S. S. College, Jehanabad

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Subject: Zoology

Topic: Gas Liquid Chromatography (GLC)

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GAS LIQUID CHROMATOGRAPHY

Gas liquid chromatography (GLC) is an analytical chromatographic technique where by the components of a mixture or sample in the gaseous state are separated as the sample passes over a stationary liquid phase and a gaseous mobile phase. It is used to determine the presence or absence and/or quantity of presence of sample that is usually organic in nature or gases.

It is basically a type of gas chromatography (GS) where stationary phase is used as liquid. In gas chromatography mobile phase used is always a gas or mixtures of sample in gaseous state, whereas stationary phase may be a liquid or solid. With this respect, there are two types of gas chromatography; gas liquid chromatography and gas solid chromatography. Therefore, in the GLC or gas liquid chromatography, the components need to be volatile for a successful analysis. The sample component should also be below 1.25 KD (1250 Da) in their molecular weight and thermally stable so they don't degrade in the GC system. It is widely used technique across most industries: for quality control in the manufacture of many products from cars to chemicals to pharmaceuticals; for research purposes from the analysis of meteorites to natural products; and for safety from environmental to food to forensics. Further to identify the chemical components, mass spectrometers (GC-MS) is coupled with gas chromatography.

It is due to certain advantages due to gaseous nature of mobile phase such as rapid movement of sample in the stationary phase (liquid or solid) and less time to diffuse in other directions, it has better resolution compared to other chromatography techniques involving liquid as mobile phase (liquid chromatography) and we get narrower bands and a sharper separation. Martin and James published the first article on gas chromatography (GC) in 1952 and thereafter, the first commercial instruments appeared in in 1956.

Principle of GLC

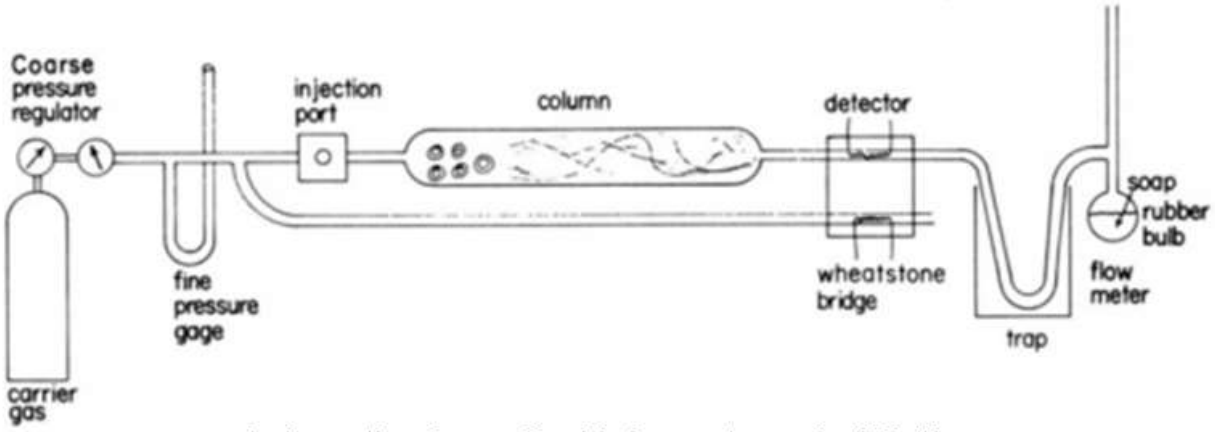
It is a type of partition chromatography and therefore the principle of separation in gas liquid chromatography (GLC) is partition and the components of the sample will partition (i.e. distribute) between the two phases: the stationary phase and the mobile phase. Gas is used as mobile phase and liquid which is coated on a solid support is used as a stationary phase. The mixture of components to be separated is converted to vapor and mixed with gaseous mobile phase. The component which is more soluble in stationary phase travel slower and eluted later and the components which are less soluble travel faster and eluted first. Hence the components are separated according to their partition coefficient. Therefore, a GLC analysis depends upon following criteria;

- ***Volatility:*** Unless a compound is volatile, it cannot be mixed with mobile phase, and therefore compounds that don't have volatile property, cannot be separated out.
- ***Thermostability:*** The compounds which are in solid or liquid form, first need to be converted to gaseous form so they have to be heated to a higher temperature. At that temperature, the compounds have to be thermostable.

Compounds that have a greater affinity for the stationary phase spend more time in the column and thus elute later and have a longer **retention time (Rt)** than samples that have a higher affinity for the mobile phase. Affinity for the stationary phase is driven mainly by intermolecular

interactions and the polarity of the stationary phase can be chosen to maximize interactions and thus the separation. Ideal peaks are Gaussian distributions[1] and symmetrical, because of the random nature of the analyte interactions with the column.

In the GLC analysis, a sample containing the solutes is injected into a heated block where it is immediately vaporized and swept as a plug of vapor by the carrier gas stream into the column inlet. The solutes are adsorbed by the stationary phase and then desorbed by a fresh carrier gas. The process is repeated in each plate as the sample is moved toward the outlet. Each solute travels at its own rate through the column. Their bands are separated into distinct zones depending on the partition coefficients, and band spreading. The solutes are eluted one after another in the increasing order of their kD and enter into a detector attached to the exit end of the column. Here they register a series of signals resulting from concentration changes and rates of elution on the recorder as a plot of time versus the composition of carrier gas stream. The appearance time, height, width, and area of these peaks can be measured to yield quantitative data.



Schematic of a gas liquid chromatography (GLC)

Source: https://doi.org/10.1007/978-1-4615-6998-5_23

Instrumentation of the GLC

Gas liquid chromatography (GLC) system consists of carrier gas, flow regulator and flow meter, injection device, columns, temperature control device, detectors, and recorders and integrators. The details of these components are as follows;

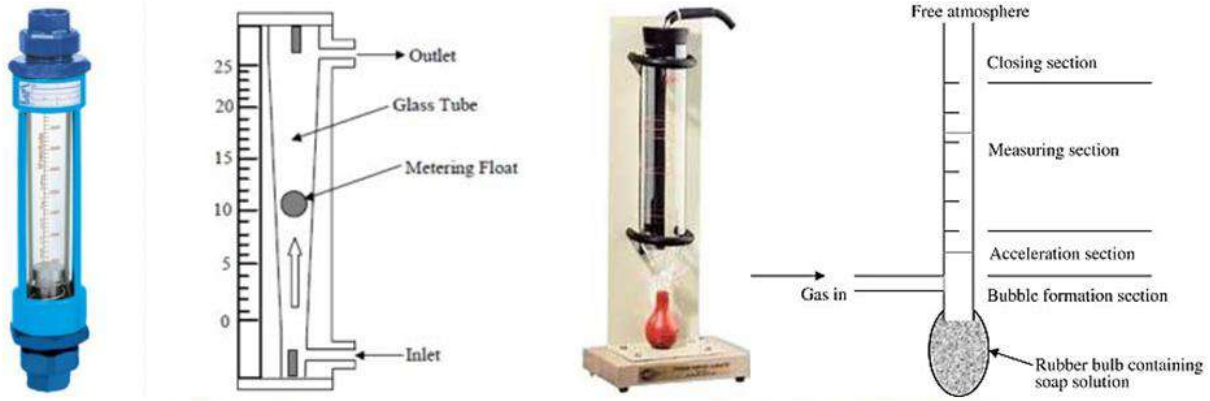
Carrier Gas: The type of carrier gas to be employed presents some choice, but is usually dictated by the type of detector used. The choice of carrier gas determines the efficacy of chromatographic separation. The most common carrier gases (mobile phase) are helium (He), nitrogen (N_2), hydrogen (H), and argon (Ar). Generally, helium is preferred for thermal conductivity detectors because of its high thermal conductivity relative to that of most organic vapors. Nitrogen is preferable when a large consumption of carrier gas is employed. It is filled in tank from where it passes through a toggle valve, a flow meter (range is 1 – 1000ml/min),

[1] Gaussian distribution, also known as normal distribution, is a bell-shaped probability distribution curve that is symmetric about the mean.

capillary restrictors, and a pressure gauze with a range of 1 – 4atm. Rate of flow of carrier gas is adjusted by means of a needle valve mounted on the base of the flow meter and controlled by capillary restrictors. The operating efficiency of the GLC is directly dependent on the maintenance of constant flow of carrier gas. The carrier gas must be inert, less expensive, suitable for detectors, high purity, compressible and should not cause the risk of fire. It is stored under high pressure in cylinder and used when required.

Flow regulators: As carrier gases are stored under high pressure, flow regulators are used to deliver the gas with uniform pressure or flow rate. Flow meter is used to measure the flow rate of carrier gas. The examples of flow meter include rotameter and soap bubble meter.

- ***Rotameter:*** It is a device used to measure gas or fluid flow, in which a float rises in a tapered vertical tube to a height dependent on the rate of flow through the tube. In this type of meter, gas enters the tapered tube, some of which strikes directly the float and some of the gas passes from side. Here two forces act on the float namely up thrust force i.e. buoyancy, and the weight of the float. When equilibrium is established, the float comes to rest.

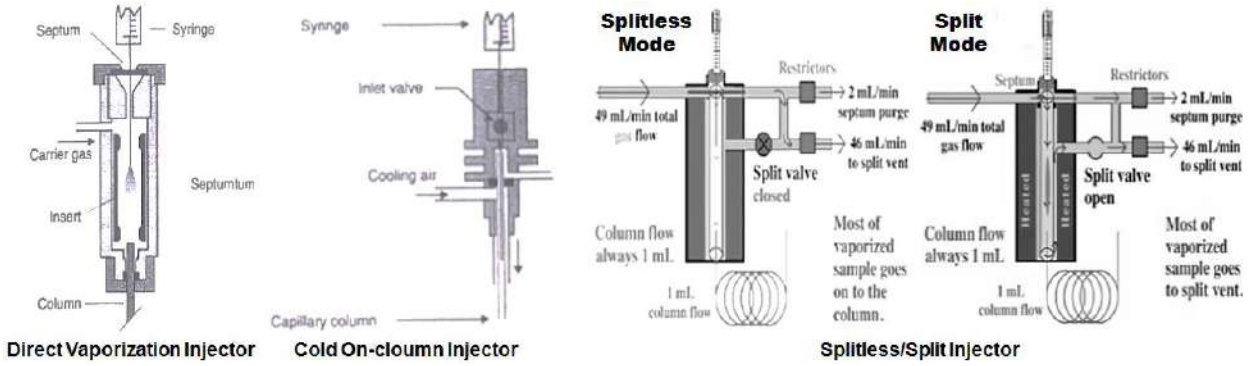


Rotameter Soap Bubble Flow Meter

- ***Soap bubble flow meter:*** In this type of gas flow meter, soap liquid is used in the rubber bulb in the base due to which bubbles are formed when gas is flown through the tube. A glass tube with a inlet for gas is placed at the bottom of flow meter. When the bulb is gently pressed of soap solution is converted into a bubble by the pressure of a carrier gas and travel up. The time required for the soap film to move between two graduations on the burette is then measured and converted to flow rate. It is an accurate device for reproducing the rate of the carrier gas.

Sample injection system: Liquid samples are injected by a micro-syringe with a needle inserted through a self-scaling, silicon-rubber septum into a heated metal block by a resistance heater. Gaseous samples are injected by a gas-tight syringe or through a by-pass loop and valves. Typical sample volumes range from 0.1 to 0.2 ml. The sample is usually introduced in the form of solution at ~0.5ml into the injector and then injected into the column. It has dual role; it provides an inlet for the sample and vaporizes the sample and mixes it with mobile phase. Modes of injection vary according to their types, which are mainly of three types; direct vaporization injection, cold on-column injection, and split/splitless injection.

- ***Direct vaporization injection:*** In direct flash vaporization injection, a liquid sample is injected via a syringe into a heated injection port. The sample is rapidly vaporized in the injection port, and then transferred to the column.
- ***Cold on-column injection:*** In this injection method, the sample is injected directly on column and vaporization occurs after the injection. Needle penetrates the column kept at 4°C before raising it to normal operating temperature. It is very useful for thermolabile components.
- ***Split/splitless column:*** This device is designed to maintain the constant flow of carrier gas and to control the amount of sample enters into the column. If the split vent is closed via a computer-controlled split valve, then all of the sample introduced into the injector vaporizes and goes into the column then it is called as splitless mode. But, if the split vent is open then most of the vaporized sample is thrown away to waste via the split vent and only a small portion of the sample is introduced into the column, the it is called as split mode.



Temperature controlled device: For vaporization of sample to occur, there may be the use of repeaters or thermostatically controlled oven. A repeater is used to convert the samples into vapour form and mix with mobile phase, while a thermostatically controlled oven is used since partition coefficient as well as solubility of a solute depends upon temperature, temperature mainlining in a coloumn is highly necessitated for efficient separation. There are two types of thermal programming in the GLC system; namely isothermal programming and linear programming.

The separation column: The heart of the gas chromatography is the column which is made of glass or metals bent in U shape or coiled into an open spiral or a flat pancake shape. Copper is useful up to 250°. Swagelok type fittings[2] make column insertion easy. Several sizes of columns are used depending upon the requirements. The column used in the GLC system may be of different types. The classification of separation column is following;

- ***Classification of coloumn on the basis of their use:*** It is of two types;
    - *Analytical column:* These columns are meant for qualitative analyses. The eluents from the analytical column may not have to be collected.

[2] Swagelok type fitting is a type of metal tube connection or fittings which make the fitting leak-tight, gas-tight seal in an easy-to-install, disassemble and reassemble form.

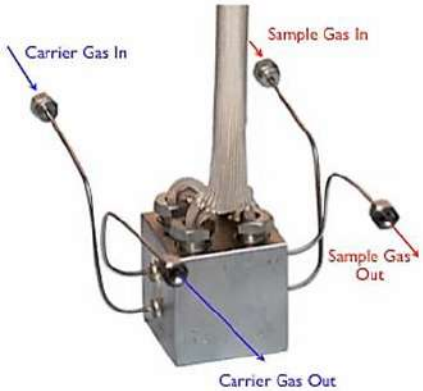
- *Preparative column:* These columns are meant for isolating compounds from natural extracts. It is meant for purifying compounds at a large scale, such as milligram or gram.

- ***Classification of columns on the basis of its nature:*** Based on nature of columns, it is of three kinds, which are;

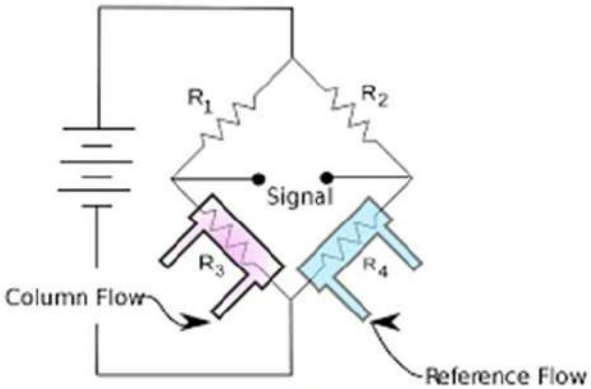
  - *Packed column:* These are available in packed manner commercially and hence they are called as packed column. Different columns ranging from nonplolar to polar are available today.
  - *Open tubular/capillary column:* They are made up of stainless steel and are in the form of a coil of long capillary of tubing of 30 – 90 meter in length with diameter of 0.025 to 0.075cm. The inner wall of the capillary is coated with stationary phase liquid in the form of a thin film. More samples in this column cannot be loaded.
  - *Support coated open tubular column:* These columns are made by depositing a micron size layer of support material on the inner wall of the capillary column and then coated with a thin film of liquid phase. These columns also have low resistance to flow of carrier gas but offer the advantage of more sample load or capacity.

Detectors: These are most important part of GLC instrument. A good detector should have the quality of applicability to wide range of sample, high sensitivity to even small concentration, rapidity of response, linearity, nondestructive to sample, simple and easy to maintain. There are mainly three types of detectors, thermal conductivity detector, flame ionization detector, electron capture detector.

Thermal conductivity detector (TCD): The principle of TCD is based upon the thermal conductivity difference between carrier gas and sample components. TCD has two platinum wire of uniform dimension which form a Wheatstone bridge[3].



A Thermal Conductivity Detector

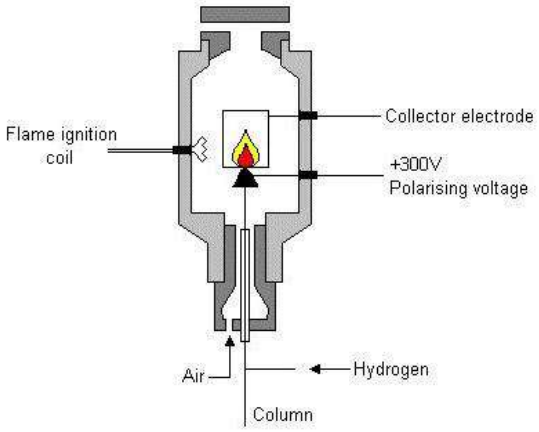


A Circuit of a Thermal Conductivity Detector

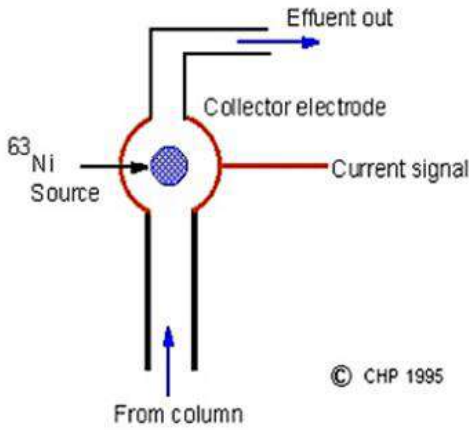
[3] Wheatstone bridge is a simple circuit for measuring an unknown resistance by connecting it so as to form a quadrilateral with three known resistances and applying a voltage between a pair of opposite corners.

Flame ionization detector (FID): In tis detector, carrier gas used is hydrogen. The detector has inlets for hydrogen and air or oxygen to burn the hydrogen and the effluent gas from the chromatograph. Hydrogen emerges through a hollow needle and is burnt as it emerges giving a colourless flame. The effluent gas is mixed with hydrogen.

Electron capture detector (ECD): It is a basically a modification of the ionization chamber used for detection of radiations. This type of detector has two electrodes. One of the electrodes is treated with a radioactive isotope which emit electron as it decays (anode). The effluent from the chromatographic column is exposed to slow electrons generated by the ionization of the carrier gas, which is either argon or nitrogen, by a constant flux of beta rays from a radioisotope.



A Flame Ionizer Detector



A Electron Capture Detector

Recorder: It is used to record the responses obtained from detector after amplification. They record the baseline and all the peaks obtained with respect to time.

Liquid phases: An infinite variety of liquid phases are available limited only by their volatility, thermal stability and ability to wet the support. No single phase will serve for all separation problems at all temperatures. The liquid phase may be of following types;

- ***Nonpolar:*** Parafin, squalane, silicone greases, apiezon L, silicone gum rubber. These materials separate the components in order of their boiling points.
- ***Intermediate polarity:*** These materials contain a polar or polarizable group on a long nonpolar skeleton which can dissolve both polar and nonpolar solutes, for example diethyl hexyl phthalate is used for the separation of high boiling alcohols.
- ***Polar:*** Liquid phases with a large proportion of polar groups, such as carbowaxes.
- ***Hydrogen bonding:*** Polar liquid phases with high hydrogen bonding e.g. glycol.
- ***Specific purpose phases:*** Relying on a chemical reaction with solute to achieve separations. e.g $AgNO_3$ in glycol separates unsaturated hydrocarbons.

Procedure

Gas liquid chromatography or GLC is a type of chromatographic technique which involves multistep process. These processes are as follows;

Step 1: Sample injection and vaporization

1. A small amount of liquid sample to be analyzed is drawn up into a syringe.
2. The syringe needle is positioned in the hot injection port of the gas chromatograph and the sample is injected quickly.
3. The injection of the sample is considered to be a "point" in time, that is, it is assumed that the entire sample enters the gas chromatograph at the same time, so the sample must be injected quickly.
4. The temperature is set to be higher than the boiling points of the components of the mixture so that the components will vaporize.
5. The vaporized components then mix with the inert gas mobile phase to be carried to the gas chromatography column to be separated.

Step 2: Separation in the Column

1. Components in the mixture are separated based on their abilities to adsorb on or bind to, the stationary phase.
2. A component that adsorbs most strongly to the stationary phase will spend the most time in the column (will be retained in the column for the longest time) and will, therefore, have the longest retention time (Rt). It will emerge from the gas chromatograph last.
3. A component that adsorbs the least strongly to the stationary phase will spend the least time in the column (will be retained in the column for the shortest time) and will, therefore, have the shortest retention time (Rt). It will emerge from the gas chromatograph first.
4. If we consider a 2 component mixture in which component A is more polar than component B then:

    - component A will have a **longer retention time** in a polar column than component B
    - component A will have a **shorter retention time** in a non-polar column than component B

Step 3: Detecting and Recording Results

1. The components of the mixture reach the detector at different times due to differences in the time they are retained in the column.
2. The component that is retained the shortest time in the column is detected first. The component that is retained the longest time in the column is detected last.
3. The detector sends a signal to the chart recorder which results in a peak on the chart paper. The component that is detected first is recorded first. The component that is detected last is recorded last.

Applications of GLC

1. GC analysis is used to calculate the content of a chemical product, for example in assuring the quality of products in the chemical industry; or measuring toxic substances in soil, air or water.
2. Gas chromatography is used in the analysis of:

    - air-borne pollutants
    - performance-enhancing drugs in athlete's urine samples
    - oil spills
    - essential oils in perfume preparation

3. GC is very accurate if used properly and can measure picomoles of a substance in a 1ml liquid sample, or parts-per-billion concentrations in gaseous samples.

Advantages of GLC

- The use of longer columns and higher velocity of carrier gas permits the fast separation in a matter of a few minutes.
- Higher working temperatures up to 500°C and the possibility of converting any material into a volatile component make gas chromatography one of the most versatile techniques.
- GC is popular for environmental monitoring and industrial applications because it is very reliable and can be run nearly continuously.
- GC is typically used in applications where small, volatile molecules are detected and with non-aqueous solutions.
- GC is favored for non-polar molecules.
- Gas Chromatography is used extensively in forensic science. Disciplines as diverse as solid drug dose (pre-consumption form) identification and quantification, arson investigation, paint chip analysis, and toxicology cases, employ GC to identify and quantify various biological specimens and crime-scene evidence.

Limitations of GLC

- Compound to be analyzed should be stable under GC operation conditions.
- They should have a vapor pressure significantly greater than zero.
- Typically, the compounds analyzed are less than 1,000 Da, because it is difficult to vaporize larger compounds.
- The samples are also required to be salt-free; they should not contain ions.
- Very minute amounts of a substance can be measured, but it is often required that the sample must be measured in comparison to a sample containing the pure, suspected substance known as a reference standard.

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