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HPLC GC MS SPE IR

Theory and Instrumentation of GC

Introduction

Wherever you see this symbol, it is important to access the on-line course as there is interactive material that cannot be fully shown in this reference manual.

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1. Aims and Objectives

Aims

- Outline a Brief History of Gas Chromatography (GC)
- Compare and contrast GC with other analytical techniques – primarily High Performance Liquid Chromatography (HPLC)
- Explain the function of each major component of the GC system
- Explain the terms and appearance of a typical Chromatogram
- Outline the fundamental basis for separation in GC
- Indicate the major advantages of GC and the application areas in which it is used

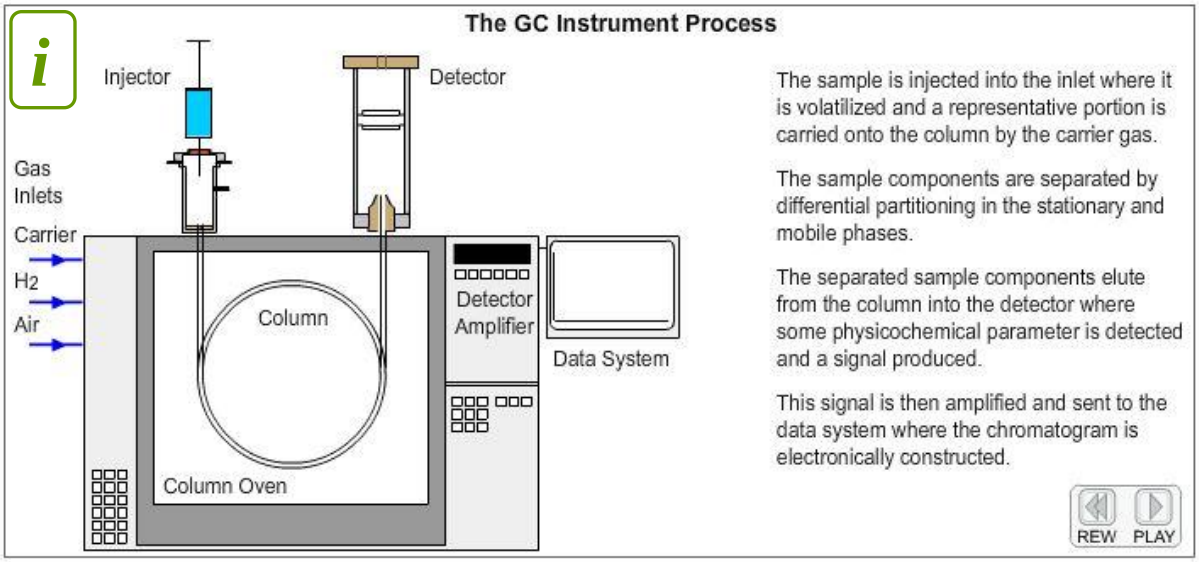


Figure 1: Gas chromatography (GC) chromatographic process.

Objectives

- at the end of this unit you should be able to-
- Identify analytes suitable for GC analysis from physicochemical data
- Describe the function of the various components of a Gas Chromatograph
- Explain the fundamental basis of separation in GC in terms of solubility and vapor pressure of analytes
- Recognize when the use of GC might be applicable to solving analytical problems

2. Origins 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.[1]

Archer J. P. Martin
1910-2002

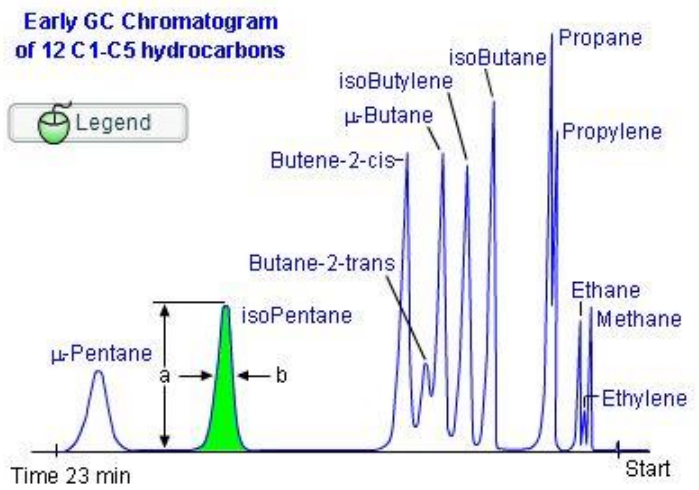
Richard L. M. Synge
1914-1994

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.[2]

They demonstrated the technique by separating and quantitatively determining the twelve components of a C_1-C_5 fatty acid mixture (Figure 2). The importance of GC was recognized almost immediately by petrochemical laboratories, which faced the challenge of analyzing complex hydrocarbon mixtures.



The chromatogram show in the separation of 12 C_1-C_5 hydrocarbons in 23 minutes was used in the first advertising of the Perkin-Elmer Model 154. A 2 m long column containing 30% triisobutylene on Celite was used at room temperature.

Figure 2: GC separation of twelve C_1 to C_5 fatty acids.

3. Why Choose Gas Chromatography

The two main chromatographic techniques used in modern analytical chemistry are Gas Chromatography (GC) and High Performance Liquid Chromatography (HPLC).

HPLC uses a liquid mobile phase to transport the sample components (**analytes**) through the column, which is packed with a solid stationary phase material.

HPLC was first proposed by the Russian botanist Mikhail Tswett who first used the term 'Chromatography' (Latin for 'colored drawing') in 1906, to describe the separation that occurred when solutions of plant pigments were passed through columns of calcium carbonate or alumina, using petroleum ether.

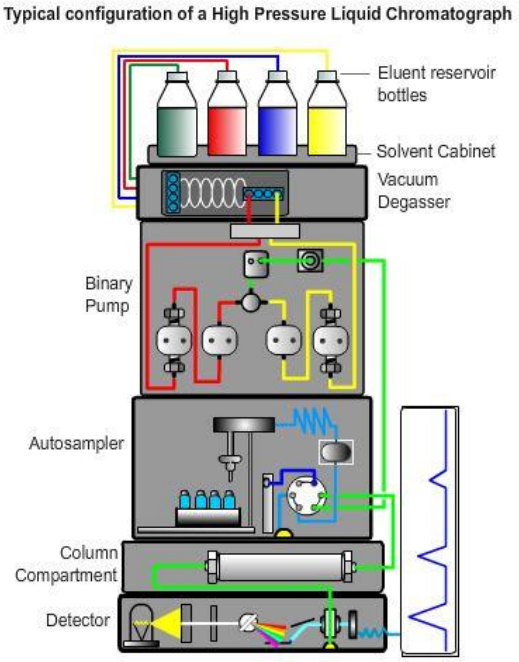


Figure 3: High Performance Liquid Chromatography (HPLC) instrument.

In contrast, gas chromatography uses a gaseous mobile phase to transport sample components through either packed columns or hollow capillary columns containing a polymeric liquid **stationary phase**. In most cases, GC columns have smaller internal diameter and are longer than HPLC columns. GC has developed into a sophisticated technique since the pioneering work of Martin and James in 1951, and is capable of separating very complex mixtures of volatile analytes.

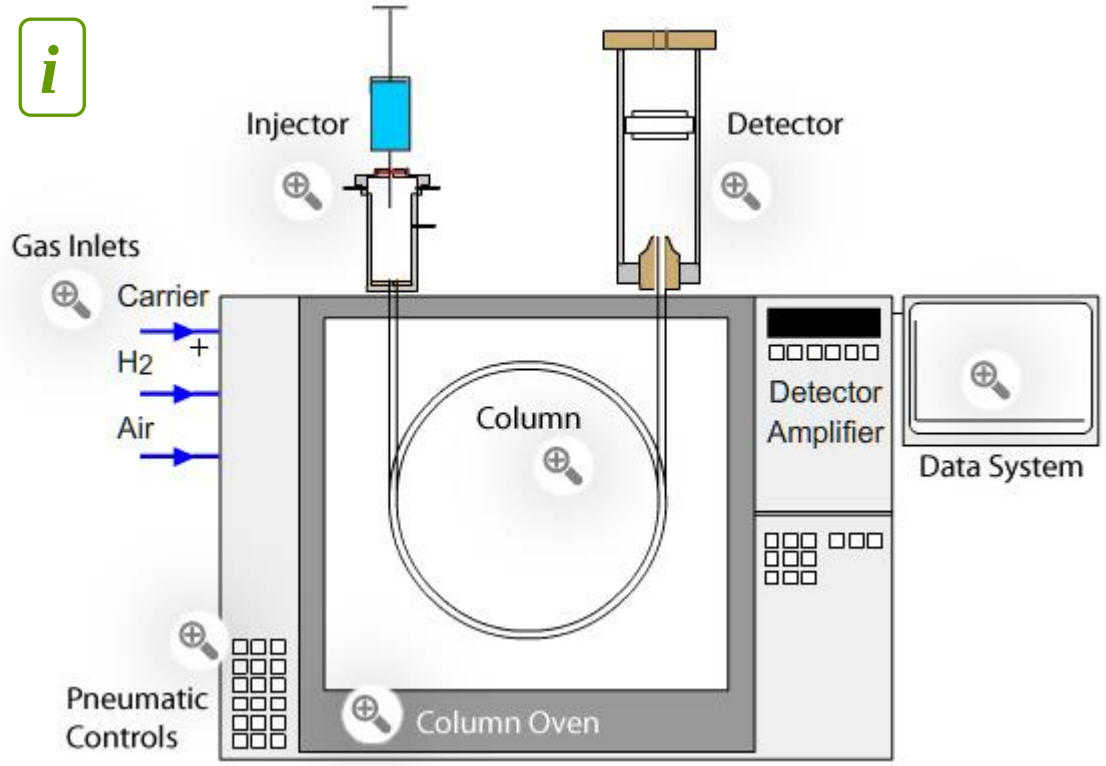


Figure 4: GC instrument.

Gas Inlets:

Gas is fed from cylinders through supply piping to the instrument. It is usual to filter gases to ensure high gas purity and the gas supply may be regulated at the bench to ensure an appropriate supply pressure (Figure 5).

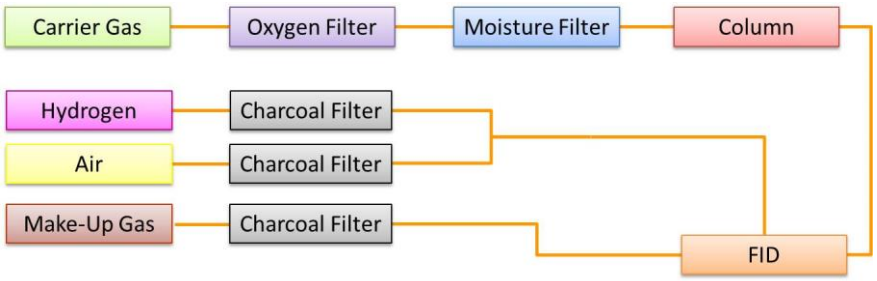


Figure 5: Gas filters required for a GC instrument with Flame Ionization (FID) detector.

Required gases might include:

Carrier - (H_2, He, N_2)
Make-up gas - (H_2, He, N_2)
Detector Fuel Gas - (H_2 & Air, Ar or Ar & CH_4, N_2) depending on the detector type

Pneumatic controls:

The gas supply is regulated to the correct pressure (or flow) and then fed to the required part of the instrument. Control is usually required to regulate the gas coming into the instrument and then to supply the various parts of the instrument. A GC fitted with a Split/Splitless inlet, capillary GC column and Flame Ionization detector may have the following different gas specifications:

Carrier gas supply pressure, column inlet pressure (column carrier gas flow), inlet split flow, inlet septum purge flow, detector air flow, detector hydrogen flow, detector make-up gas flow.

Modern GC instruments have Electronic Pneumatic pressure controllers – older instruments may have manual pressure control *via* regulators (Figure 4).

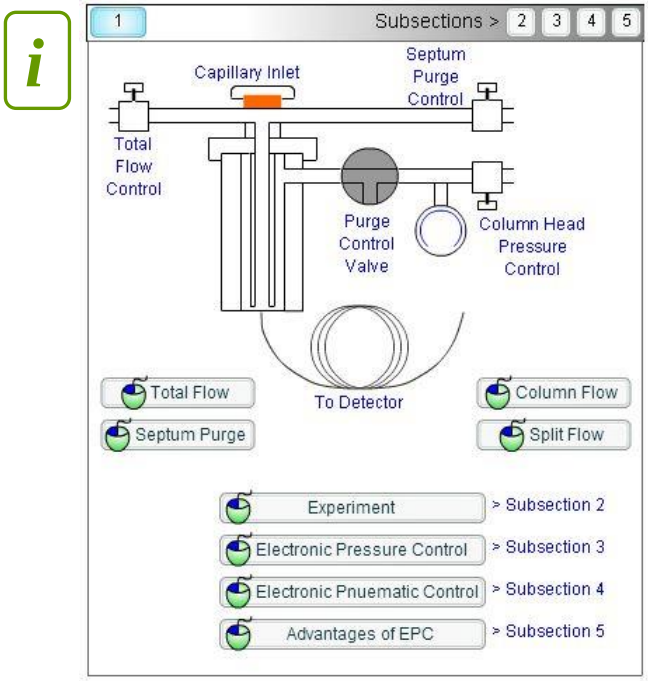


Figure 6: GC pneumatic controls.

Injector:

Here the sample is volatilized and the resulting gas entrained into the carrier stream entering the GC column.

Many inlet types exist including:

- Split / Splitless
- Programmed Thermal Vaporizing (PTV)
- Cool-on-column (COC) etc.

The COC injector introduces the sample into the column as a liquid to avoid thermal decomposition or improve quantitative accuracy.

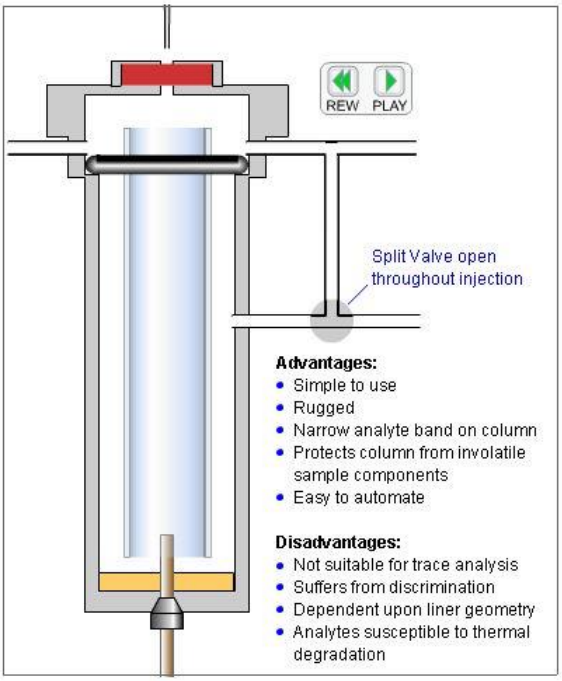


Figure 6: Split/splitless inlet.

Column:

In GC, retention of analyte molecules occurs due to stronger interactions with the stationary phase than the mobile phase. This is unique in GC and, therefore, interactions between the stationary phase and analyte are of great importance. The interaction types can be divided into three broad categories:

- Dispersive
- Dipole
- Hydrogen bonding

The sample is separated into its constituent components in the column. Columns vary in length and internal diameter depending on the application type and can be either packed or capillary. Packed columns (typical dimension 1.5 m x 4 mm) are packed with a solid support coated with immobilized liquid stationary phase material (GLC). Capillary columns (typical dimension 30 m x 0.32 mm x 0.1 mm film thickness) are long hollow silica tubes with the inside wall of the column coated with immobilized liquid stationary phase material of various film thickness.

Many different stationary phase chemistries are available to suit a host of applications. Columns may also contain solid stationary phase particles (GSC) for particular application types.

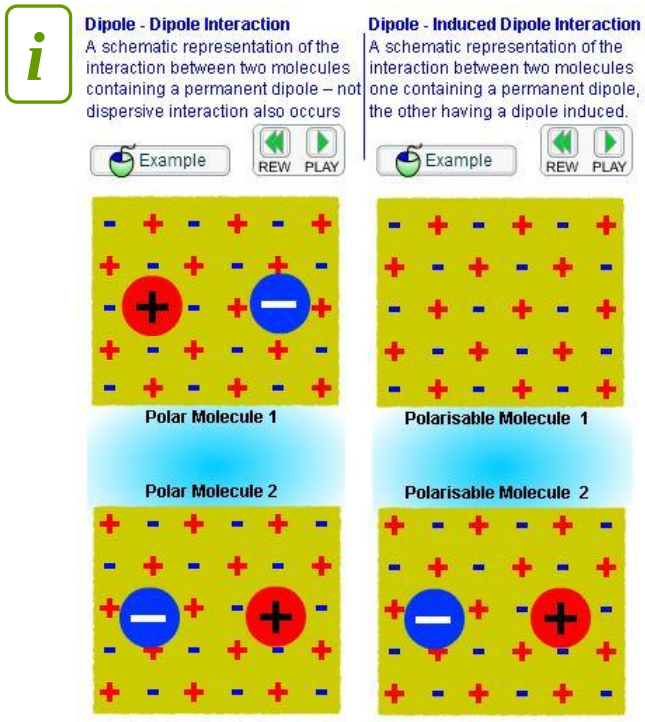


Figure 7: Dipole-dipole and dipole-induced dipole interactions.

Column Oven:

Temperature in GC is controlled via a heated oven. The oven heats rapidly to give excellent thermal control. The oven is cooled using a fan and vent arrangement usually at the rear of the oven.

A hanger or cage is usually included to support the GC column and to prevent it touching the oven walls as this can damage the column.

The injector and detector connections are also contained in the GC oven. For Isothermal operation, the GC is held at a steady temperature during the analysis. In temperature programmed GC (pTGC) the oven temperature is increased according to the temperature program during the analysis.

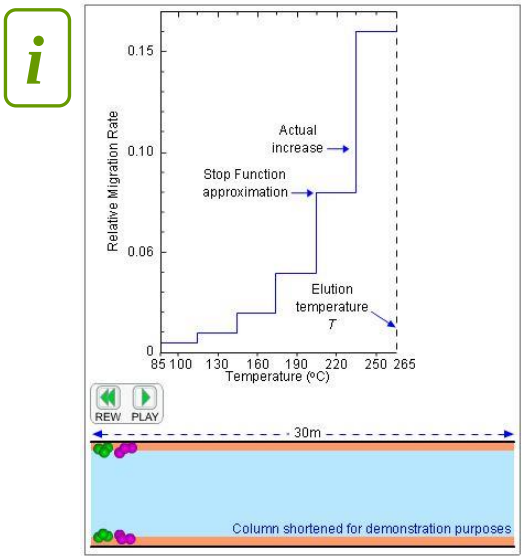


Figure 8: GC temperature programming.

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)

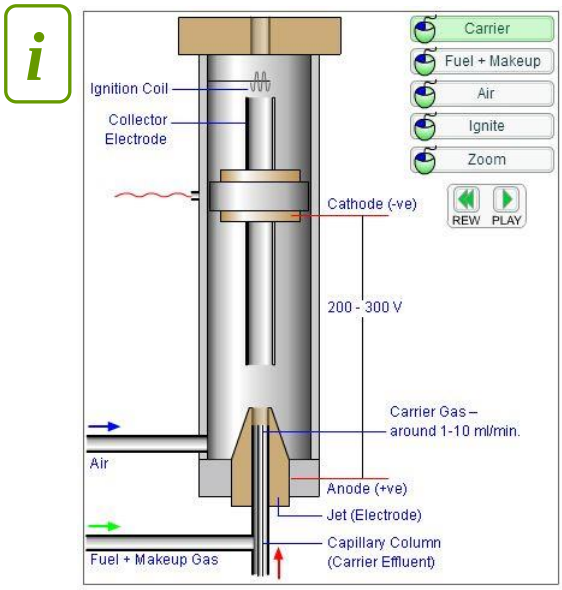


Figure 9: Flame ionization (FID) detector.

Data System:

The data system receives the analogue signal from the detector and digitizes it to form the record of the chromatographic separation known as the 'Chromatogram' (Figure 10). The data system can also be used to perform various quantitative and qualitative operations on the chromatogram – assisting with sample identification and quantitation.

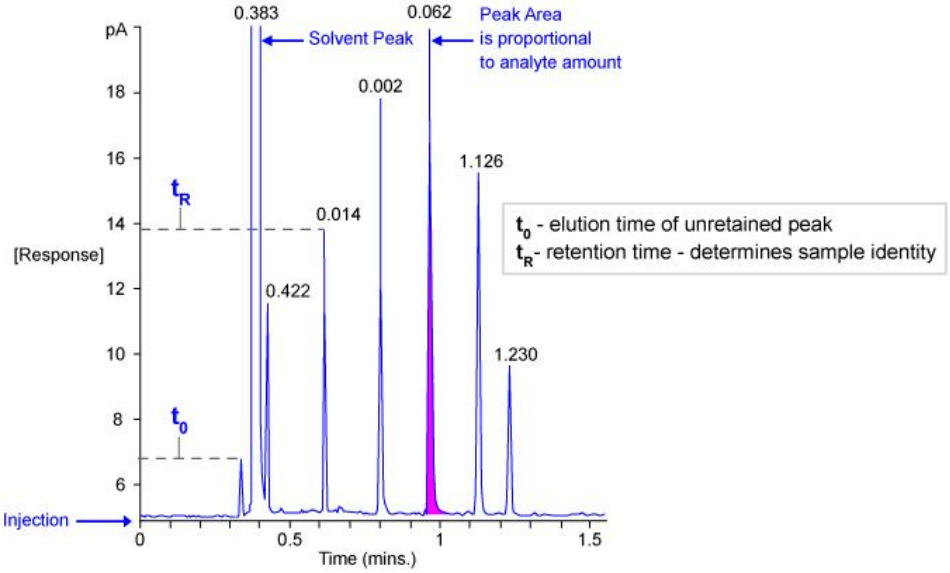


Figure 10: GC chromatogram.

The following information gives an indication of the type of sample (analyte) analyzed by either GC and HPLC and relative strengths and limitations of each technique.

GC

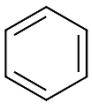
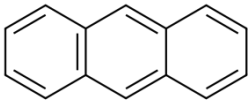
- Samples analyzed by GC must be volatile (have a significant **vapor pressure** below 250 °C)
- **Derivatization** to increase volatility is possible but can be cumbersome and introduces possible **quantitative** errors
- Most GC analytes are under 500 Da Molecular Weight for volatility purposes
- Highly polar analytes may be less volatile than suspected when dissolved in a polar solvent or in the presence of other polar species due to intermolecular forces such as hydrogen bonding.

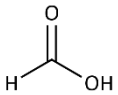
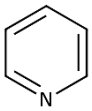
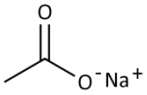
HPLC

- HPLC analysis has no volatility issues; however the analyte must be soluble in the mobile phase.
- HPLC can analyze samples over a wide **polarity** range and is able to analyze ionic samples. Mobile phase components are selected to ensure sample solubility.
- HPLC has no real upper molecular weight limit and large proteins of many thousands of Daltons may be analyzed.

Under what circumstances would we choose GC to separate our sample components?

Let's look at which of the following analytes/samples are suitable for GC analysis (Table 1).

	**Hexane** C_6H_{14}, M.Wt. 86.2 Boiling Point (°C) 69 Vapor Pressure 130 kPa @ 25 °C	**Hexane IS suitable for GC analysis** – it has low boiling point (and is therefore volatile) and generates a significant vapor pressure under the conditions shown. Hexane is often used as a solvent to prepare samples for GC analysis.
	**Benzene** C_6H_6, 78.1, Boiling Point (^oC) 80.1, Vapor Pressure 12.7 kPa @ 25 ^oC	**Benzene IS suitable for GC analysis** - it has low boiling point (and is therefore volatile) and generates a significant vapor pressure under the conditions shown. HOWEVER, benzene is carcinogenic and great care is required when analyzing this substance. Split vent traps should be fitted and the FID effluent directed into a fume cupboard.
	**Anthracene** $C_{14}H_{10}$, 78.1, Boiling Point (^oC) 340, Vapor Pressure n/a kPa @ 25 ^oC	Anthracene is involatile and does not generate a significant vapor pressure under the conditions shown. Anthracene is a solid at room temperature – this can often be a clue as to the suitability of an analyte to GC analysis. **Anthracene MAY be analyzed by GC** if high temperature columns (metal clad) and fittings are used.

	**Formic Acid** CH_2O_2, M.Wt. 46.0, Boiling Point (^oC) 100.7, Vapor Pressure 23 kPa @ 25 ^oC	**Formic acid IS suitable for GC analysis** under certain conditions. Analytes that are ionic in solution are usually much less volatile than expected – and are often difficult to dissolve in volatile organic solvents ready for injection into the GC. However, the analyte may be extracted using Headspace sampling or Solid Phase Micro-Extraction (SPME) – several literature applications exist.
H_2SO_4	**Sulfuric Acid** H_2SO_4, M.Wt. 98.1, Boiling Point (^oC) 280, Vapor Pressure n/a kPa @ 25 ^oC	**Sulfuric acid IS NOT suitable for GC analysis**. It is ionized in solution, non-volatile and highly destructive towards GC columns.
	**Pyridine** C_5H_5N, M.Wt. 79.10, Boiling Point (^oC) 115.2, Vapor Pressure 16 kPa @ 25 ^oC	**Pyridine IS suitable for GC analysis**. The analyte is semi-volatile and can be dissolved in a range of organic solvents for introduction into the GC using a split / splitless inlet. Again care must be taken when analyzing pyridine as it is toxic.
	**Sodium Acetate** $C_2H_3NaO_2$, M.Wt. 82.0, Boiling Point (^oC) n/a, Vapor Pressure n/a kPa @ 25 ^oC	**Sodium Acetate IS NOT suitable for GC analysis**. This highly ionic substance is non-volatile and cannot be dissolved in a suitable organic solvent prior to analysis (water is rarely used as a solvent for GC analysis as will be studied in subsequent sections).
K^+Cl^-	**Potassium Chloride** KCl, M.Wt. 74.5, Boiling Point (^oC) 1420, Vapor Pressure n/a kPa @ 25 ^oC	**Potassium Chloride IS NOT suitable for GC analysis**. This highly ionic substance is non-volatile and cannot be dissolved in a suitable organic solvent prior to analysis (water is rarely used as a solvent for GC analysis as will be studied in subsequent sections).

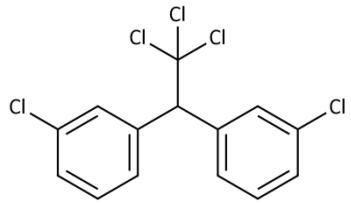
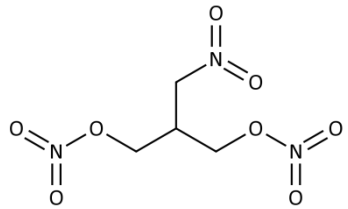
	**DDT** $C_{14}H_9C_{15}$, M.Wt. 354.5, Boiling Point (°C) 260, Vapor Pressure n/a kPa @ 25 °C	**DDT IS suitable for analysis by GC**. Although its boiling point is high – high temperature injection methods may be used for sample introduction into the GC. Several literature methods are reported and most require the use of an internal standard to ensure the analyte does not degrade in the high temperature injection port.
	**Nitroglycerin** $C_3H_5N_3O_9$, M.Wt. 227.1, Boiling Point (°C) n/a, Vapor Pressure n/a kPa @ 25 °C	Perhaps surprisingly – **Nitroglycerin IS suitable for analysis by GC**. Although the analyte is ionic – it may be extracted from the sample into certain organic solvents (acetonitrile is widely reported in literature), prior to injection using a spilt / splitless inlet.

Table 1: GC analyte molecular properties.

4. Gas Chromatography Separation Mechanism

In Gas Chromatography (GC) the mobile phase is a gas and the stationary phase is either a solid - Gas solid chromatography (GSC) or an immobilized polymeric liquid - Gas Liquid Chromatography (GLC). Of the two types of GC, GLC is by far the most common as will be seen.

The movie below (Figure 11) shows a typical separation process in GC. Each sample component 'partitions' between the gaseous mobile phase and liquid stationary phase (often coated onto the inner wall of a long thin capillary tube). The rate and degree of partitioning depends upon the chemical affinity of the analyte for the stationary phase and the analyte vapor pressure – which is governed by the column temperature.

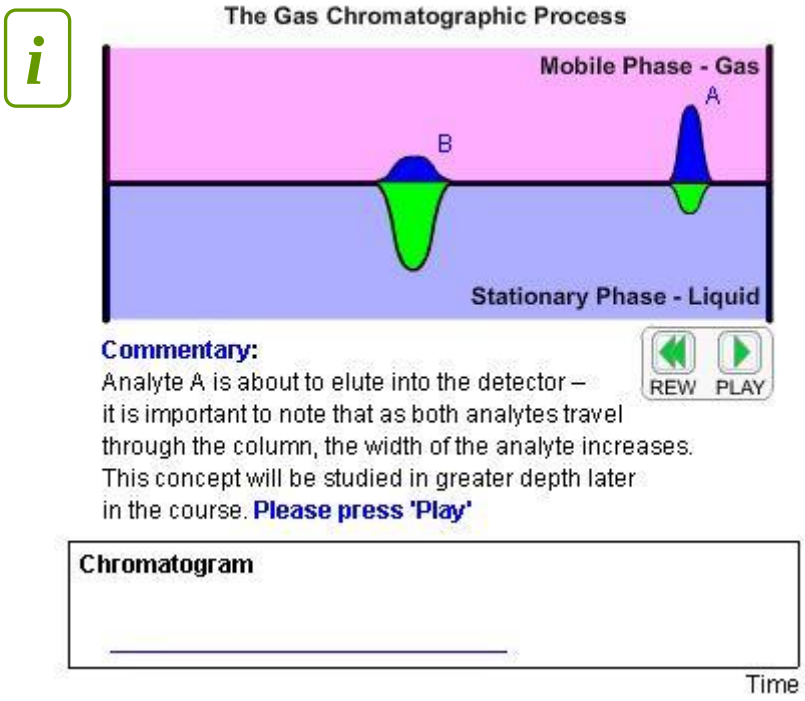


Figure 11: Gas chromatographic process.

From the movie it can be seen that component A has a lower affinity for the stationary phase and therefore is moved through the column more quickly than component B, which spends more of its time in the stationary phase – in this way separation is achieved.

In GC, analyte separation is achieved by optimizing the differences in stationary phase affinity and the relative vapor pressures of the analytes. In practice these parameters are manipulated by changing the chemical nature of the stationary phase and the column temperature.

The Distribution Coefficient (Partition Coefficient) (K_c)

The 'distribution coefficient' measures the tendency of an analyte to be attracted to the stationary phase (Equation 1). Large K_c values lead to longer retention analyte times. The value of K_c can be controlled by the chemical nature of the stationary phase and the column temperature.

$$K_c = \frac{[C_S]}{[C_M]} \quad (1)$$

Where:

C_S = concentration of analyte in the stationary phase

C_M = concentration of analyte in the mobile phase

5. The Gas Chromatograph

Instrumentation for Gas chromatography has continually evolved since the inception of the technique in 1951 and the introduction of the first commercial systems in 1954.

Most modern commercial GC systems operate in the following way (Figure 12):

- An inert carrier gas, such as helium, is supplied from gas cylinders to the GC where the pressure is regulated using manual or electronic (pneumatic) pressure controls
- The regulated carrier gas is supplied to the inlet and subsequently flows through the column and into the detector
- The sample is injected into the (usually) heated injection port where it is volatilized and carried into the column by the carrier gas
- The sample is separated inside the column - usually a long silica based column with small internal diameter. The sample separates by differential partition of the analytes between the mobile and stationary phases, based on relative vapor pressure and solubility in the immobilized liquid stationary phase
- On elution from the column, the carrier gas and analytes pass into a detector, which responds to some physicochemical property of the analyte and generates an electronic signal measuring the amount of analyte present
- The data system then produces an integrated chromatogram
- Gas chromatography uses ovens that are temperature programmable. The temperature of the GC oven typically ranges from 5 °C to 400 °C but can go as low as -25 °C with cryogenic cooling

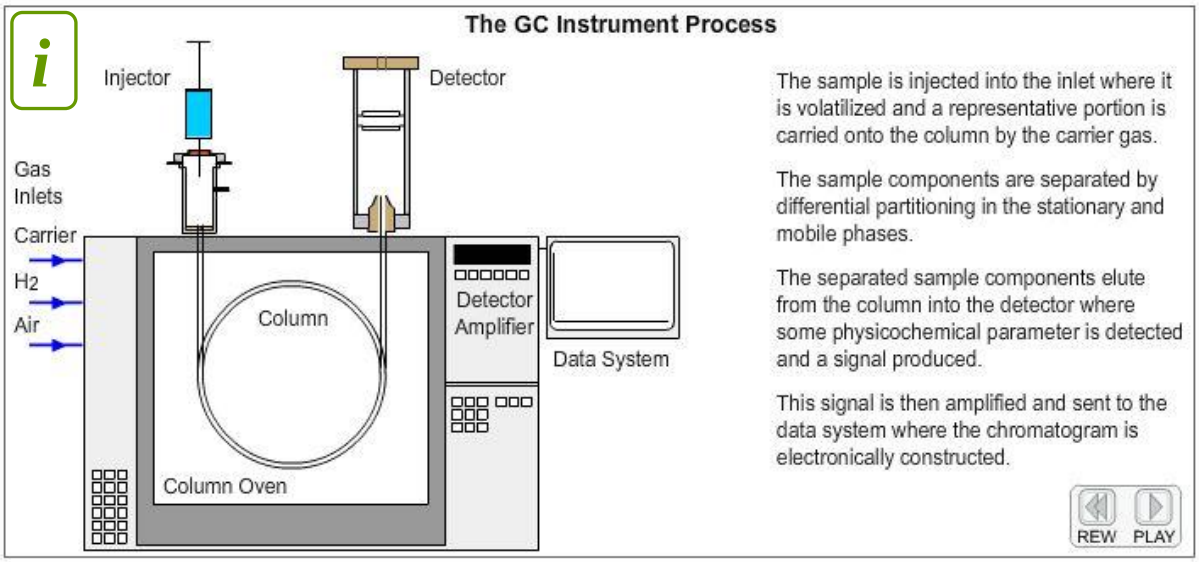


Figure 12: Gas chromatography (GC) chromatographic process.

6. The Chromatogram

As the components elute from the column they pass into a detector – where some physicochemical property of the analyte produces a response from the detector. This response is amplified and plotted against time – giving rise to a 'chromatogram' (Figure 13).

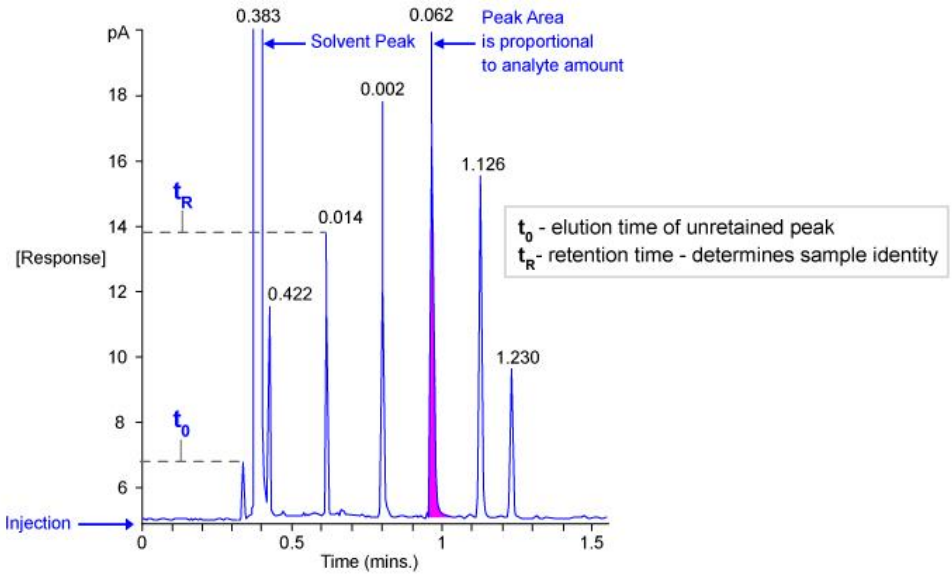


Figure 13: GC chromatogram.

Components (such as the injection solvent) that are not retained within the column **elute** at the 'dead time' or 'hold up time' $\mathbf{t_0}$. There are various ways of measuring this parameter using unretained compounds such as methane or hexane.

Those compounds (analytes and sample components) that are retained elute as approximately '**Gaussian'** shaped peaks later in the chromatogram. Retention times provide the **qualitative** aspect of the chromatogram and the retention time of a compound will always be the same under identical chromatographic conditions. The chromatographic peak height or peak area is related to the quantity of analyte. For determination of the actual amount of the compound, the area or height is compared against standards of known concentration.

7. GC Advantages and Disadvantages

Gas chromatography has several important advantages which are listed opposite.

GC techniques produce fast analyses because of the highly efficient nature of the separations achieved – this will be studied further in the Band Broadening Section. It can be argued that modern GC produces the fastest separations of all chromatographic techniques. A column has been produced to separate 970 components within a reasonable analysis time - proving that very complex separations may be carried out using GC.[2]

By using a combination of oven temperature and stationary phase chemistry (polarity) very difficult separations may also be carried out – including separations of chiral and other positional isomers.

GC is excellent for quantitative analysis with a range of sensitive and linear detectors to choose from.

GC is however limited to the analysis of volatile samples. Some highly polar analytes can be derivatized to impart a degree of volatility, but this process can be difficult and may incur quantitative errors.

A practical upper temperature limit for conventional GC columns is around 350-380 °C. Analyte boiling points rarely exceed 400 °C in GC analysis and the upper Molecular Weight is usually around 500 Da.

Advantages

- Fast analysis
- High efficiency – leading to high resolution
- Sensitive detectors (ppb)
- Non-destructive – enabling coupling to Mass Spectrometers (MS) - *an instrument that measures the masses of individual molecules that have been converted into ions, i.e. molecules that have been electrically charged*
- High quantitative accuracy (<1% RSD typical)
- Requires small samples (<1 mL)
- Rugged and reliable techniques
- Well established with extensive literature and applications

Disadvantages

- Limited to volatile samples
- Not suitable for samples that degrade at elevated temperatures (thermally labile)
- Not suited to preparative chromatography
- Requires MS detector for analyte structural elucidation (characterization)
- Most non-MS detectors are destructive

8. Typical GC Applications

Since the development of GC instruments in the early to mid 1950's, GC has found applications in a host of industrial, environmental, pharmaceutical and biotechnology analytical laboratories.

Modern GC techniques are able to sample from a wide variety of matrices, including solids, liquids and permanent gases.

High temperature applications using specially designed columns are able to analyze relatively non-volatile substances and Cool-on-Column injection techniques allow the sampling of moderately thermally labile materials.

Purge and trap and headspace autosampling techniques are now well established and are able to desorb or extract samples collected in the most inhospitable of environments, such as the emission stacks of industrial plants.

Detector technology for GC is able to detect very small amounts of pesticides for example, from environmental samples and GC-MS techniques allow structural elucidation of even the most complex analytes.

Pharmaceutical

In the pharmaceutical industry GC is used to analyze residual solvents in both raw materials (drug substance) and finished products (drug product). Biopharmaceutical applications include urine drug screens for barbiturates and underivatized drugs and for ethylene oxide in sterilized products such as sutures.

Food/Flavors/Fragrances

The food industry uses GC for a wide variety of applications including quality testing and solvents testing. The Flavors and Fragrances industries use GC for quality testing and fingerprinting of fragrances for characterization.

Petrochemical

GC applications include natural gas analysis or refineries, gasoline characterization and fraction quantitation, aromatics in benzene, etc. Geochemical applications include mapping of oil reserves and tracing of reservoirs etc.

Chemical/Industrial

Chemical / Industrial uses include determination of product content, determination of purity, monitoring production processes, etc. GCs are used to detect organic acids, alcohols, amines, esters, and solvents.

Environmental

Environmental GC applications include detection of pollutants such as pesticides, fungicides, herbicides, purgeable aromatics, etc. Industrial environmental protection applications include stack and waste emissions as well as water discharges.

9. Glossary

Mobile phase - A liquid or gas which percolates through or over the stationary bed in a definite direction. It may be a liquid (liquid chromatography, LC) or a gas (gas chromatography, GC). In gas chromatography the expression 'carrier gas' may be used for the mobile phase. In elution (liquid) chromatography the expression 'eluent' is also used for the mobile phase.

Efficiency - Also called plate number (N), describes the broadening of the chromatographic band by using the chromatographic peak width. Plate number is indicative of column performance and can be calculated as follows:

$$N = 16\left(\frac{t_R}{w_b}\right)^2$$

Where:

t_R = peak retention time

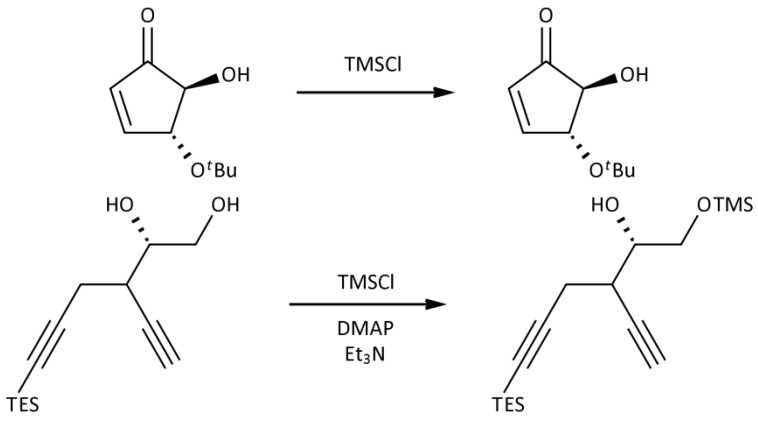
w_b = width of the peak at the base measured using tangents to the peak sides

Analyte - The compound of interest to be analyzed by injection into and elution from an HPLC or GC column.

Stationary phase - The stationary phase is one of the two phases forming a chromatographic system. It may be a solid, a gel, or an immobilized polymeric liquid. If a liquid, it may be distributed on a solid. This solid may or may not contribute to the separation process. The liquid may also be chemically bonded to the solid (bonded phase). The expression *chromatographic bed* or *sorbent* may be used as a general term to denote any of the different forms in which the stationary phase is used. A stationary phase which is covalently bonded to the support particles or to the inside wall of the column tubing is known as a *bonded phase*.

Vapor pressure - the absolute pressure at which the vapor contained in a substance is at equilibrium with its liquid or solid phase.

Derivatization - To chemically alter an analyte to impart favorable chromatographic characteristics (e.g. increase volatility). A typical derivatization reaction scheme may be:



Quantitative - Chemical analysis undertaken to determine (measure) the quantity or amount of each analyte within a mixture.

Polarity - The greater the difference in electron affinity (electronegativity) between atoms ina covalent bond the more polar the bond. Partial negative charges are found on the most electronegative atoms, the others are partially positive. The molecular electrostatic potential is the potential energy of a hydrogen ion at a particular location near a molecule.

Negative electrostatic potential corresponds to partial negative charges.

Positive electrostatic potential corresponds to partial positive charges.

Elute - To chromatograph by elution chromatography. The process of elution may be stopped while all the sample components are still on the chromatographic bed or continued until the components have left the chromatographic bed.

t_0 - The volume of the mobile phase (or the corresponding time) required to elute a component the concentration of which in the stationary phase is negligible compared to that in the mobile phase. In other words, this component is not retained at all by the stationary phase. Thus, the hold-uip volume (time) is equal to the retention volume (time) of an unretained compound. The hold-up volume (time) includes any volumes contributed by the sample injector, the detector, and connectors.

$$t_0 = \frac{V_M}{F_c}$$

Where:

V_M = column volume

F_c = flow rate

Gaussian - Following the shape of a normal distribution as described by Gauss. Used to describe a symmetrical bell shaped peak.

Qualitative - Involving distinctions based on qualities. Qualitative analysis attempts to determine the chemical identity of a substance or mixture.

10. References

1. Martin, A. J. P.; Synge, R. L. M. *Biochem. J.* **1941**, *35*, 1358-1368.
2. Berger, T. A. *Chromatographia* **1996**, *42*, 63-71.