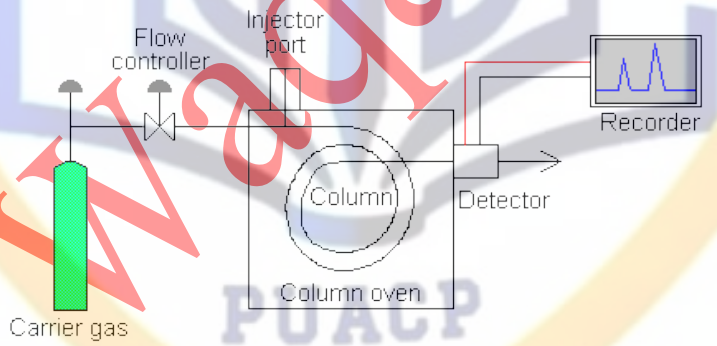
Origin of Gas Chromatography

The development of GC as an analytical technique was pioneered by **Martin and Synge 1941**; they suggested the use of gas-liquid partition chromatograms for analytical purposes. When dealing with liquid-liquid partition chromatography, they predicted that the mobile phase need not be a liquid but may be a vapor. Very refined separations of volatile substances on a column in which a permanent gas is made to flow over a gel impregnated with a non-volatile solvent would be much faster and thus, the columns much more efficient and separation times much shorter. The concept of gas chromatography was envisioned in the early forties but unfortunately little notice was taken of the suggestion. It was left to Martin himself and his co-worker A. T. James to bring the concept to practical reality some years later in 1951, when they published their epic paper describing the first gas chromatograph. They demonstrated the technique by separating and quantitatively determining the twelve components of a C1-C5 fatty acid mixture. The importance of GC was recognized almost immediately by petrochemical laboratories, which faced the challenge of analyzing complex hydrocarbon mixtures.

Introduction

Gas chromatography - specifically gas-liquid chromatography - involves a sample being vaporized and injected onto the head of the chromatographic column. The sample is transported through the column by the flow of inert, gaseous mobile phase. The column itself contains a liquid stationary phase which is adsorbed onto the surface of an inert solid.



There are two types of gas chromatography called Gas-Solid Chromatography (GSC) and Gas-Liquid Chromatography (GLC).

(i) Gas-Solid Chromatography (GSC): GSC is based upon a solid stationary phase in which process involve adsorption. In GSC, the column is packed with an adsorbent and the components of the sample distribute themselves between the gas phase and the adsorbed phase that is, on the surface of the solids. However, GSC can not be used at higher temperatures due to limitations of volatility and instability of liquid coatings at higher temperatures. GSC is more often used for analysis of gases having no active functional groups which interact with the adsorbent surface.

(ii) Gas-Liquid Chromatography (GLC): In gas-liquid chromatography separation takes place by partition of analyte between a gas mobile phase and a liquid phase immobilized on the surface of an inert support. If stationary phase is a liquid coated on solid particles then it is GLC. Components of the mixture distribute between the gas phase and the stationary liquid phase according to their partition coefficients. The solid functions only as a support for the liquid

stationary phase. GLC has several advantages over GSC such as large range of liquid coatings provides a large range of separations and good resolution between peaks in shorter analysis time.

Instrumental components

Carrier gas

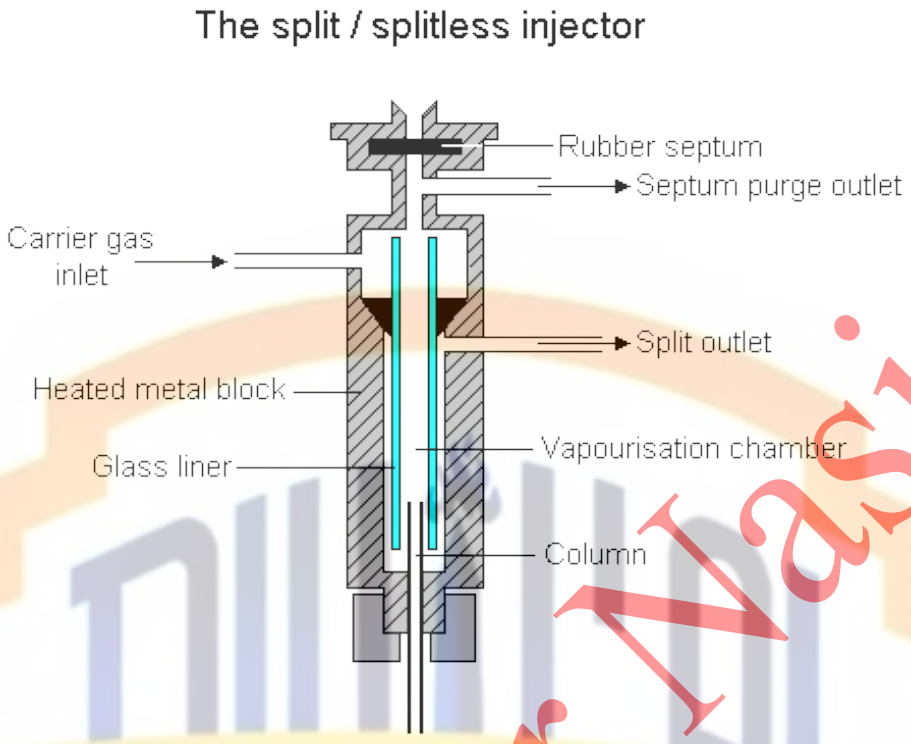
The choice of carrier gas is often dependent upon the type of detector which is used. The carrier gas system also contains a molecular sieve to remove water and other impurities.

- Helium, N_2, H_2, Argon and CO_2 are used as carrier gases.
- Helium is preferred for thermal conductivity detectors because of its high thermal conductivity relative to that of most organic vapours.
- N_2 is preferable when large consumption of carrier gas is employed.
- Carrier gas from the tank passes through a toggle valve, a flow meter, (1-1000 ml / min), capillary restrictors, and a pressure gauge (1-4 atm).
- Flow rate 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 gas chromatograph is directly dependant on the maintenance of a constant gas flow.

Sample injection port/ Pneumatic controls

For optimum column efficiency, the sample should not be too large, and should be introduced onto the column as a "plug" of vapour - slow injection of large samples causes band broadening and loss of resolution. The most common injection method is where a micro-syringe is used to inject sample through a rubber septum into a flash vapourizer port at the head of the column. **The temperature of the sample port is usually about 50 °C higher than the boiling point of the least volatile component of the sample.** For packed columns, sample size ranges from tenths of a microliter up to 20 microliters. Capillary columns, on the other hand, need much less sample, typically around 10^{-3} mL. For capillary GC, split/splitless injection is used. Have a look at this diagram of a split/splitless injector;

The injector can be used in one of two modes; split or splitless. The injector contains a heated chamber containing a glass liner into which the sample is injected through the septum. The carrier gas enters the chamber and can leave by three routes (when the injector is in split mode). The sample vaporizes to form a mixture of carrier gas, vaporized solvent and vaporized solutes. A proportion of this mixture passes onto the column, but most exits through the split outlet. The septum purge outlet prevents septum bleed components from entering the column.



The separation column

- The heart of the gas chromatography is the column which is made of metals bent in U shape or coiled into an open spiral or a flat pancake shape.
- Copper is useful up to 250^0C.
- Sewage lock fittings make column insertion easy.
- Several sizes of columns are used depending upon the requirements.

Capillary GC Columns

Columns can be in any shape that will fill the heating oven. Column 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. Well-packed columns may have 1000 plates/m, and so a representative 3-m column would have 3000 plates. Short columns can be made of glass or glass/silica-lined stainless steel, but longer columns may be made of stainless steel or nickel so they can be straightened for filling and packing. Columns are also made of Teflon. For inertness, glass is still preferred for longer columns. The resolution for packed columns increases only with the square root of the length of the column. Long columns require high pressure and longer analysis times and are used only when necessary (e.g., analytes that are poorly retained require more stationary phase to achieve adequate retention). Separations are generally attempted by selecting columns in lengths of multiples of 3, such as 1 or 3 m. If a separation isn't complete in the shorter column, then the next longer one is tried. The column is packed with small particles that may themselves serve as the stationary phase (adsorption chromatography) or more commonly are coated with a nonvolatile liquid phase of varying polarity (partition chromatography). Gas–solid chromatography (GSC) is useful for the separation of small gaseous species such as H_2, N_2, CO_2, CO, O_2, NH_3, and CH_4 and volatile

hydrocarbons, using high surface area inorganic packings such as alumina (Al_2O_3) or porous polymers (e.g., Porapak Q—a polyaromatic cross-linked resin with a rigid structure and a distinct pore size). The gases are separated by their size due to retention by adsorption on the particles. Gas–solid chromatography is preferred for aqueous samples. The solid support for a liquid phase should have a high specific surface area that is chemically inert but wettable by the liquid phase. It must be thermally stable and available in uniform sizes. The most commonly used supports are prepared from diatomaceous earth, a spongy siliceous material. They are sold under many different trade names. Chromosorb P is a pink-colored diatomaceous earth prepared from crushed firebrick. Chromosorb W is diatomaceous earth that has been heated with an alkaline flux to decrease its acidity; it is lighter in color. Chromosorb G was the first support expressly developed for GC, combining the good efficiency and handling characteristics of Chromosorb G while having the low adsorptive properties of Chromosorb W. Generally, all of the above are available in non-acid washed, acid washed, and silanized with dimethylchlorosilane (DMCS, this greatly reduces polarity) and in high-performance versions (HP, controlled uniform fine particles). Chromosorb 750 is a very inert and efficient support that is acid washed and DMCS treated. Chromosorb T is useful for separating permanent gases and small molecules, it is largely based on fluorocarbon (Teflon) particles. Chromosorb P is much more acidic than Chromosorb W, and it tends to react with polar solutes, especially those with basic functional groups. Column-packing support material is coated by mixing with the correct amount of liquid phase dissolved in a low-boiling solvent such as acetone or pentane. About a 5 to 10% coating (wt/wt) will give a thin layer. After coating, the solvent is evaporated by heating and stirring; the last traces may be removed in a vacuum. A newly prepared column should be conditioned at elevated temperature by passing carrier gas through it for several hours, preferably before connecting detectors or other downstream components. The selection of liquid phases is discussed below. Particles should be uniform in size for good packing and have diameters in the range of 60 to 80 mesh (0.25 to 0.18 mm), 80 to 100 mesh (0.18 to 0.15 mm), or 100 to 120 mesh (0.15 to 0.12 mm). Smaller particles are impractical due to high pressure drops generated.

Columns

Packed columns	Capillary columns
Stationary phase is coated directly in the column	Stationary phase is coated with the inner wall of the column
Applicable for both GSC and GLC	Applicable only for GLC
Liquid phase is adsorbed onto the surface of the beads in a thin layer or onto the solid inert packing	Liquid stationary phase is immobilized on the capillary tubing walls

Although capillary columns were introduced in 1959, they did not gain popularity until 1980. At present, it is estimated that more than 80% of all applications are run on capillary columns due to the fast and efficient separation they afford.

Capillary chromatographic columns are not filled with packing material; instead, a thin film of liquid phase coats the inner wall. Because the tube is open, its resistance to flow is very low, and it is thus referred to as an open tubular column.

Open tubular columns can be divided into three groups and are described in the next sections.

Porous Layer Open Tubular Column

Porous layer open tubular (PLOT) columns, first suggested by Golay in the late 1950s, have been successfully developed and commercialized. PLOT columns contain a porous layer of a solid adsorbent such as alumina, molecular sieves, or Porapak. PLOT columns are well suited for the analysis of light, fixed gases, and other volatile compounds. The typical structure of a porous layer open tubular column is shown in Figure 2.

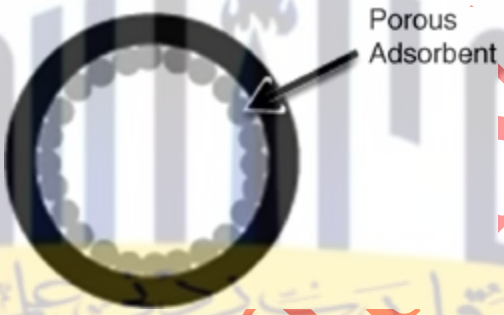


Figure 2

Wall-Coated Open Tubular Column

In 1957, Golay demonstrated the superiority of wall-coated open tubular (WCOT) columns (a 100-fold or higher increase in efficiency) relative to packed columns; yet, it took a quarter century before this efficiency was realized in practice. In WCOT columns, the wall is directly coated with the stationary-phase layer at a film thickness of 0.05–3 µm. A typical wall-coated open tubular column is shown in Figure 3.

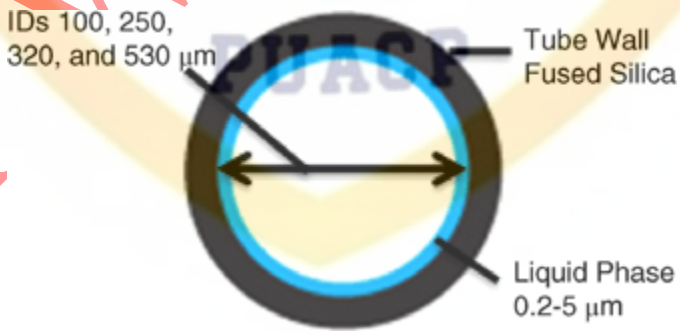


Figure 3

Support-Coated Open Tubular Column

Support-coated open tubular (SCOT) columns were introduced in 1963 by Halász and Horváth. These columns contain an adsorbed layer of a very fine solid support (such as Celite) coated with the liquid phase. SCOT columns can hold more liquid phase and have a higher sample capacity

than the thin films of early wall-coated open tubular (WCOT) columns had. With the introduction of cross-linking techniques, the use of stable, thick films in WCOT columns has become possible, thereby making SCOT columns redundant. A typical support-coated open tubular column is shown in Figure 4.

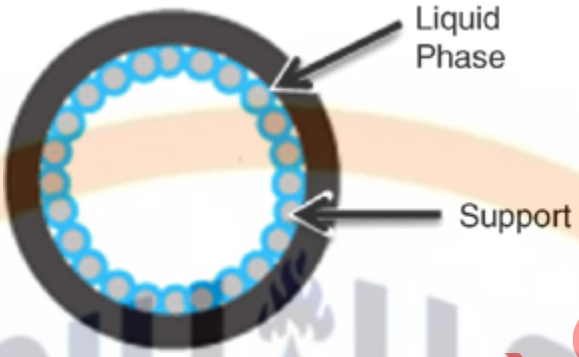


Figure 4

Fused Silica Open Tubular Column

Stainless steel was utilized early as a material for capillary GC. However, due to the lack of efficiency and high reactivity with compounds, including steroids, amines, and free acids, the steel capillary has been outdated. Glass columns suffer from the drawback of being fragile. Thus, fused silica was introduced in 1979, and almost all capillary columns are made of fused silica. The cross-section of a fused silica open tubular (FSOT) column is depicted in Figure 5.

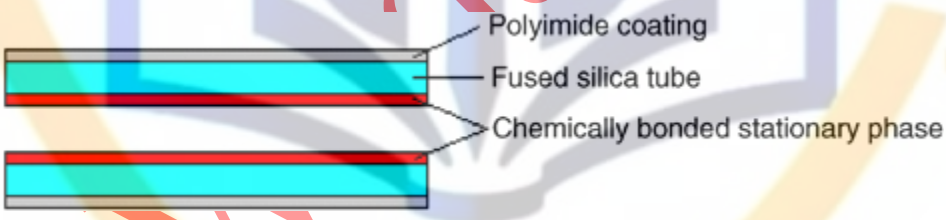


Figure 5

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The fused silica tubes have much thinner walls than glass capillary columns, and are strengthened by the polyimide coating. These columns are flexible and can be wound into coils. They offer the advantages of physical strength, flexibility, and low reactivity.

Capillary columns offer certain advantages relative to packed columns. Capillary columns are coated with a thin, uniform liquid phase. Because of the smooth, inert surface of fused silica, high efficiency can be achieved, typically 3000–5000 theoretical plates per meter. In contrast, packed columns have thicker, often non uniform films, and generate only 2000 plates per meter. Thus, the total plates available in long capillary columns range from 180 000 to 300 000, whereas packed columns typically generate only 4000 plates and have much lower resolution. Due to the small pressure drop associated with open tube capillary columns, long columns of up to 60 m can easily be used. However, packed columns are tightly filled with solid support and suffer from greater pressure drops; thus, it is impossible to use packed columns much longer than 2 m.

Optimizing GC separation requires fine-tuning of a number of variables and their interactions. Both physical (internal diameter, length, and stationary phase) and parametric (temperature and flow velocity) column variables affect the separation process.

Column temperature and oven

The column temperature must be high enough to provide sufficient vapor pressure for components of sample to be eluted in a suitable time. Column temperature depends upon the boiling point of the sample and kept at about 10 °C above to the boiling point of the highest boiling component of the sample mixture. For control of column temperature, different thermostats have been used like electrically heated air baths, liquid baths, vapor jackets, or metal blocks. Temperature in gas chromatography is controlled via a heated oven. The oven heats rapidly to give excellent thermal control. The injector and detector connections are also contained in gas chromatographic oven. The latest development is programmed heating. In temperature programmed GC, the oven temperature is increased according to the temperature program during the analysis. Typically the temperature of oven ranges from 5°C - 400 °C and may decrease up to -25 °C with cryogenic cooling. The detector is kept at a higher temperature than the column temperature so components of sample will be gaseous state.

Flow Meters

The flow of gas from reservoir is smoothly regulated by flow meters. Different flow meters such as soap bubble flow meter, capillary flow meter, float flow meters are used.

Detector

The detector responds to a physicochemical property of the analyte, amplifies this response and generates an electronic signal for the data system to produce a chromatogram. Many different detector types exist and the choice is based mainly on application, analyte chemistry and required sensitivity – also on whether quantitative or qualitative data is required. Detector choices include:

Flame Ionization (FID)
Electron Capture (ECD)
Flame Photometric (FPD)
Nitrogen Phosphorous (NPD)
Thermal Conductivity (TCD) and
Mass Spectrometer (MS)

Gas Chromatography Detectors

Since the initial experiments with gas chromatography were begun, a large number of 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. We describe some of the more widely used detectors. Table lists and compares some commonly used detectors with respect to application, sensitivity, and linearity.

1-Thermal conductivity detectors: The original GC detector was the thermal conductivity, or hot wire, detector are inexpensive and exhibit universal response, but they are not very sensitive. (TCD). As a gas is passed over a heated filament wire, the temperature and thus the resistance of

the wire will vary according to the thermal conductivity of the gas. Typically it is deployed in a referenced configuration: The pure carrier 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 generates a voltage as the resistance of the sensing filament changes. So long as there is only carrier gas in the effluent, the resistance of the wires will be the same. But whenever a sample component elutes, a small resistance change will occur in the effluent arm. The change in the resistance, which is proportional to the concentration of the sample component in the carrier gas, is registered by the data system. The TCD is particularly useful for the analysis of gaseous mixtures, and of permanent gases such as CO_2. Hydrogen and helium carrier gases are preferred with thermal conductivity detectors because they have a very high thermal conductivity compared with most other gases, and so the largest change in the resistance occurs in the presence of sample component gases (helium is preferred for safety reasons). The thermal conductivity of hydrogen is 53.4×10^{-5} and that of helium is 41.6×10^{-5} cal/˚C-mol at 100 ˚C, while those of argon, nitrogen, carbon dioxide, and most organic vapors are typically one-tenth of these values. The advantages of thermal conductivity detectors are their simplicity and approximately equal response for most substances. Also, their response is very reproducible. They are not the most sensitive detectors, however.

2-Flame Ionization Detector (FID): Most organic compounds form ions in a flame, generally cations such as CHO^+. The flame ionization detection is both general and sensitive. It is the most commonly used general detector. This forms the basis of an extremely sensitive detector, the flame ionization detector (FID). The ions are measured (collected) by a pair of oppositely charged electrodes. The response (number of ions collected) depends on the number of carbon atoms in the sample and on the oxidation state of the carbon. Those atoms that are completely oxidized do not ionize, and the compounds with the greatest number of low oxidation state carbons produce the largest signals. This detector gives excellent sensitivity, permitting measurement of components in the ppb concentration range. The FID is about 1000 times more sensitive than the TCD. However, the dynamic range is more limited, and samples of pure liquids are generally restricted to 0.1 μL or less. The carrier gas is relatively unimportant. Helium, nitrogen, and argon are most frequently employed. The flame ionization detector is insensitive to most inorganic compounds, including water, and so aqueous solutions can be injected (but only if you have a compatible column). If oxygen is used as the flame support gas in place of air, then many inorganic compounds can be detected because a hotter flame is produced that can ionize them. When sulfur and phosphorus compounds are burned in an FID-type flame, chemiluminescent species are produced that produce light at 393 nm (sulfur) and 526 nm (phosphorous). An optical interference filter passes the appropriate light to a photomultiplier tube, a sensitive photon detector. These detectors are known as flame photometric detectors (FPD).

3-Flame Photometric Detectors (FPD): In the FPD for sulfur, light emission takes place from the excited diatomic sulfur (S_2^*) species. As a result, the emission signal is proportional to the square of the concentration. This unusual dependence of the signal on a power function of the concentration leads to some unusual procedures to increase sensitivity. The sensitivity in detecting sulfur compounds actually increases if the carrier gas is deliberately doped with a small concentration of a sulfur compound, e.g., 1 ppb of sulfur hexafluoride (SF_6). Consider that without any sulfur doping, with zero sulfur in the background, the background would be zero and

the response from a sample containing 1 and 3 ppb S will be 1 and 9 arbitrary units. If I have 1 ppb S in the carrier gas, the background will be 1 unit and when the 1 and 3 ppb samples elute, the total concentrations of 2 and 4 ppb S will produce signals of 4 and 16 units, the net signals being 3 and 15 units, much greater than those obtained with no doping. Such a beneficial effect of deliberately contaminating the background is unique.

4-Electron Capture Detector (ECD): The electron capture detector (ECD) is extremely sensitive for pesticides that contain electronegative atoms and is selective for these. It is similar in design to the β-ray detector, except that nitrogen or methane doped with argon is used as the carrier gas. These gases have low excitation energies compared to argon and only compounds that have high electron affinity are ionized, by capturing electrons. Many ECDs operate with helium as the carrier gas, using nitrogen as a makeup gas in the detector. The detector cathode consists of a metal foil impregnated with a β-emitting element, usually tritium or nickel-63. The former isotope gives greater sensitivity than the latter, but it has an upper temperature limit of 220 ˚C because of losses of tritium at high temperatures; nickel-63 can be used routinely at temperatures up to 350 ˚C. Also, nickel is easier to clean than the tritium source; these radioactive sources inevitably acquire a surface film that decreases the β-emission intensity and hence the sensitivity. The β sources are used in a sealed form for safety reasons. The cell is normally polarized with an applied potential, and electrons (β rays) emitted from the source at the cathode strike gas molecules, causing electrons to be released. The resulting cascade of thermal electrons is attracted 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 negative ion. Such negative ions are much larger than an electron and have mobilities in an electric field about 100,000 times less than electrons. Thus, analytes passing through the ECD are detected by a decrease in standing current.
Relatively few compounds show significant electronegativities, and so 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, certain condensed ring aromatics, and certain metals. The ECD is widely used for pesticides and polychlorinated biphenyls (PCBs). Electron capture has very low sensitivity for hydrocarbons other than aromatics. Many analytes of interest are not directly detectable by an ECD but may be determined by preparing appropriate derivatives. Most biological compounds, for example, possess low electron affinities. Steroids such as cholesterol can be determined as the chloroacetates. Trace metals, e.g., Al, Cr, Cu, Be, etc., have been determined at pg-ng levels by preparing volatile trifluoroacetylacetone chelates. Methylmercuric chloride, present in contaminated fish, can be determined at the nanogram level.

5-Atomic Emission Detector (AED): The gas chromatograph may be interfaced with atomic spectroscopic instruments for specific element detection. Chromatography is used to separate different forms of an element, and atomic spectroscopy detection identifies the element. This powerful combination is useful for speciation of different forms of toxic elements in the environment. For example, a helium microwave induced plasma atomic emission detector (AED) can be used to detect volatile methyl and ethyl derivatives of mercury in fish, separated by GC. The AED can simultaneously determine the atomic emissions of many elements. The emitted light passes through a monochromator and is detected by an array detector. Also, gas chromatographs are interfaced to inductively coupled plasma–mass spectrometers (ICP–MS) in

which atomic species from the plasma are introduced into a mass spectrometer, for very sensitive simultaneous detection of species of several elements and even to differentiate between different isotopes of the same element.

6-GC-MS:

Gas Chromatography-Mass Spectrometry (GC-MS) is a very powerful analytical tool that combines all the characteristics of Gas Chromatography (GC) and Mass Spectrometry (MS) to identify different components of a sample. Actually gas chromatographs are linked to a mass spectrometer which acts as the detector. In GC-MS. GC separates the components of the sample mixture and these components are analyzed and identified by MS. The sample mixture is subjected into the GC inlet where it is vaporized due to high temperature and passed on column by carrier gas. The components of sample are distributed between the stationary phase and mobile phase which swept through GC column at different rates. So separation of sample components takes place inside column based on retention time. The separated components of the sample exit from the GC column, entering the ionization chamber of the MS in such a way that most of the carrier gas is eliminated. In this high vacuum ionization camber all components, are ionized and these ionized fragments are separated, accelerated and detected using their mass to charge ratio (m/e).Clear separation, identification and quantification of various components in a sample mixture became possible by this hyphenated technique in a best way.

Applications

GC analysis is used to calculate 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.

Gas chromatography is used in the analysis of:

(a) air-borne pollutants

(b) performance enhancing drugs in athlete's urine samples

(c) oil spills

(d) essential oils in perfume preparation.

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

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.

Advantages of Gas Chromatography

- Use of longer columns and higher velocity of carrier gas permits the fast separation in a matter of few minutes.
- Higher working temperatures up to 500 ˚C and 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.

Limitations of Gas Chromatography

- 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.

Applications of GC

Gas chromatography is frequently used for separation of hydrocarbons and refinery gases in petroleum industries.
It is useful for determination of minute quantities of herbicides, fertilizers and pesticides (organophosphates, halogenated compounds) in vegetables, fruits and animal tissues.
It is helpful to analyze environmental hazards substances like automobile exhaust gases, blood, human breathe, saliva, urine, ambient air, volatile, trace elements substances in waste water and environmental toxins.
Gas chromatography analyze and separate aromas of flowers, beverages, ingredients, contents of Food and flavor.
It is used extensively in pharmaceutical industries especially to check intermediates, purity of samples and drugs assay as well.
It is most important technique for forensic and clinical analysis, toxicological cases, fatty acids, steroids, biological specimens and body secretions.
It is used to ensure the quality of various industrial as well as agricultural products.
It is used to study reaction mechanism.
This technique has also been used to analyze detergents, soap, rubber products resins, plastics, binders, coatings and plasticizers and polymers.
The high degree of resolution of GLC allows purity of a sample to be checked.
It has also been used in the separation of radioactive products.

Limitations of GC

This technique is used for separation of only gaseous substances or substances which can be made volatile at high temperature up to 300°C but most of organic substances decompose at high temperature. This is the main drawback of this technique. Compared with the other chromatographic methods, gas chromatography requires fairly complicated and expensive apparatus, but the advantages more than compensate for these drawbacks.