GAS CHROMATOGRAPHY

Versatile technique for analyzing complex mixture of substances. An inert gas is used as carrier. The compound (migrate) at different speeds and carrier gas leads these through a column, packed with specially treated surface active adsorbent.
The novel idea that a gas can also be used as a mobile phase was first introduced by Martin and Synge in 1941.
In 1962 A.T.James and A.J.P.Martin performed first chromatographic separation of mixture of Amino acid or Fatty acid by using gas as mobile phase.
The development of liquid column and especially the introduction of capillary or open tubular column in 1958 by Golary revolutionized the analysis of complex mixture of volatile compounds.
The use of GS-MS and GC-IR system has made the application of gas liquid chromatography (GLC) most important in recent years.
Micro-particulate columns (5-10μm) with chemically bounded liquid phases and the microcell detectors used now a day.

Basic principle of gas chromatography:

All chromatographic methods are based on the distribution of the sample between two phases, Stationary and Mobile. Gas chromatography is a separation process and stationary phase is an active adsorbent, it is called Gas-solid chromatography.
Gas-liquid chromatography, on the other hand has a stationary phase that is liquid fixed on an inert solid support.
G.C is one of the fastest and most useful separation techniques available in laboratory. G.C analysis is basically limited to organic compounds that are volatile and not thermally labile (decomposable).
Both types of Gas-chromatography require that sample be converted into or exist in the vapor state and be transported by an inert carrier gas through a column packed with either a liquid phase coated on a solid support or simply a solid adsorbent with no liquid phase coating.
A sample is subjected into a heating block where it is immediately vaporized and swept as a concentrated vapor into a column. Separation occurs as the various compounds vapors are selectively adsorbed by the stationary phase and then desorbed by fresh carrier gas.
The sorption-desorption occurs separately as the compounds move through the column towards a detector. The compounds will be eluted from the column with those having high affinity for the column packing being slower than those with little affinity.

Instrumentation:

A typical gas chromatography consists the following

1. A carrier gas supply
2. Sample injection port
3. Column
4. Column oven
5. Detector
6. Recorder / integrator system

FIGURE

1. **Carrier gas**:

The carrier gas is used to transport the sample molecules from the injection part to the detector and provide the means for partitioning the sample molecule from the stationary phase. The most common carrier gases are Helium and Nitrogen. These gases are supplied in high pressure tanles which require the two stage pressure regulator for reducing the inlet gas pressure and controlling the gas viscosity through the column.
The gas must be high purity with minimal moisture or other contaminants present to reduce erroneous detector signals.
Various commercial gas purifiers are available for removing carrier gas contaminants, especially oxygen and water. Refillable in-line traps are also available that contain molecular siene 5 A, charcoal, etc for removing oxygen, moisture and hydrocarbon contaminants. These purifiers should be checked and reconditioned periodically, especially if drifting bare line is being experienced.

2. **Sample injection**:

A sample inlet system must be provided that allows liquid sample in the range 1-10 µl to be injected with a micro syringe through a septum into a block that is heated to a temperature in excess of the compounds "boiling point" the liquid sample is immediately vaporized as a "plug" and swept through the column by the carrier gas. Septum (plural: septa) is a self releasing rubber or elastomeric material that forms a leak proof entrance into the injection pot. Septa are manufactured in disc ranging in size from 5 to 16 mm to fit injection inlets and are available in very high puncture tolerance/ preconditioned types or in less expensive simple rubber stock. Leakage in septum is one of the common sources of trouble in G.C.
Gas and liquid samples can also be introduced using a gastight valve and calibrated volume loop system. Samples can be introduced using a programmable injector for that continuous operation of chromatographic system.

- Special injector splitters are used with capillary columns, which usually require sample less than 1 microlitre. These injector splitters mix the vaporized sample and split (ratio between 1/10 and 1/100) the original injection volume by venting the excess.

3. **Columns**:

Two types of basic types of columns are generally used

I. Packed column
II. Capillary column

Packed column will usually have 1000 to 3000 plates per meter, while capillary column can exceed 4000 plates per meter.

Packed column: are normally made of copper, stainless steel, or glass with common bores of 1.6, 3.2, 6.4 or 9.5mm and length of 1-3 m. glass columns and glass injection-port lines are necessary when dealing with labile compounds that might react or decompose on contact with metal surface, especially at elevated temperature.
These columns have been "packed" with a coated sieved (ranging from 60mesh to 120mesh) inert solid support.
The solid support is coated (usually 1-10% by weight) with a highly viscous liquid phase.

Capillary column: do not contain solid support coatings and simply have the liquid phase (less than 1 micrometer thick) coated directly onto the interior walls of the column. This wall coated open tubular technique provides an open, unrestricted carrier gas path through the typical 0.25 mm diameter column.
Since capillary column present very little restriction to gas flow, they can be made extremely long (50 to 150 meters) for greater compound resolution.
A never form of capillary column technology called support coated open tubular has recently been developed. A layer of solid support is adsorbed onto the interior of the capillary tubing walls and the liquid phase applied. The primary advantage of this surface area increasing technique is to increase the sample capacity of the column, and, in some cases, sample splitting is not necessary as was the case with conventional capillary columns.

4. **Column oven**:

Operating the column at the constant temperature during an analysis is critical for reproducible results. Temperature programming column oven in which temp. can be changed during analysis to separate out all components of sample properly.
Good resolution is obtained for low boiling components at moderate oven temp., however analysis will require excess time for the evaluation of any high boiling compounds and chromatographic peak will be too broad for proper quantitative interpretation.
Temperature programming can reduced this problem by allowing the low boiling compounds to elute at initially low temp. and column oven is then increased at a reproducible rate to elute the high boiling compounds in a reasonable time.

5. **Detectors used in Gas chromatography**:

Each chromatograph has a detection device at exist of each column. The purpose of the detector is to monitor the carrier gas as it emerges from the column and response to changes in its composition as the solutes are eluted. An ideal detector should have the following characteristics;

i. Rapid response (even less than a second)
ii. High sensitivity: it should be able to detect ρpm and ρpb
iii. A wide range of linear response
iv. It should be stable
v. It should have small internal volume
vi. Low noise
vii. Simple, inexpensive, robust and safe to operate
viii. Low detection limit

Detector evaluation:

The response R is plotted versus the quantity measure. The limit of detection Q_o corresponds to twice the noise level 2RN of the detector. The slope of $\Delta R/\Delta Q$ determines the sensitivity. The region where the curve begins to deviate from a straight line defines the limit of linear response, with Q_L the upper limit.

FIGURE

Types of detectors:

There are two types of detectors:

1. Integral type:
It accumulates the instantaneous response or signal and gives the total amount which has been measured up to a given instant.

2. Differential type:

These are more common. They give no response when pure carrier gas is following through them but as any component from the column passes through they give a response which is directly proportional either to quantity or concentration of that component. When the component has passed the director again give zero response, until a further component emerges. e.g. thermal conductivity detector.

figure

Detector can also be classified as destructive or non destructive depending whether or not the sample component can be collected unchanged for further study.

- **Hydrogen flame detector: (ionization detector)**

Ionization detectors are based upon the principle that the electrical conductivity of the gas is directly proportional to the concentration of the charged particles within it. Effluent gas from the column passes between two electrodes across, where a dc potential is applied. The carrier gas is partially ionized allowing a steady current to flow between the electrodes and through a resistor where a corresponding voltage drop is amplified and fed to a recorded. When a sample component is eluted from the column, it is also ionized in the electrode gap there by increasing the conductivity and producing a response in the detector circuit.

One of the simple detection is the hydrogen flame detector. Hydrogen must be used as a carrier gas. It is burned as it emerges from the column through a hollow needle, yielding a nearly colorless flame. When an organic compound emerges the flame becomes yellow. The retention time can be measured with a stop watch. The amount of the component is roughly proportional to the height or luminosity of the flame. A photocell can be used to measure the luminosity of the flame.

- **Flame ionization detector:**

It is one of the most widely use detectors. The effluent gas from the column is mixed with hydrogen and burned at a small metal jet. The jet form one electrode from of an electrolytic cell, the other being a loop of wire placed just above the flame. The potential difference applied across the plates is almost 200 v. sample is ionized as it enters the flame.
The ion current produced is approx. proportional to number of carbon atoms entering the flame. More precisely the response depends upon the state of oxidation of carbon atoms. The FID responds to virtually all organic compounds except formic acid, air and other inorganic gases. It is especially worth that it does not respond to water vapor or air. The FID is relatively simple, extremely sensitive and has a wide range of linear response.
For inorganic compounds a converter is required which converts inorganic compounds into organic ones, i.e. CO_2 to CH_4, and then the remaining part is burnt in a second flame and detected. These can detect up to 10^{-12} g of the sample.

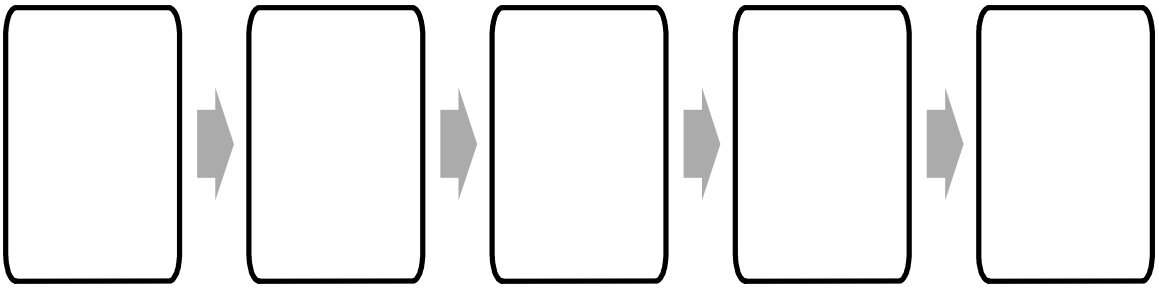
DIAGRAM

Flame ionization detector

- **Thermal conductivity detector:**

(Original GC detector also known as hot wire detector)
a gas is passed over a heated filament wire, the temperature and thus the resistance of wire will vary according conductivity of the gas.
The pure carrier gas passed over one filament and the effluent gas containing the sample is passed over another. These filaments are in opposite arms of a Wheatstone bridge that measure the difference in their resistance. If there is no sample in effluent gas, there is no difference in resistance of wires. As the sample along with effluent gas reach there, a small resistance change will occur in the effluent arm. Change in resistance is proportional to the concentration of analyte and recorded as signal on recorder.
TCD is useful for the analysis of gaseous mixture, and of permanent gases such as CO_2.

GC-MS:
Mass spectrophotometer is used as detector. Effluent from the G.C will introduce into the mass-spectrophotometer and then after fragmentation, the mean path of those separated component can be determined.



Applications of gas chromatography:

Any series of organic or inorganic compounds which has a reasonable vapour pressure can be separated by this technique. One of the most useful applications in the automated elemental (C, H, N) analysis organic compounds.
The alcohol components of alcohol beverages have been qualitatively determined by this technique.
Compounds having low vapour pressure are first converted to their more volatile derivatives and then put to analysis by gas chromatography.
Gas chromatography itself cannot solve all the problems but the following examples give some idea of its scope.

1. **Fuel gases:**

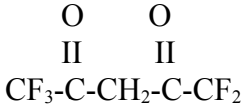
The energy crises have stimulated efforts to manufacture fuel gases from sewage and wastes. The analysis of products is best handled by using gas chromatographic methods.

2. **Auto exhaust gas:**

Air pollution caused in large part by the automobile engine a continuing major concern. The analysis of exhaust gases by gas chromatography has helped us to understand the combustion process and to improve all parts of the fuel system.

3. **High purity metals:**

Most metals and their compounds are not sufficiently volatile to be determined directly by gas chromatography.
They can however be converted to their volatile compounds. The most useful complexes for this purpose are those formed by trifluoro and hexafluoro acetyl acetone.



DOUBLE BOND

The higher sensitivity of the electron capture detector for fluoro complexes makes this technique ideal for trace impurities in metals.

4. Identification of natural products:

The flavours and aromas of the natural products are the result of the unique combination of trace quantities of hundreds of organic compounds. Strawberry flavor was one of the first to be investigated by gas-chromatography.

The objective is to be able to duplicate rare or expensive flavours and aromas with synthetic chemicals. Another objective is to make a positive identification of a particular variety.

In a recent study, the oils obtained from a number of che?eses were chromatographed. Adultration can also be detected by this technique**.**

5. Preparative scale:

The remarkable separation power of gas chromatography could be used in the manufacture of high purity chemicals on commercial scale. But unfortunately due to increase in the sample amount and the increase in column diameter, revolution is drastically reduced. This is mainly due to the reason that a uniform flow rate cannot be maintained across the column. Furthermore to construct a sophisticated apparatus for large scale separation is very expensive.

However, gas chromatography can be used on relatively small scale for this purpose (upto 1-5 liter sample for which one foot column is used).

6. Process control:

Chromatography is not a suitable technique for controlling processes in which fast action is desired as it does not give an immediate and continuous signal.

However, in the case of complex mixtures the interferences can be removed by using gas chromatography, after which the mixture can be subjected to other methods of analysis. The detector signal can be sent directly to a computer system, which controls the addition of reagents, temperature, and pressure and so on to maximize the production.

7. Separation of volatile substances:

High molecular weight fatty acids are first converted to their ester derivative which are volatile and then separated by gas chromatography.

8. Separation of homologous series:

Gas chromatography is sensitive to small differences in molecular weight so, a homologous series can be separated by using this technique. E.g. methane ethane, propane…..

9. Biomedical applications:

Gas chromatography can be used to analyse te human body fluids. Scientists believe that in future samples of breath, blood and urine could be analysed in a few minutes. This would give a complete diagnosis of the state of health and prescription to cure any problem.

Linus pauling and coworkers have concluded that gas chromatography is best instrument for this purpose.