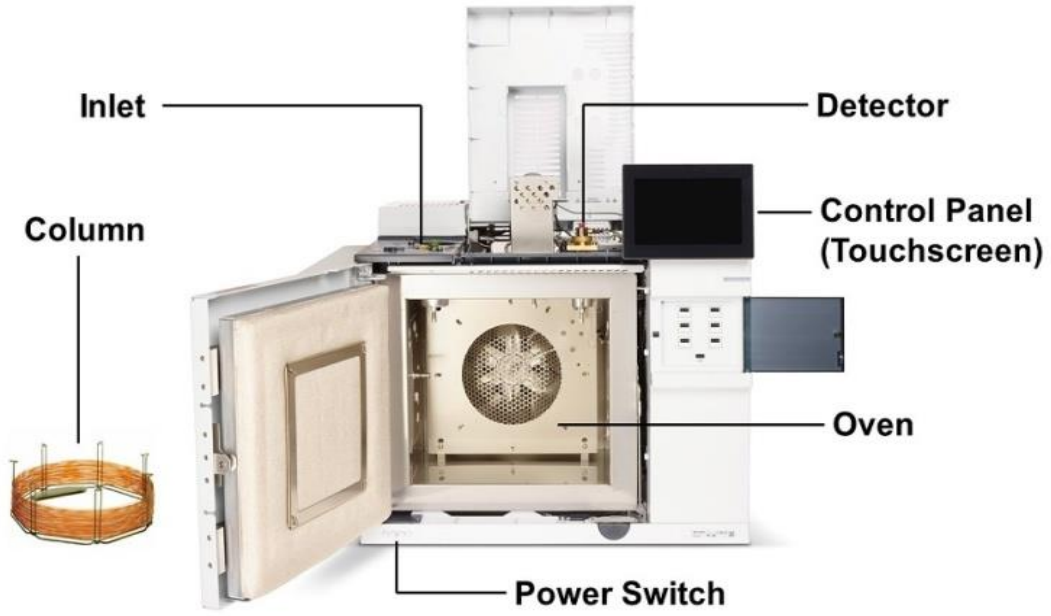
detected and tested as they exit the end of the column. As each component exits the segment at a different time, the stationary stage in the column has the ability to isolate numerous individual components. Different parameters that can be utilized to change the order or time of retention are the flow rate of carrier gas, length of column and the temperature[10-17].A precise known volume of vaporous or fluid analyte is fed into the "entry" (head) of the column during a gas chromatography analysis, typically using a microsyringe. The adsorption of the analyte atoms onto the segment relies or pushing elements in the segment prevents the carrier gas from clearing the analyte particles through the column[18-28]. The quality of adsorption, which in turn depends on the type of atom and the stationary stage materials, determines how quickly the particles advance along the segment. The varied segments of the analyte mixture are separated as they advance along the section and reach the end of the segment at different times because each type of particle has a different rate of growth. The moment at which each component reaches the outlet and the measure of that segment can be determined by using an indicator to track the outlet stream from the segment. Substances are often identified by the direction in which they rise from the location and by the duration of the analyte in section [29- 34].



Types of Gas Chromatography

The two major types of Gas Chromatography:

1) Gas-Solid Chromatography : In the type, the stationary phase in solid (absorbance like alumina, silica, active carbon, etc. are used.) This method provides a key column life time; however catalytic changes are observed in this technique.

2) Gas- Liquid Chromatography : In the type, the stationary phase is an immobilized liquid coated on solid support . In this method the liquid gradually blees off, and this is the disadvantage of this method .

Advantages

Gas chromatography has the following advantages:

1) It is reliable technique and provide rapid analysis.

2) It is highly efficient and lead to high resolution.

3) It utilities sensitive detectors.

4) It required small sample (<1ml)

5) It is non-destructive as it enables the coupling of mass spectrometer, which measures the masses of individual molecules converted into ions, i.e. molecules that have been electrically charged.

6) It provide high quantitative accuracy.

7) It is a well established technique with extensive literature and applications.

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Disadvantage

Gas chromatography has the following disadvantage:

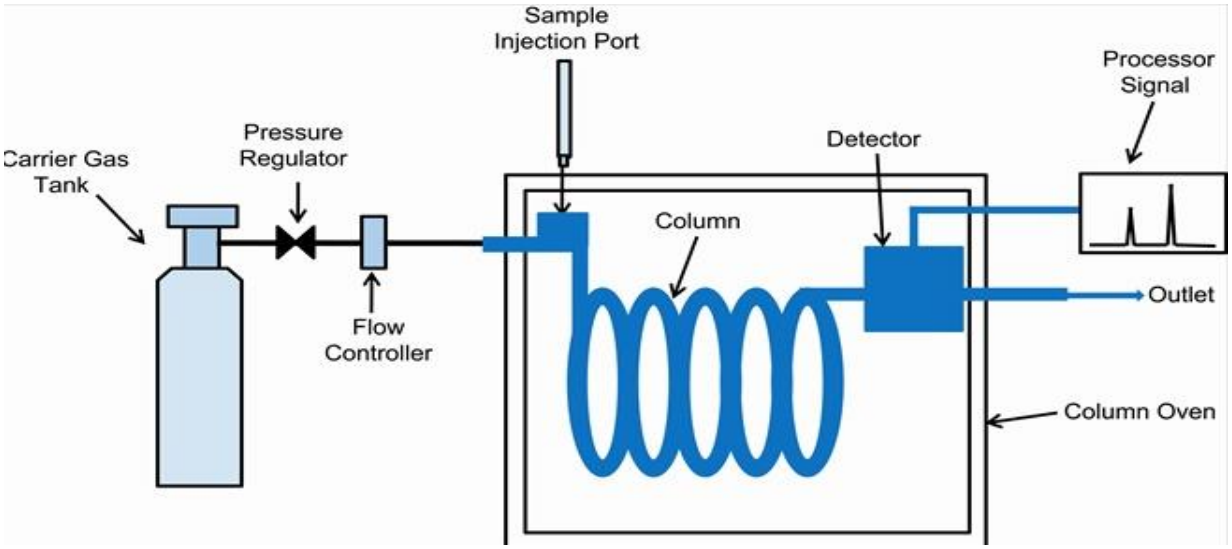
1) It is limited to volatile sample.

2) It is not suitable for thermolabile samples (that degrade at elevated temperature).

3) It is suited to preparative chromatography.

4) It requires MS detecter for structural elucidation of the analyte, since most of the Non- MS detectors are distructive.

Working of Gas Chromatography



Like for all other Chromatographic techniques, a mobile and a stationary phase are required. The mobile phase is comprised of an inert gas e.g. helium, argon, nitrogen, etc. The stationary phase consists of a packed column where the packing or solid support itself act as stationary phase, or is coated with the liquid stationary phase. More commonly used in many instruments are capillary columns, where the stationary phase coats the walls of a small- diameter tube directly

The main reason why different compounds can be separated this way is the interaction of compound with the stationary phase. The stronger the interaction is the longer the compound remains attached to the stationary phase, and the more time it takes to go through the column [9].

The following components make up the instrumentation of gas chromatography

1) Carrier gas maintained at a high pressure and delivered at a rapid and reproducible rate

2) Sample injector

3) Separation columns,

4) Detectors.

5) Thermostated chambers for regulating the temperature of column and detectors,

6) Amplifier and recorder system.

Separation in gas chromatography is carried out in a tubular column (of glass, metal, or Teflon) filled with an adsorbent (the stationary phase). The adsorbent is packed as fir size graded powder, while the liquids before being packed in the column are either cod as a fine film on the column wall or are coated over an inert size graded porous support (such as firebrick powder). A carrier gas (the mobile phase) is continuously made to be through the column so that the sample components are distributed in the column.

The sample vapour is introduced in the column through the carrier gas entrance end. The different sample components absorb stationary phase to different extents, and this depends on their distribution coefficients. The carrier gas immediately sweeps further portion of each component in the gas phase. Thus, a fraction of the adsorbed amount desorb out to maintain the value of distribution coefficient . Simultaneously out of the amount swept away, some amount again goes into the adsorbent at the next point in the column to maintain the

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distribution coefficient value. This process continues and band for each component moves further in the column attaining the shape of Gaussian distribution.[9]

1) Carrier gas

Hydrogen, helium, nitrogen, and air are the most widely used carrier gases. Hydrogen in comparison to other gases is more advantageous and also dangerous to use. Helium is the next best gas, and is used because of its exceptional thermal conductivity, inertness, low density, and greater flow rates; but it is expensive. Nitrogen is inexpensive but reduces sensitivity. Air is used only when the atmospheric oxygen is useful to the detector or separation.

The following considerations should be kept in mind while selecting a carrier gas:

1) It should be inert, i.e. it should not react with the sample, stationary phase, or contacted hardware.

2) It should be suitable for the detector used and the type of sample being analysed.

3) It should be available in high purity. It should give best column performance reliable with required speed of analysis.

5) It should not be expensive.

6) It should not cause any fire or explosion hazard.

Flow Controllers

A constant pressure applied to a column does not ensure a constant flow of mobile phase though the chromatographic system, particularly if the column is being temperature programmed. Raising the temperature of a gas causes the viscosity to increase, and at constant inlet pressure, the flow rate will fall. The reduction in flow rate will be related to the temperature program limits and to a certain extent on the temperature gradient. To obviate the flow rate change, mass controllers are used which ensure a constant mass of mobile passes through the column in unit time irrespective of the system temperature.

2) Injection Devices

In gas chromatography two basic types of sampling system are used, those suitable for packed columns and those designed for open tubular columns. In addition, different sample injectors are necessary that will be appropriate for alternative column configurations. It must be stressed, however, that irrespective of the design of the associated equipment, the precision and accuracy of a GC analysis will only be as good as that provided by the sample injector. The sample injector is a very critical part of the chromatographic equipment and needs to be well designed and well maintained.

A) spilt injections: It splits the volume of sample stream into two unequal flows by mean of a needle valve, and allow the smaller flow to pass on the Columns & bigger past is allowed to be vented to the atmosphere. This technique is not suitable when highest sensitivity is required.

B) Spitless injectors: They allow all of the sample to pass through the Column for loading. Sample should be very dilute to avoid overland of the Column and a high capacity Column such as SCOT or heavily coated WCOT columns should be used

C) On Column injectors: A syringe with a very fine quartz needle is used. A cooled to 20 degree Celsius b.p. of the Sample after then the warmer air is circulated to vaporized sample.

D) Automatic injectors: For improving the reproducibility and if a large number of Samples are to be analyzed operation is required without an attendant, automatic injectors are used.The solid samples are introduced as a solution or in a sealed glass ampoule, crushed in the gas steam with the help of gas tight plunger, and the sample gets vapourized and flows into column under the influence of Carrier gas.[9]

3) Separation of column

The columns used are made of glass or metal tubing, and have a diameter of 4.8mm. They may be of any length ranging from a few centimetres to a hundred meters. They may be coiled, bent, or straight.

The following six types of analytical columns are used in gas chromatography:

1) Packed Columns: These columns are prepared by packing metal or glass tubing with granular stationary phase. In gas liquid chromatography, the packing is prepared by coating a size graded inert solid support with the liquid phase.

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2) Open Tubular or Capillary or Golay Columns: These columns are made of long capillary tube have uniform and narrow internal diameter. They are of stainless steel (most popular), copper, nylon, glass, etc. The liquid phase is coated over the inner wall of capillary tubing as a thin (0.5-1micron) and uniform film. Since there is no packing in these columns, the flow of carrier gas experience least resistance.

3)Support Coated Open Tubular Columns: These columns are prepared by coating the inner wall of a capillary column with a micron size porous layer of support material follow by coating with liquid phase as thin film.

4) Wall Coated Open Tubular Columns: These columns are prepared by coating the unmodified smooth inner wall of the tube with the liquid stationary phase.

5) Porous-Layer Open-Tubular (PLOT) Columns: These columns are prepared by coating the inner wall with a porous layer. Porosity can be achieved either by chemical methods (e.g., etching) or by depositing porous particles on the wall form a suspension. The porous layer either provides support to the liquid stationary phase acts as the stationary phase itself.

6) Support-Coated Open-Tubular (SCOT) column: In these columns the porous layer consist of support particles and was deposited from a suspension.[9]

4) Detectors :

There are many detectors which can be used in gas chromatography. Different detectors will give different types of selectivity. A non-selective detector responds to all compounds except the carrier gas, a selective detector responds to a range of compounds with a common physical or chemical property and a specific detector responds to a single chemical compound. Detectors can also be grouped into concentration dependant detectors and mass flow dependant detectors. The signal from a concentration dependant detector is related to the concentration of solute in the detector, and does not usually destroy the sample Dilution of with make-up gas will lower the detectors response. Mass flow dependant detectors usually destroy the sample, and the signal is related to the rate at which solute molecules enter the detector. The response of a mass flow dependant detector is unaffected by make-up gas. Have a look at this tabular summary of common **GC detectors:**

Detectors	Type	Support gases	Selectivity	Detectability	Dynamic range
Flame Ionisation (FID)	Mass Flow	Hydrogen and Air	Most organic compounds	100pg	10^7
Thermal conductivity (TCD)	Concentration	Reference	Univarsal	1ng	10^7
Electron Capture Detectors (ECD)	Concentration	Make-up	Halides, nitrates, nitriles, peroxides, anhydrides, organometallic	50fg	105
Nitrogen - Phosphorus	Mass Flow	Hydrogen and Air	Nitrogen, Phosphorus	10pg	10^6
Flame photometric (FPD)	Mass Flow	Hydrogen and Air possibly oxygen	Sulphur, Phosphorus, tin, boron, arsenic, germanium, selenium, chromiun	100pg	10^3
Photo-Ionisation (PID)	Concentration	Make-up	Aliphatics, aromatics, ketones, esteers, aldehydes, animes, heterocyclics, organosulphurs, someorganometallics	2pg	10^7
Hall	Mass flow	Hydrogen,	Halides, nitrogen,	-	-

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eletrolytic conductivity	oxygen	nitrosamines, Sulphur

1) Flame Ionisation Detectors

The effluent from the column is mixed with hydrogen and air, and ignited. Organic compounds burning in the flame produce ions and electrons which can conduct electricity through the flame. A large electrical potential is applied at the burner tip, and a collector electrode is located above the flame. The current resulting from the pyrolysis of any organic compounds is measured. FIDS are mass sensitive rather than concentration sensitive; this gives the advantage that changes in mobile phase flow rate do not affect the detector's response. The FID is a useful general detector for the analysis of organic compounds; it has high sensitivity, a large linear response range, and low noise. It is also robust and easy to use, but unfortunately, it destroys the sample.[36]

2) Thermal conductivity detectors

This is less sensitive than the FID (105-10g/s, linear range: 103-10), but is well suited for preparative applications, because the sample is not destroyed. It is based on the comparison of two gas streams, one containing only the carrier gas, the other one the carrier gas and the compound. Naturally, a carrier gas with a high thermal conductivity e.g. helium or hydrogen is used in order to maximize the temperature difference (and therefore the difference in resistance) between two thin tungsten wires. The large surface-to-mass ratio permits a fast equilibration to a steady state. The temperature difference between the reference cell and the sample cell filaments is monitored by a Wheatstone bridge circuit.[36]

3) Electron Capture Detectors

The detector consists of a cavity that contains two electrodes and a radiation source that emits ß-radiation (e.g. "Ni, 3H). The collision between electrons and the carrier gas (methane plus an inert gas) produces a plasma containing electrons and positive ions. If a compound is present that contains electronegative atoms, those electrons are "captured" and negative ions are formed, and the rate of electron collection decreases. The detector is extremely selective for compounds with atoms of high electron affinity (10-14 g/s), but has a relatively small linear range (~102-103). This detector is frequently used in the analysis of chlorinated compounds e.g. pesticides, polychlorinated biphenyls, which show are very high sensitivity.[36]

4) Flame photometric

Flame photometric (FPD) is a method for identifying the spectral lines of mixtures as they burn in a fire by using a photomultiplier tube. Compounds that elute from the column are fed into a hydrogen-energized fire, which excite specific molecular components (such as P, S, halogens, and some metals), which then emit light with specific wavelength characteristics. A photomultiplier tube separates and detects the light that is being emitted. In particular, sulphur discharge occurs at 394 nm and phosphorus emission occurs between 510 and 536 nm.[36]

Parameters Used in Gas Chromatography

- Retention time : Retention time is the difference in time between the point of injection and appearance of peak maxima. Retention time is the time required for 50% of a component to be eluted from a column. Retention time is measured in minutes or seconds. Retention time is also proportional to the distance moved on a chart paper, which can be measured in cm or mm.
- Retention Volume (Vr): Retention volume is the volume of carrier gas required to elute 50% of the component from the column. It is the product of retention time and flow rate.

Retention volume = Retention time x flow rate

- Separation factor (S): Separation factor is the ratio of partition co-efficient of the two components to be separated. It can be expressed and determined by using the following equation:

$$S = K_b/K_a = K`_a/K`_b = (t_b - t_0)/(t_a - t_0)$$

Where,

t_0 = Retention time of unretained substance

K_b,K_a = Partition coefficients of b and a

t_b,t_a= Retention time of substance b and a

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S= Depends on liquid phase, column temperature

If there is more difference in partition coefficient between two compounds, the peaks are far apart and the separation factor is more. If the partition coefficients of two compounds are similar, then the peaks are closer and the separation factor is less.

- Resolution: Resolution is a measure of the extent of separation of two components and the baseline separation achieved. It can be determined by using the following formula:

$$Rs= 2(Rt_1-Rt_2)/w_1+w_2$$

Factors influencing the separation

1) Polarity of the stationary phase: Polar compound interact strongly with a polar stationary phase, hence have a longer retention time then non- polar columns. Chiral stationary phases based on amino acid derivatives, cyclodextrins, chiral silanes, etc. Are capable to separate enantiomers, because one form is slightly stronger bonded than the other one, often due to steric effects.

2) Temperature: The higher the temperature, the more of the compound is in the gas phase. It foes interact less with the stationary phase, hence the retention time is shorter, but the quality of separation deteriorates.

3) Carrier gas flow: If the carrier gas flow is high, the molecules do not have a chance to interact with the stationary phase. The result is same as above.

4) Column length: The longer the column is the better the separation usually is. The trade-off is that the retention time increases proportionally to the column length. There is also a significant broadening of peaks observed, because of increased back diffusion inside the column.[36]

II. CONCLUSION

In analytical science, gas chromatography (GC) is a popular type of chromatography that is used to separate and study compounds that can be vaporised without disintegrating. Based on the retention time, different types of detectors are used to analyse the product. An expansive variety of tests can be analysed the until the compounds are adequately thermally steady and reasonably volatile.

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