Gas Chromatography (GC)

Fundamentals, Application, Troubleshooting,

Method Development & Validation

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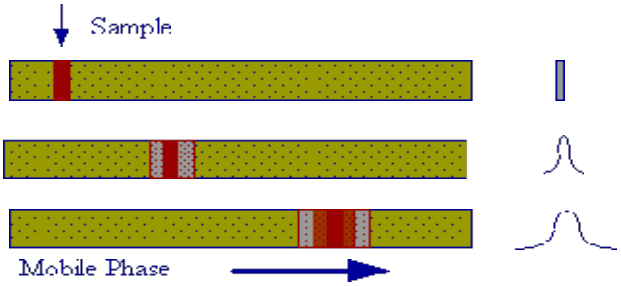
Introduction

The term "chromatography" is the general name for a wide range of physicochemical separation processes in which the components to be separated are distributed between a stationary and a mobile phase. Chromatography is a separations method that relies on differences in partitioning behavior between a flowing mobile phase and a stationary phase to separate the components in a mixture.

Chromatography involves a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase then forced through an immobile, immiscible stationary phase. The phases chosen such that components of the sample have differing solubilities in each phase.

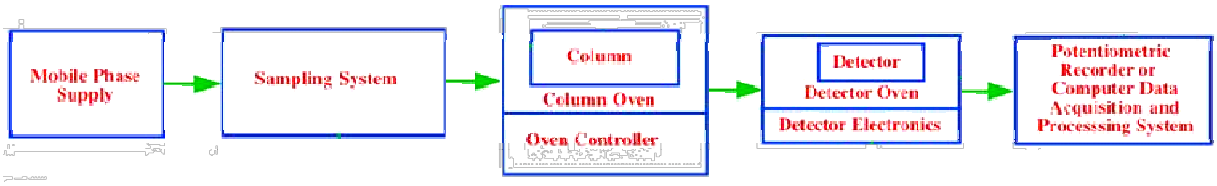
A component, which quite soluble in the stationary phase will take longer to travel through it than a component, which is not very soluble in stationary phase but very soluble in the mobile phase. Because of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase.



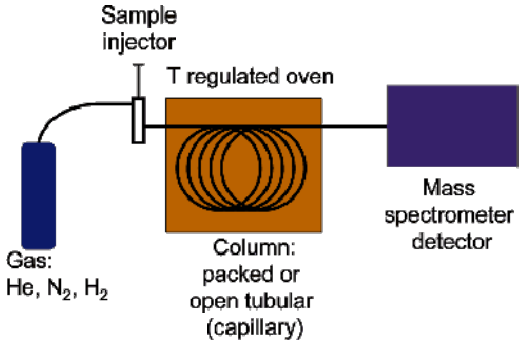
Techniques such as H.P.L.C. (High Performance Liquid Chromatography) and G.C. (Gas Chromatography) use columns - narrow tubes packed with stationary phase, through which the mobile phase is forced. Sample transported through the column by continuous addition of mobile phase. This process called elution. The average rate at which an analyte moves through the column is determined by the time it spends in the mobile phase.

A column (or other support for TLC, see below) holds the stationary phase and the mobile phase carries the sample through it. Sample components that partition strongly into

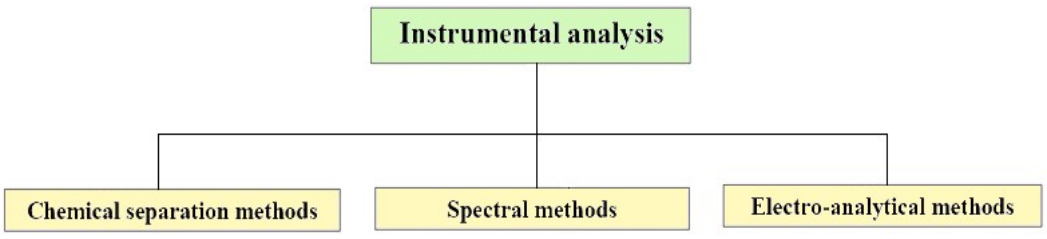
the stationary phase spend a greater amount of time in the column and are separated from components that stay predominantly in the mobile phase and pass through the column faster.



As the components elute from column they can be quantified by a detector and collected for further analysis. An analytical instrument can be combined with a separation method for on-line analysis, like gas and liquid chromatography with mass spectrometry (GC-MS and LC-MS), Fourier-transform infrared spectroscopy (GC-FTIR), and diode-array UV-VIS absorption spectroscopy (HPLC-UV-VIS).



Separation methods are an important part of analysis, and chromatography has developed into the premier analytical separation technique. Chromatography's rapid development can be attributed to its relative simplicity and the successful application of theory to practice. Furthermore, when equipped with sensitive detectors, chromatographs are capable of performing highly accurate quantitative analyses.



A brief history

Although chromatography entered a new phase in the early 1950s, the Russian botanist ***Tswett*** is generally referred to as ***father of chromatography***. His work, originally presented in 1903 and then published in 1906, described separation of plant pigments by column liquid chromatography, defined the terms, and demonstrated the technique so well that it was used in its original form for about 40 years. The original article is of significant historical interest and serves as a fitting introduction to a discussion of the concepts of chromatography; fortunately, it has been translated into English and is readily available.

It can be argued that modern HPLC began with publication of Martin and James's article on gas chromatography in 1952. In the period between 1906 and 1952 there were some developments of importance. Earliest was the use of paper as a plane support, but when thin layers of silica gel were introduced as an alternative in the late 1950s, the field of thin-layer chromatography (TLC) was born and became so popular that it largely replaced the older technique. Column chromatography developments accelerated in the 1940s. Martin and Synge published their Nobel Prize-winning article in which they introduced liquid-liquid (or partition) chromatography and accompanying theory that became known as plate theory.

GC, was found to be simple and fast and capable of producing separations of volatile materials that were impossible by distillation. It was natural to attempt to apply the successful results from GC to the older technique of LC, liquid chromatography. In 1963 Giddings published an article entitled “LC with operating conditions analogous to those of GC,” setting off a revolution in LC that led to its achieving a level of efficiency comparable to that achieved in GC. Because these new conditions for operating LC columns required high pressure, HPLC was used to describe high-pressure LC. The use of high pressure also produced the expected high-performance separations, so HPLC also denotes high-performance LC. In either case, HPLC is usually used to distinguish between the new, modern mode of operation as opposed to the old Tswett method. As noted earlier, USP uses LC to denote low-pressure or gravity feed LC and HPLC to denote high-pressure LC.

Separations in everyday life

We utilize separation methods virtually all the time. For example, we use air filters to allow us to breathe clean air indoors. Depending on the type of filter used, we can remove relatively large dust particles or microscopic pollen from plants that may produce many types of allergies. Similarly, our municipal drinking water goes through various purification steps to separate out the impurities. It is important for us to understand the chemistry of separations to better deal with our purification problems in various human endeavors.

Chemistry is a discipline that is based on a variety of separation processes. It plays a major role in our lives. Dutch word for chemistry, means the art of separation. From time immemorial, humanity has made efforts to better understand its environment (air, water, food, and other elements that affect us directly or indirectly), to protect our health, and to make changes that would benefit people at large. These efforts have been closely linked to the separation of chemical substances.

It may be interesting to review a number of examples of historical interest that relate to some process of chromatographic or non chromatographic separation listed below.

- Distillation of alcohol for various purposes is a simple separation process.
- Isolation of dyes to add color to various materials to add sparkle to our life depends on various separation processes that include chromatography.
- Extraction of natural products to provide useful drugs.
- Isolation of metals in metallurgy is based on various techniques of separation.

Industrial and scientific revolutions have brought about the need for development of a variety of separation methods, and this need is being met admirably by separation science. As a matter of fact, most advances in chemistry, chemical technology, and life sciences have been related to advances in separations and chromatography:

- Automobile industry owe their success to effective use of separation processes used in petroleum industry; they have truly revolutionized our lives today.
- Atomic age owes its progress to the resolution of the difficult problem of separation of 235U from 238U.
- The studies on separation processes in the laboratory are of great significance in understanding the phenomena such as selective permeation of cell membranes. These studies can help us determine how diseases are transmitted and how we can treat them better with suitable medicines.
- Our understanding of a variety of biochemical processes occurring in human body became possible after development of separation methods such as ultracentrifugation, chromatography, and electrophoresis.
- The studies on the human genome and proteomics owe their success to separation science. This knowledge will lead to better treatment of diseases.
- The use of the artificial kidney is a notable example of the use of separation processes in modern medicine.
- To assure the preparation of contaminant-free materials needed for various industrial processes, it is necessary to use highly sensitive chromatographic methods for trace and ultra trace analysis.
- Forensic analysis to monitor arson or explosives used by terrorists also demands the use of trace and ultra trace chromatographic analysis.
- The future solutions of environmental problems will depend to a great extent on finding economical ways to solving the corresponding separation problems.

It goes without saying that the removal of contaminants from the soil, water, air, food, or other items consumed by human beings will continue to require application of separation processes on a large scale. At the same time, it will be necessary to monitor and control the introduction of new pollutants by rigorous development of trace and ultra trace monitoring methods based on separations.

Simple separation procedures

A large number of separation methods are available that utilize select characteristics as means of separation. Each of these methods can be further divided into different techniques with unique characteristics. The need for a great variety of separation procedures can be attributed to the following factors:

- Different separation goals
- Diversity of the mixtures to be separated
- Variety of physicochemical phenomena involved in separation

A common objective of various separation methods is adequate segregation of constituents of interest with maximum speed and minimum effort, and with as large a capacity as possible. Separation plays an important role in the analysis of mixtures, as exemplified in the following examples.

- Removal of interfering constituents before determination of one or more compounds
- Isolation of unknown compounds for further characterization
- Analysis of complex unknown mixtures by separation into individual constituents

Mixtures to be separated can vary greatly with respect to molecular weight, volatility, or other properties. They may range from mixtures of atomic species through organic molecules. In some cases the properties of the individual components are so different that very simple separation techniques can be used (e.g., salt can be recovered from seawater by simply evaporating water, or it can also be collected by condensation in a distillation unit).

The complexity of the sample mixture with respect to the number of constituents or their physical and chemical properties affects the difficulty of separation and the type of technique that can be utilized. Most important, the choice of a separation technique depends on the amount of mixture to be separated, which can range from a few molecules that would require micro techniques suitable for ultra trace analysis to tons of material in an industrial-scale process that would require preparative-scale separations.

- **Evaporation**

Evaporation simply entails vaporizing the solvent by using heat or by utilizing air currents in a manner that the material concentrates to a solid state. For example, common salt (sodium chloride) in a reasonably pure form can be separated from seawater by the simple process of evaporation, using an open container for a period of time or by suitable application of heat.

Evaporation procedures are commonly used in separations to concentrate solutions as a prelude to other separation steps, or they can be the final step for obtaining an isolated material as a residue. The process of evaporation and concentration frequently leads to the formation of crystals that come out of solution.

Often, crystalline materials are obtained in a chemistry laboratory or chemical industry by simply concentrating a solution in the selected solvent(s) and allowing the solution to cool. The separation process that leads to formation and isolation of crystals is called crystallization. Evaporation procedures have undeniably led to more useful separation procedures, such as precipitation, crystallization, and distillation, discussed later.

- **Precipitation**

Changing the concentration of a solute in a solution so that it exceeds solubility in a given solvent can bring about precipitation of the solute. As mentioned earlier, when this process is carried out in an appropriately selected solvent and in a controlled manner, it can lead to crystallization. This allows us to obtain pure crystalline materials. Precipitation can be achieved by a number of different methods. Some of the methods utilized are listed here.

A. *Solvent Precipitation:* Precipitation can be achieved by adding another miscible solvent such that solubility of the component of interest would be at a low level in the mixed solvent. This procedure is called solvent precipitation.

B. *Precipitation via Chemical Reaction:* It is possible to obtain a precipitate by chemical means. In this case a change in chemical composition is brought about to obtain

a desired material; for example, a precipitate of barium sulfate can be obtained by adding sulfuric acid to an aqueous solution of barium chloride

C. *Precipitation by Adjustment of pH:* Some organic compounds can be precipitated by suitable adjustment of pH. For example, it is possible to precipitate most of the weakly basic organic compounds from aqueous solution by addition of a strongly basic solution. Similarly, the organic acids can be precipitated by acidification of the solution with strong acids.

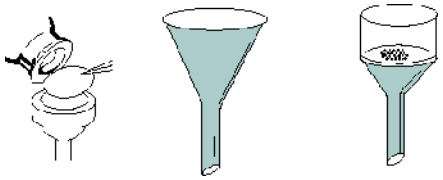
- **Crystallization**

Crystallization is an important procedure used by chemists to obtain pure material in the crystalline state. This involves concentrating a solution containing the component of interest by heating it and then allowing it to stand (i.e., cooling it) until the crystals are obtained from the solution. This process can allow separation of fairly pure material and is utilized for preparation of a large number of chemicals; however, related materials can crystallize simultaneously and thus lead to poor separation.

So it becomes necessary to design specialized crystallization procedures from optimal solvents that minimize such contamination. However, contamination from other materials crystallizing under the same conditions still remains a major consideration.

- **Filtration**

We can reasonably assume that the filtration process has been used from time immemorial to separate solids from liquids. Initially, some porous material (similar to a filter paper or cloth) was most likely used. The primary requirement of such a material is that it should allow liquid, i.e., the solvent, to pass through while retaining the solids. This concept has eventually led to a large variety of filter papers and membranes of various pore sizes.



Separation of particles that are visible to the naked eye with a filter paper is called filtration; however, filtration of submicron particle size is also possible. Simple filter papers are made from cellulose and exhibit particle retention levels down to 2.5 mm (e.g., Whatman grade 5 filter paper). These cellulose filter papers are used for general filtration. A wide choice of flow-rate combinations can be used, depending upon application.

A simple filtration process entails pouring the liquid over commonly available fluted filter paper (e.g., Whatman grade 1 is suitable for particles larger than 11 mm) or a filter paper folded into quadrants that has been placed in a conical glass filter funnel, then collecting the filtrate at the other end of the funnel. In this case gravitational force is adequate to achieve the separation of solids from the liquid. Sometimes, extra force is necessary to obtain separation. This is achieved by laying the filter paper at on a perforated plate and applying vacuum to the collection. This type of funnel is called a Buchner funnel.

Membrane filters with various pore sizes provide wide choices in filtration. Membrane holders may incorporate either sealed-in sintered glass or removable stainless steel mesh supports. Membrane filters made from various materials can offer various pore sizes that allow separations down to 0.02-mm level. The unique properties of borosilicate glass microfiber allow the manufacture of filters with retention levels extended to the sub-micron range (e.g., Whatman grade GF/F). These filters can withstand temperatures up to 550 ^{o}C.

- **Membrane separations**

It should be noted that most permeable barrier separation processes of practical importance utilize semi permeable membranes as the restrictive interface. These membranes allow passage of certain chemical species completely, while preventing or strongly retarding the permeation of others. Synthetic membranes have been developed that can be used successfully for a number of applications listed here.

- Produce drinkable water from salt water
- Remove urea from blood
- Separate azeotropic mixtures such as alcohol and water

- Recover helium from natural gas
- Remove sulfur dioxide from stack gases

Membranes have also been used as separators in batteries and fuel cells, and as ion-selective tools in analytical chemistry. The familiar separation methods referred to as ultra filtration, dialysis, electro dialysis, and reverse osmosis are based on membrane separations; they have gained increasing attention because they are convenient methods for purification and separation of molecular solutions.

As mentioned previously, a semi permeable membrane allows movement of particular chemical species completely while stopping or strongly restricting the permeation of others. At the same time, the transport rate of permeation has to be high enough to help achieve reasonably rapid separations. Good mechanical and chemical stability are important in these membranes. Numerous applications have been found for membrane separations. Large scale purification of saline water is most common application of *reverse osmosis* membrane. It may not be most economical process for seawater desalination; however, it can be very useful for brackish water purification.

A dialyzer: is an apparatus in which one or more solutes are transported from one fluid to another through a membrane under a concentration driving force. Two independent factors determine the overall efficiency of a dialyzer: the ratio of flow rate of two fluids and the rate constant of the solute transport between the fluids. The latter is controlled by the characteristics of the membrane, the membrane area, the local fluid velocities, and the fluid channel geometry.

An efficient ***artificial kidney*** is an excellent example of the application of dialysis. Low molecular weight toxins in the patient's blood, such as urea, creatinine, and uric acid, move across the membrane into a dialyzate solution having a composition such that the osmotic pressure is the same as that of blood, thereby controlling the rate of transport of certain salts. The ''cleaned'' blood is then returned to the patient.

Electro dialysis: In electro dialysis, electrically charged membranes are used to separate components of an ionic solution under the driving force of an electric current. This process has been used for desalination of water, recovery of salt from seawater, de-ashing of sugar solutions, and de-acidification of citrus juices.

- **Distillation**

The origin of distillation can be related to evaporation. Distillation can be distinguished from evaporation in that distillation is the separation of a mixture in which all the components of interest are volatile, whereas in evaporation volatile components are separated from nonvolatile ones.

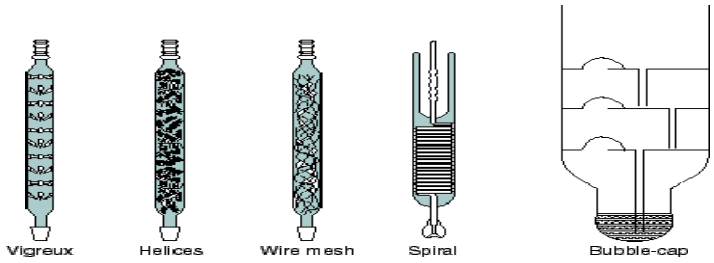
Most of these volatile components separated by distillation are generally liquids; however, distillation is not necessarily limited to volatile liquid components. At times, it is possible to volatilize a solid component directly by sublimation, which can be described as a highly specialized distillation method where a solid is directly vaporized without passing through a liquid phase.

Distillation is used mainly for separation of the components of liquid mixtures, and it depends on the distribution of constituents between the liquid mixture and vapor in equilibrium with the mixture. The two phases exist by formation of the vapor phase through partial evaporation of the liquid mixture.

Each phase can be recovered separately, with the more volatile components concentrated in the vapor, while the less volatile ones are in greater concentration in the liquid. Various forms of distillation such as fractional distillation, flash distillation, vacuum distillation, steam distillation, and azeotropic distillation are briefly described below.

Fractional Distillation: Distillation evolved into fractional distillation, which proved to be a boon to the petroleum industry in its quest to isolate various fractions of petroleum. This method is based on the return of a portion of condensate to the distillation unit under conditions such that this condensate is continuously and counter currently in contact with the vapors. The liquid portion returned to the distillation unit is referred to as reflux, and the

method is called fractional distillation or rectification. This method provides great enrichment of vapors of the more volatile component than produced in simple distillation. It requires the use of multiplate columns, commonly called fractionating columns. Fractional distillation is not restricted to the petroleum industry. It can be useful wherever volatile fractions have to be separated from one another.



Fractionating columns.

Fractional distillation, for example, can be carried out with a greater degree of success in a bubble cap column. The original mixture is heated in a pot to its boiling point. The vapors pass through plate 1 and are defected and condensed by the bubble cap. Plate 1 is maintained at the boiling point of the mixture at this stage, which is somewhat lower than the boiling point of the original mixture in the pot.

The vapors formed at plate 1 are condensed at plate 2, and this process continues to the end of the column, where a condenser is placed. The vapors at each plate are progressively richer in the more volatile component. This means that if we have adequate plates, we can separate any two volatile components in a mixture to the required degree of purity. It may be desirable to mention here that the concept of using plates to evaluate efficiency of a column has been carried into chromatography.

Flash Distillation: Flash distillation consists of instantaneous and continuous vaporization of a definite fraction of the liquid mixture in such a way that the total vapor produced is in equilibrium with the residual liquid.

Vacuum Distillation: Distillation under decreased pressure is called vacuum distillation and is used to separate high-boiling mixtures or materials that decompose below their normal boiling points. Low pressure reduces the boiling temperatures.

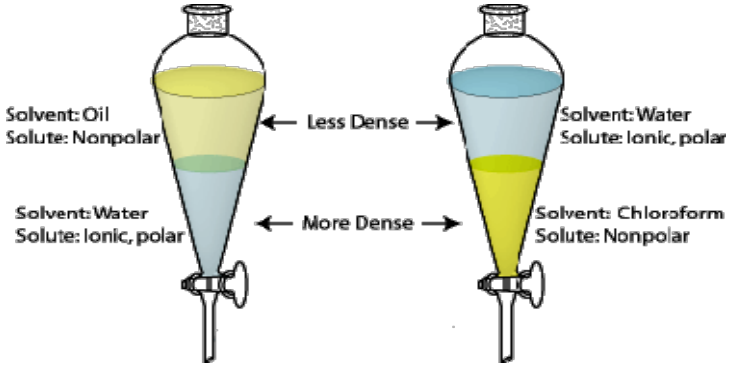
Steam Distillation: Steam distillation is utilized to separate high-boiling mixtures or to separate a material from a nonvolatile impurity. The boiling temperature of the mixture is reduced by vaporizing it into a stream of carrier vapor (steam), which, upon condensation, is immiscible with the original mixture and thus can be easily separated from it.

Azeotropic/Extractive Distillation: Azeotropic/extractive distillation methods are used for mixtures that are difficult to separate. The relative volatility of the components in the mixture is altered by adding another substance. These methods are useful in the separation of mixtures whose components boil too close together for economic fractional distillation.

Distillation as the Precursor to Gas Chromatographic Methods: Fractional distillation can be considered a precursor to the development of gas chromatographic methods, where volatile components are separated on a packed or capillary column. The need to separate nonvolatile components led to the discovery of liquid chromatographic methods such as HPLC that are based on partition.

- **Extraction**

Extraction is a relatively simple type of separation process in which a solute is distributed between two immiscible solvents. An important point to remember is that the distribution coefficient is related to relative solubilities of the solute in the two solvents. Frequently, one of the solvents is aqueous and the other solvent is organic, and it is essential that the selected organic solvent.



Chromatography Separation

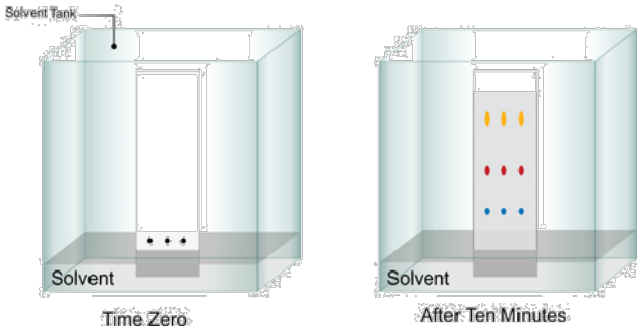
- **Definition of Chromatography**

Separation is a familiar word, and it is unlikely that any confusion will arise from its use in reference to chemical analysis. Nevertheless, there is some merit in considering a precise, general definition such as that suggested by Rony:

"Separation is the hypothetical condition where there is complete isolation, by separate macroscopic regions, for each of the chemical components which comprise a mixture. In other words, the goal of any separation process is to isolate the chemical components, in their pure forms, into separate vessels, such as glass vials or polyethylene bottles."

The adjective hypothetical is used for two reasons. In the first place, it is theoretically impossible to accomplish the complete separation of the components of a mixture. Consider, for example, the process of analyte transfer in a liquid-liquid extraction. If a single extraction removes most of the analyte from phase 1 into phase 2, say 90%, each successive step will also remove 90% of the analyte that remained after the previous step. Thus 10% of the analyte from the previous step remains in the first phase and subsequent steps can remove only a fraction (0.9) of it.

Clearly, all of the analyte cannot be removed; of course, when the fraction removed approaches 1, say 0.9999, we consider that virtually all of it has been removed. The second reason for using the term hypothetical is that the separated components often are not actually isolated into vessels but rather are detected and their presence recorded (on chart paper or in a computer data file).



Principals and Theory of Chromatography

Most separation methods employ two phases. In chromatography, they are called the mobile phase and the stationary phase. In liquid-liquid extraction, they are called the extractant and the raffinate. The extractant in a multistage liquid-liquid extraction has a function similar to the mobile phase in liquid chromatography, and comparisons of these two techniques will help in understanding both of them. In distillation, the second phase (a vapor) is formed from the first phase (a liquid) as the latter is heated.

This process differs from extraction and chromatography where the second phase is added. Obviously, the principles of distillation are different from those for chromatography. However, as has been noted, much of the development of GC was carried out by chemists who were accustomed to making their separations by distillation, so they labeled the chromatographic process with distillation terms such as theoretical plutes.

Next table lists some common separation methods providing a convenient reference for contrasting them with chromatography. A few separation methods employ only one phase; examples are field-flow fractionation and some types of electrophoresis. In these cases, a second force such as a voltage gradient is required to effect a separation. In fact, the list of processes in the third column of the table can be classified into three different categories, which will help organize variations in methods such as secondary forces.

Properties of Some Separation Methods

Name	Number of Phases	Type of Process
Gas–liquid chromatography (GLC)	2	Partition (absorption)
Gas–solid chromatography (GSC)	2	Adsorption
Liquid–liquid chromatography (LLC)	2	Partition
Liquid–solid chromatography (LSC)	2	Adsorption
Liquid–Liquid extraction (LLE)	2	Partition
Supercritical fluid extraction (SFE)	2	Partition
Solid-phase extraction (SPE)	2	Adsorption
Solid-phase microextraction (SPME)	2	Adsorption and partition
Zone electrophoresis (CZE)	1	Electromigration
Capillary electrochromatography (CEC)	2	Partition and electromigration
Field-flow fractionation (FFF)	1	Electromigration
Distillation	2	Change of state
Precipitation	2	Change of state
Zone melting	2	Change of state
Dialysis	2	Sieving (size exclusion)
Filtration	2	Sieving
Size exclusion chromatography (SEC)	2	Size exclusion

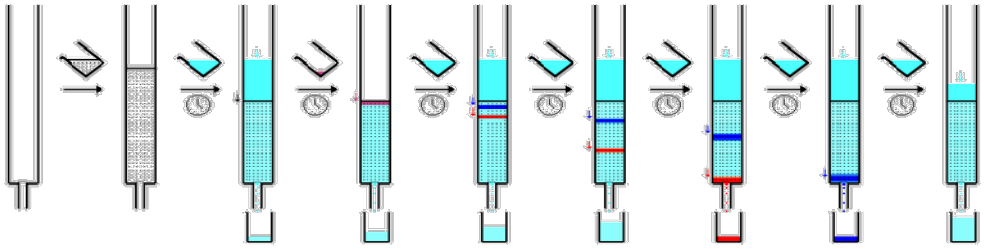
One can consider that all separation techniques depend upon a differential distribution of the analytes in a sample. The types of differential distribution are based on one of three different processes-different states, phases, or environments-as follows:

- Different chemical states of the same matter, also called phase equilibria or changes in state. For example, the distribution between liquid and vapor states is called distillation. Other separation methods in this category are sublimation, crystallization, zone melting (refining), and precipitation.

- Different chemical phases, also called phase distribution equilibria. This category includes most of the chromatographic processes listed in Table as absorption (partition) or adsorption as well as liquid-liquid extraction (LLE), solid-phase extraction (SPE), supercritical fluid extraction (SFE), and solid-phase micro extraction (SPME).

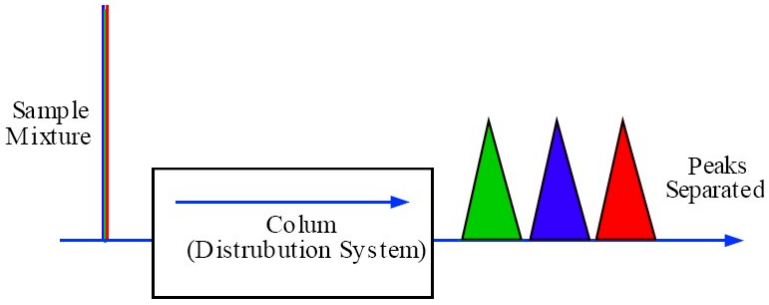
- Different chemical environments or different locations. These separations are based on differential rates of migration of analytes under the influence of a field. It is a non equilibrium process, unlike types 1 and 2, and the analytes end up in different places within the field. Some examples are:

  - Electric field: electrophoresis (of ions) and field-flow fractionation (FFF)
  - Gravitational field: centrifugation and filtration
  - Thermal field: thermal diffusion
  - Membrane (semi permeable): dialysis, osmosis, and ultra filtration



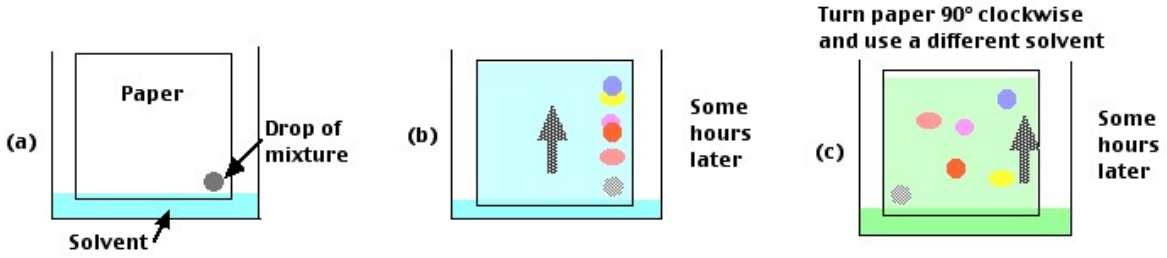
A sample that requires analysis is often a mixture of many components in a complex matrix. For samples containing unknown compounds, components must be separated from each other so that each individual component can be identified by other analytical methods.

Separation properties of components in a mixture are constant under constant conditions, and therefore once determined they can be used to identify and quantify each of components. Such procedures are typical in chromatographic and electrophoretic analytical separations.



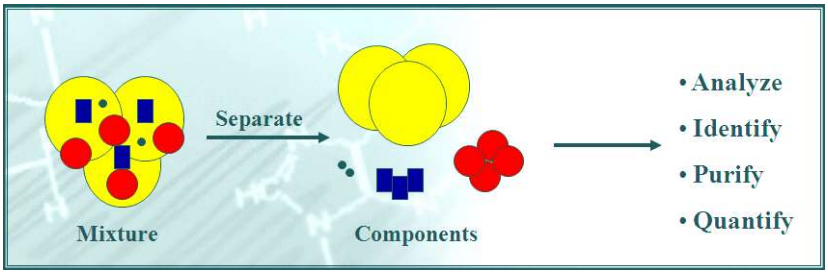
A mixture can be separated using the differences in physical or chemical properties of the individual components. As an example, dumping spaghetti and water in a colander separates the two components because the liquid water can run through the colander but the solid spaghetti cannot. Some water will stick to the spaghetti and some spaghetti may go down the drain because the colander is not 100% efficient.

An analogous example is the filtering of a solid precipitate to separate it from a solution. These separations are based on states of matter of two components; other physical properties that are useful for separations are density and size. Some useful chemical properties by which compounds can be separated are solubility, boiling point, and vapor pressure.



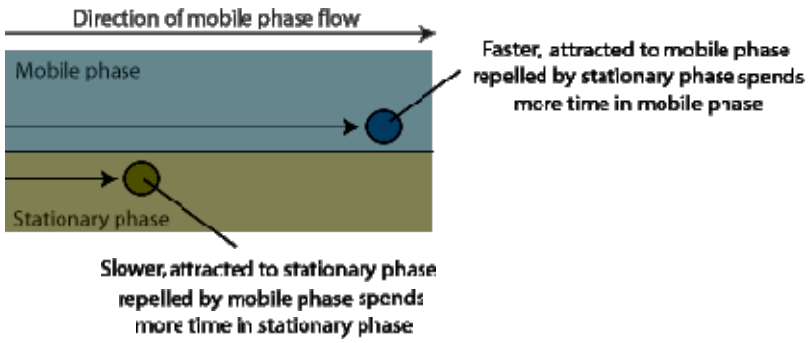
In chromatography, one phase is held immobile or stationary and the other one (the mobile phase) is passed over it, as noted above. Strictly speaking, in current practice, many stationary phases nominally listed as liquids are really chemically bonded to (or chemically polymerized and cross-linked to) a solid support [as in bonded-phase (BP) HPLC] or to the column wall (as in capillary GC). The IUPAC definition of chromatography is:

"Chromatography is a physical method of separation in which the components to be separated are distributed between two phases, one of which is stationary (stationary phase) while the other (the mobile phase) moves in a definite direction. Elution chromatography is a procedure in which the mobile phase is continually passed through or along the chromatographic bed and the sample is fed into the system in a definite slug."



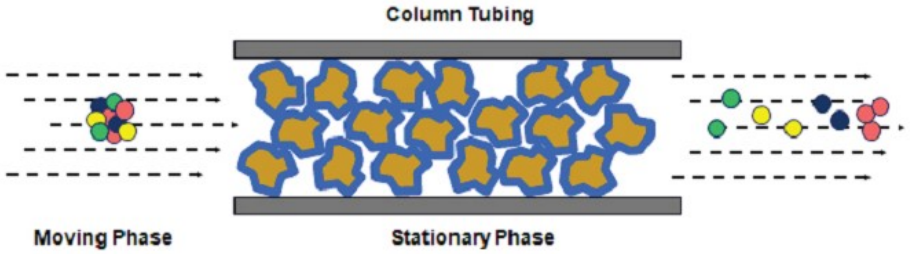
Mobile phase In HPLC, TLC, and CZE the mobile phase is a liquid that moves through /past a stationary phase and causes separation. In GC separations, the mobile phase is a gas (often called the carrier gas).

Stationary phase The retentive component(s) of the packing material. In adsorption chromatography the packing material itself is the stationary phase, whereas in partition chromatography the adsorbed liquid phase or bonded phase is the stationary phase.



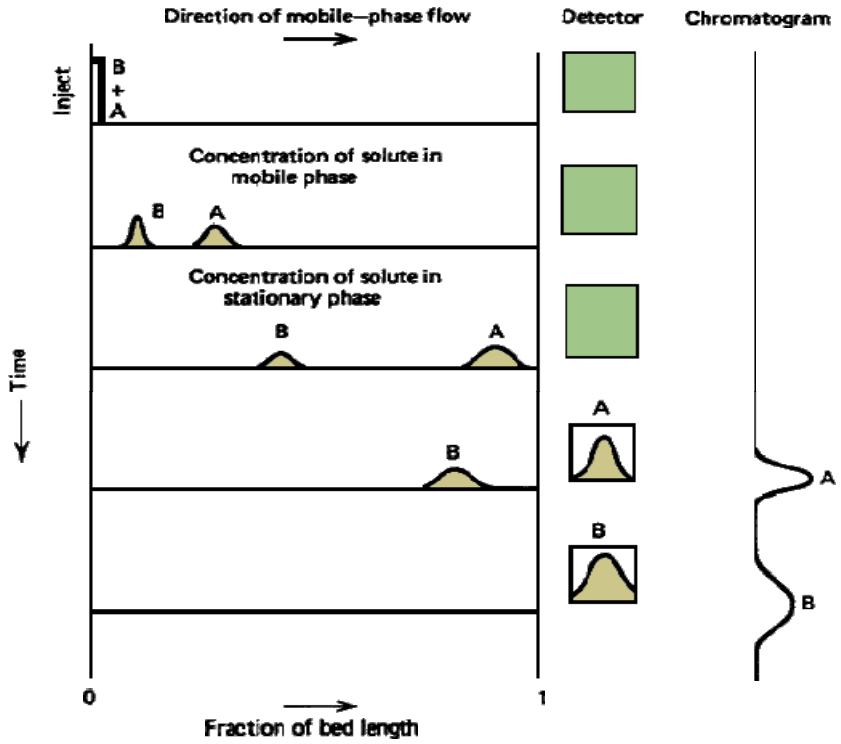
At any given time, a particular analyte molecule is either in the mobile phase, moving along at its velocity, or in the stationary phase and not moving at all in the downstream direction. The sorption-desorption process occurs many times as the molecule moves through the bed, and the time required to do so depends mainly on the proportion of time it is sorbed and held immobile.

A separation is effected if the various components emerge from the bed at different times, which are called ***retention times***. The status of the separation is shown at five different times-five snapshots of the separation. In the first one, the mixture of analytes A and B is introduced to the bed in as narrow a zone as possible.



The stationary and moving phases

The mobile phase, flowing from left to right, carries them along the bed. Since all the molecules of a particular analyte do not encounter the exact same local environment in the stationary and mobile phases, peak for that analyte will have a finite width in chromatogram.

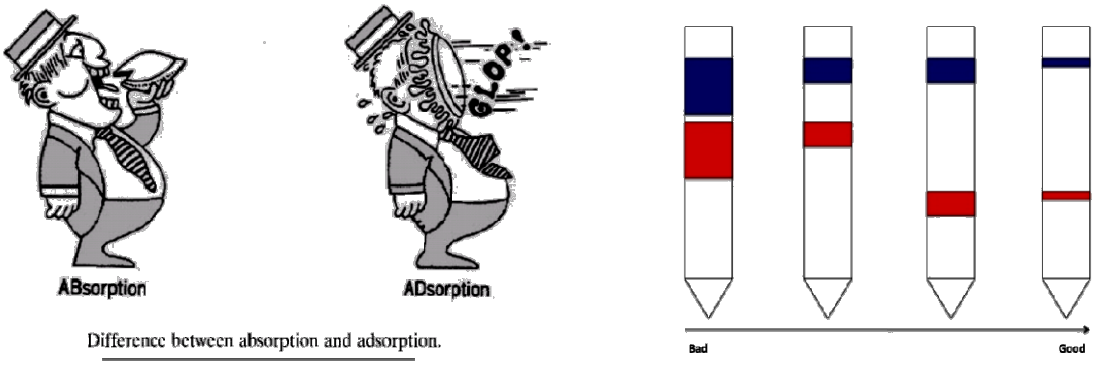


Schematic representation of the chromatographic process.

Note that the width of the peaks increases with the length of time they remain in the bed. Because analyte A has a greater affinity for the mobile phase, it spends more time in the mobile phase and travels faster and elutes before analyte B. Thus A and B become separated. Another, less popular form of chromatography is controlled by displacement. The sample is pushed through the system by displacing it from the stationary bed with other sample components and a strong mobile phase.

The chromatographic process we have defined is also known as zonal or hatch chromatography because the sample is applied to the system all at once in one narrow zone. By contrast, the sample can be applied continuously during a run; this process is called ***frontal analysis***. One application to immune affinity chromatography has been published recently, but it will not be discussed further here because of its limited use.

The mode of interaction between the sample components and the two phases can be classified into two types, although many separation processes are combinations of both. If the sample is attracted to the surfaces of the phases, commonly to the surface of a solid stationary phase, the process is called adsorption. Alternatively, if the sample diffuses into the interior of the stationary phase-for example, into the bulk of a stationary liquid chromatographers call the process partition. Actually, absorption seems to be a better name for this process because we can then speak of sorption as the general process and add the prefixes ah or ad when we want to be more specific. For this reason, the terms absorption and adsorption will be used in this monograph even though ***partition*** is the term recommended by the IUPAC.



Difference between absorption and adsorption.

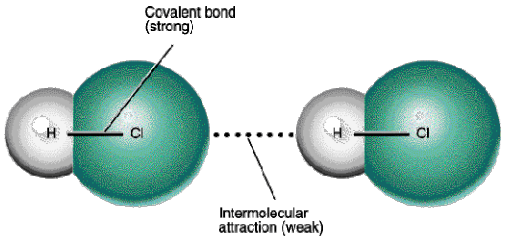
Physical forces and interactions chromatography

The process of selecting a chromatographic system for a given separation will be facilitated if we have some understanding of the forces that are operating in the system and effecting the separation. The magnitude of these forces is reflected in the numerical values of the equilibrium partition coefficients (distribution constants),

- **Intermolecular and interionic forces**

We want to explore the forces that give rise to a particular partition coefficient and also see what effect they have on peak shape. Before examining the types of forces present in chromatographic systems, let us consider some parameters that are commonly used to reflect the magnitude of intermolecular forces in general. A typical list includes:

- Ionization potential
- Electron affinity
- Electronegativity
- Molar volume
- Ionic radius
- Ionic potential
- Dipole moment
- Dielectric constant
- Polarizability
- Boiling point and vapor pressure
- Solubility
- Activity coefficients
- Hildebrand solubility parameter



In most cases these parameters define properties of a given chemical (molecules or ions) as it exists alone (neat) or perhaps in aqueous solution. For example, the high boiling point (bp) of the chemical, water, is commonly used as an illustration of effect of intermolecular hydrogen bonding, which drastically increases forces between individual water molecules

making them harder to separate (volatilize). bp is an example of a measurement of the forces between like molecules; but, in chromatography, we are more interested in the forces between unlike molecules, as in a solution with solute and solvent. This is a more difficult task and, as noted earlier, we are often forced to rely on empirical relationships.

Ionic Interactions: Forces between ions of like charge are repulsive and between ions of different charge are attractive, according to Coulomb's law. They are relatively long-range and strong forces. The main illustration of ionic forces in LC is in ion exchange where the sample is at least partly ionized and the stationary phase contains ionic sites. Ions are also attracted or repulsed by the polar ends of a dipolar molecule. These forces can be referred to as ion-dipole forces. In addition to ion exchange chromatography, they are also important in aqueous LC systems where a polar stationary phase can interact with ionic solutes.

Van der Waals Forces Three types of weaker forces have been identified and together are referred to as van der Wuals forces: dipole-dipole, dipole induced dipole, and induced dipole induced dipole. Orientation forces are easy to understand by analogy with magnetic forces. The opposite poles attract even though random molecular motion keeps the attraction small. Similarly, the induction forces can be viewed as similar to a magnet attracting nonmagnetic iron. The induction of a dipole depends on the polarizability of the non polar molecule; large molecules with easily deformed electronic clouds have large polarizabilities.

Hydrogen Bonding: formed between molecules containing a hydrogen atom bonded to an electronegative atom such as oxygen or nitrogen. Such is the case in alcohols, amines, and water, all of which can both donate and receive a hydrogen atom, forming hydrogen bonds. Other molecules such as ethers, aldehydes, ketones, and esters can only accept protons-they have no hydrogens to donate; they can form hydrogen bonds only with hydrogen donors such as alcohols. A common exception is chloroform, which is a weak proton donor. As we seen, strength of hydrogen bonds makes them very important in separation processes and may lead to nonsymmetrical chromatographic peak shapes.

Charge Transfer Finally, there is a group of specific interactions in which two molecules or ions combine by transferring an electron from one to the other. The process is called

charge-transfer and charge-transfer complex is formed from the attractive forces produced. It too has some characteristics of a bond, exhibiting an internal redox exchange. One of the most common examples in chromatography is the complex formed between Ag^+ ions and olefins, which has been used to separate olefins from paraffins.

- **Size exclusion-molecular sieving**

Although not a "force," sieving is another mechanism by which separations can be achieved in chromatography. Probably sieving is not the best term to use, but it does denote that separations are made on the basis of the sizes of the sample molecules. In fact, in their most common form, chromatographic separations based on size are achieved by controlling the size of the pores in the stationary phase so that some (small) molecules will be able to enter the pores while others (the large ones) cannot. The large molecules are excluded, which is why this process is correctly called size exclusion and the technique, size exclusion chromatography (SEC). Molecules of intermediate size will be partially excluded from the pores and can be separated from each other based on the fractional extent of their exclusion.

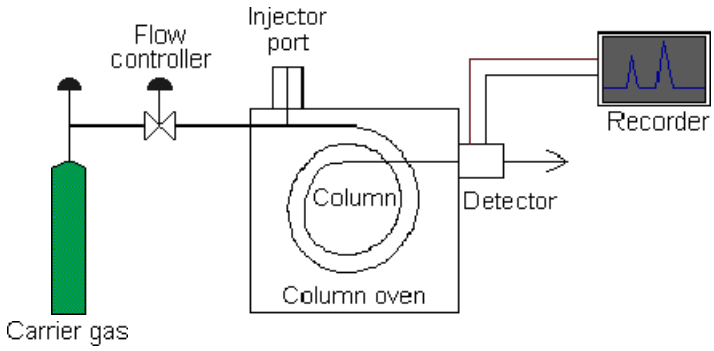
In GC this process has been called molecular sieve chromatography, although the mechanism of separation probably involves mainly adsorption as well as some size exclusion. It is used to separate fixed gases such as hydrogen, oxygen, nitrogen, methane, carbon monoxide. Ethane, carbon dioxide, and ethylene. The sieves are natural zeolites or synthetic materials of which the alkali metal alumino silicates are typical. Newer molecular sieves have been especially prepared for GC from carbon. For example, Supelco' markets its carbon molecular sieves as Carbosieve S-I1 and Carboxen 1000 for separations of permanent gases. In HPLC the main application has been the characterization of polymers using synthetically prepared stationary phases of varying pore sizes. It must be remembered that some of the solids used as stationary phases in this technique also contain some polar functional groups, and adsorption by analytes does occur in many cases. Van der Waals forces are present and help stabilize the complexes, and in some cases hydrogen bonds are involved in forming the cages. These methods are not widely used, but they can produce some exceptional separations.

Gas Chromatography (GC)

A brief history

The publication in 1952 of James and Martin's study' on gas chromatography (GC). Since that time, GC has become the premier technique for the separation and analysis of volatile compounds and gas chromatographs have been the most widely used analytical instrument in world, although HPLC is becoming more widely used.

Clearly, GC is a major analytical method and it is complemented by other major form of Chromatography, HPLC, which is capable of handling non volatiles not suited to GC. The currently accepted status between them is that GC can be used up to 350°C corresponding to an upper molecular weight limit of 600 D," and HPLC is used for higher molecular weight compounds.

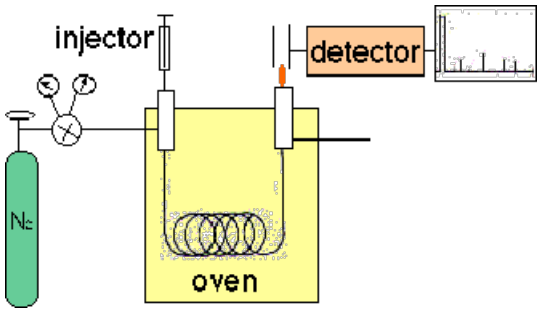


Before GC became popular in the late 1950s, the only way to separate volatile materials was by distillation, which separates materials based on differences in vapor pressure or boiling point. GC is similar, but its separations also depend on the nature of the stationary phase, which gives it much more versatility than distillation.

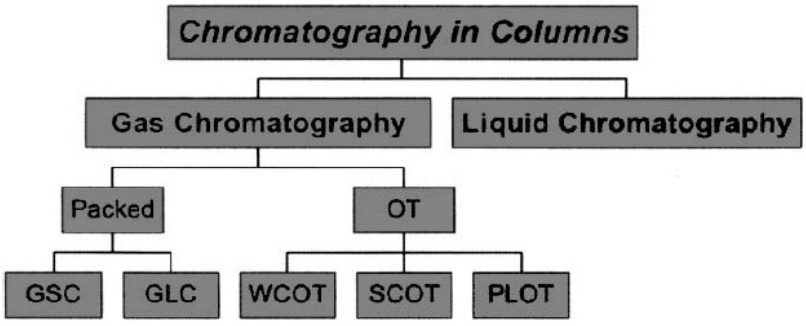
Imagine the pleasure and surprise of separations chemists who could now separate materials with close boiling points, such as benzene and cyclo-hexane (The boiling points of benzene and cyclo-hexane are nearly same, 80.1 and 81.4 °C). In addition, it was easy, fast, and not too expensive; in addition, they did not have to worry about azeotropes.

Basic Principals

Gas chromatography is a chromatographic technique that can be used to separate volatile organic compounds. A gas chromatograph consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase, and a detector. The organic compounds are separated due to differences in their partitioning behavior between the mobile gas phase and the stationary phase in the column.



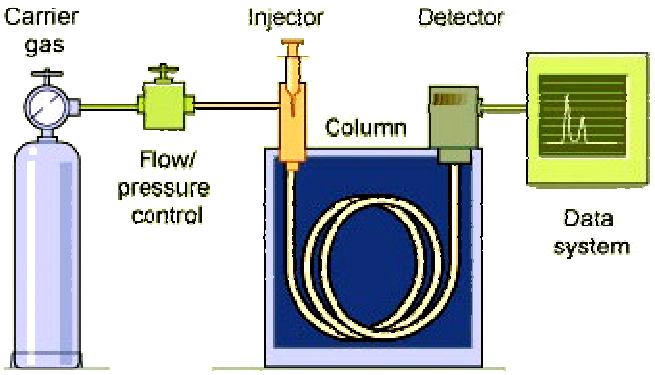
The earliest columns were packed with solids or with solid supports coated with liquids. In accordance with our naming convention, these two types of chromatography are called gas-solid chromatography (GSC) and gas-liquid chromatography (GLC).



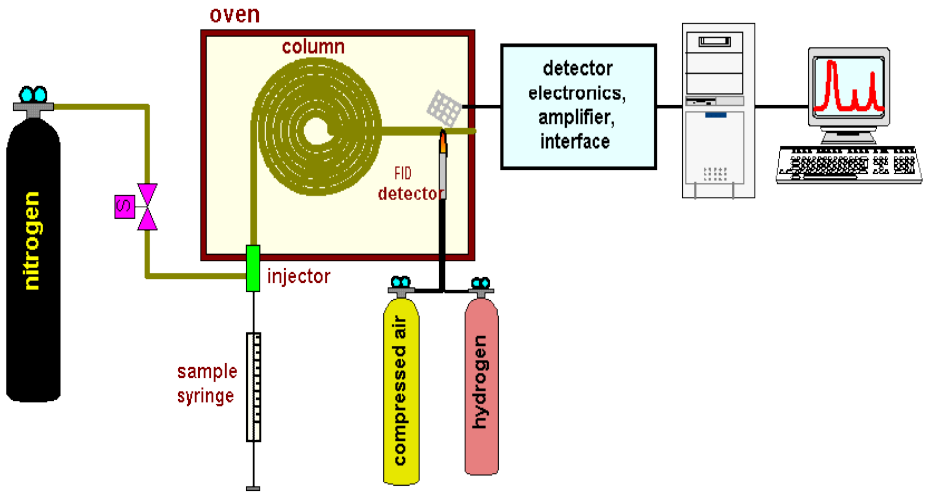
Classification of GC methods.

In 1957 Golay published his ideas for using columns that were not packed but were open tubes.' These tubes had to have small inside diameters; they were capillary columns, but the name open tubular (OT) columns is more descriptive and preferred when comparing them to packed columns. In OT columns, the SP can be coated on, or bonded to, the inside wall, and these columns are called wall-coated OT columns (WCOT).

In GC, mobile phase, usually helium is inert and does not participate in the chemistry of the chromatographic process but only serves to carry the sample through the column. Hence, the theory of GC is concerned only with the interactions between the SP and the analytes.

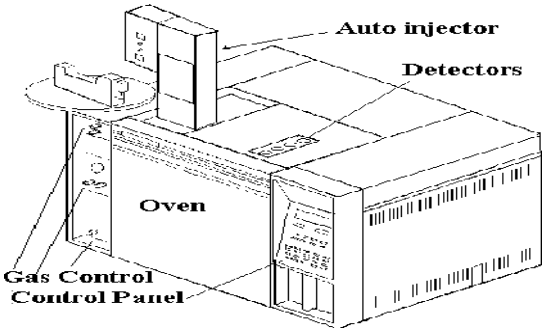


Starting with that base, the following discussion shows how GC theory has developed in an attempt to explain in more elaborate terms the effectiveness of the SP in effecting separations like the one just illustrated, benzene and cyclohexane.



Instrumentation for GC

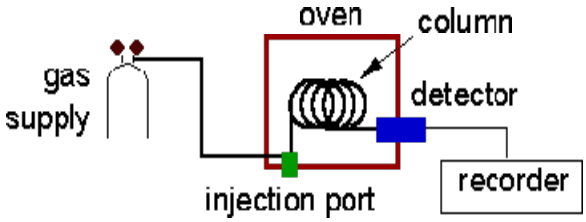
The essential parts of a gas chromatograph as shown in Figure are: carrier gas, flow or pressure regulator, injection port/splitter, column, and detector. Usually there are three separate heated zones, for the injector, the column, and detector.



Connections between these heated zones must also be kept hot enough to prevent condensation of analytes in them. Chromatographs designed for OT columns have an injection port that allows for sample splitting and a provision for some additional "makeup" gas for the detector, as shown.

- **Mobile Phase**

Mobile phases are generally inert gases such as helium, argon, or nitrogen. The most popular carrier gases are nitrogen, helium, and hydrogen. They must be very pure, and they are chosen for their inertness since, as we have seen, their only purpose is to carry the analyte vapors through the column.



Helium is the most popular because of its higher efficiency at faster flow rates, the optimum velocity for Helium is around 20-30 cm/s, but little efficiency is lost at higher velocities, so most chromatographers run their OT columns around twice optimum velocity.

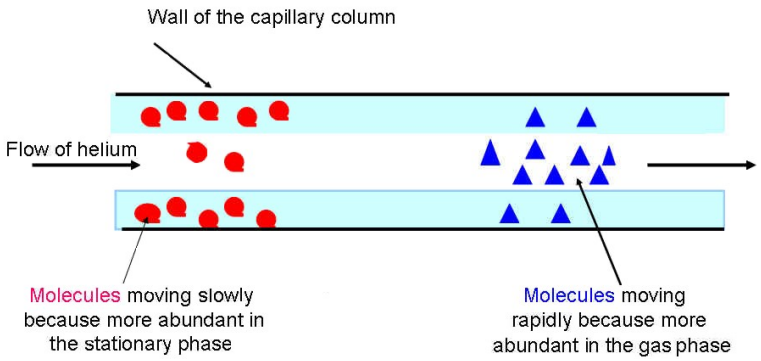
Since helium is expensive and in limited supply in some parts of the world, hydrogen is increasing in popularity, but its use requires additional safety precautions to prevent explosions. However, these advantages are not as impressive for programmed temperature GC as they are for isothermal GC, so helium remains the gas of choice for most laboratories. Sometimes the choice of carrier is dictated by the detector.

Preferred Carrier Gases for Three Detectors

Detector	Carrier Gas
Flame ionization detector (FID)	Helium
Thermal conductivity detector (TCD)	Helium, hydrogen
Electron capture detector (ECD	Very dry nitrogen

The electron capture detector (ECD) has the most unique requirements, and the thermal conductivity detector (TCD) works best with gases of high thermal conductivity.

Over the years there has been some interest in using carrier gases that are not inert but have some effect on the partitioning process. Water is the one used most often, and the technique is sometimes called steam GC. The pressure drop across an OT column is only 10-100 kPa. It is regulated with one or more valves in order to get the desired flow rate (linear velocity). We have already seen that a constant flow rate is desirable so that retention times will not vary, and flow-sensitive detectors will not become nonlinear. In the technique of programmed temperature (PT)



The viscosity of the carrier gas increases during a run, so flow rate will decrease during a PT run if it is being operated at constant pressure. On the other hand, the split injectors

used with OT columns require constant pressure regulation, so older chromatographs are run at constant pressure, and the decrease in carrier gas velocity during the run is tolerated and partially compensated by starting the run at a higher pressure. Modern instruments often have electronic pressure control (EPC), so constant flow can be achieved by choosing a mode of constant linear velocity whereby the pressure is increased during the PT run.

- **Gas Supplies**

Gases for use with gas chromatograph were originally all obtained from gas tanks or gas cylinders. However, over the past decade the use of gas generators have become more popular as it avoids having gases at high pressure in laboratory which is perceived by some as potentially dangerous.

In addition, use of a hydrogen generator avoids use of a cylinder of hydrogen at high pressure which is also perceived by some as a serious fire hazard despite the fact that they have been used in laboratories, quite safely for nearly a century.

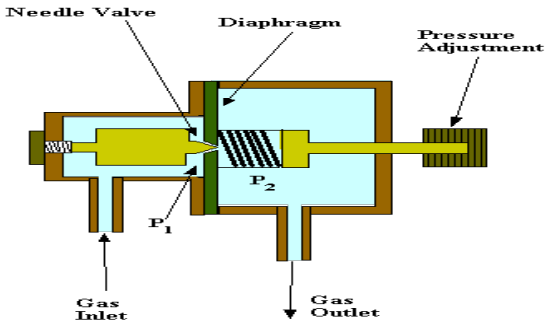
Supplies from Gas Tanks: Gasses are stored in large cylindrical tanks fitted with reducing valves that are set to supply the gas to the instrument at the recommended pressure defined by the manufacturers. The cylinders are often situated outside and away from the chromatograph for safety purposes and the gasses are passed to the chromatograph through copper or stainless steel conduits at relatively low pressure. The main disadvantage of gas tanks is their size and weight which makes them difficult to move and replace.

Pure Air Generators: Air generators require an air supply from air tanks or directly from the laboratory compressed air supply. The Packard Zero Air Generator passes the gas through a 0.5 m filter to remove oil and water and finally over a catalyst to remove hydrocarbons. The hydrocarbon free air is then passed through a 0.01 m cellulose fiber filter to remove any residual particulate matter that may be present. The manufacturers claim the resulting air supply contains less than 0.1 ppm total hydrocarbons and delivers air at 125 psi at flow rates up to 2,500 cc per min.

Pure Nitrogen Generators: The nitrogen generator can also operate directly from the laboratory compressed air supply. General contaminants are first removed with appropriate filters and adsorbents and the purified air passes over layers of polymeric hollow fiber membranes through which nitrogen selectively permeates. The residual nitrogen-depleted air containing about 30% oxygen is vented to atmosphere. The nitrogen produced by Air Products nitrogen generator contains less than 0.5 ppm of oxygen, less than 0.5 ppm of water vapor and less than 2.0 ppb of halocarbons or hydrocarbons. It can supply up to 1 l/min. at pressures from 60 to 100 psi.

Hydrogen Generators: In the Packard Hydrogen Generator, hydrogen is generated electrolytically from pure deionized water. Unfortunately, the technology used in hydrogen generators is largely proprietary and technical details are not readily available. The electrolysis unit uses a solid polymer electrolyte and thus does not need to be supplied with electrolytes, only the deionized water. The manufacturers claim the device generates 99.999% pure hydrogen with a reservoir capacity of 4 liter, and an output pressure that ranges from 2 to 100 psi. Other units can produce hydrogen flows that range from 0 to 125 ml/min. to 0 to 1200 ml/min. The oxygen, produced simultaneously with hydrogen at half the flow rate, is vented to air.

Pressure Controllers: The first control on any gas line is afforded by a simple pressure controller. There are a number of pressure controllers associated with a gas chromatograph. The reducing valves on the gas tanks are examples of simple pressure controllers and the flow controllers that are used for detector and column flow control often involve devices based on the same principles. A diagram of a pressure controller is shown in figure.



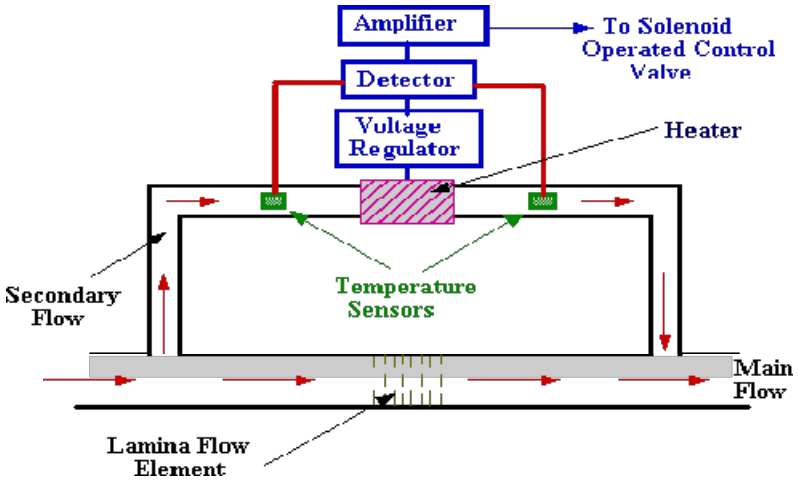
Pressure controller

The pressure controller consists essentially of two chambers separated by a diaphragm, in the center of which is a needle valve that is actuated by the diaphragm. The diaphragm is held down by a spring that is adjustable so that the pressure in the second chamber, and thus the outlet flow, can be set at any chosen value.

When gas enters the lower chamber, the pressure on the lower part of the diaphragm acts against the spring setting, and opens the valve. Gas then passes into the upper chamber and pressure is built up in the upper chamber to the value that has been set at which time the diaphragm moves downward closing the valve. If the pressure falls in the upper cylinder, the diaphragm again moves upward due to the pressure in the lower chamber, which opens the valve and the pressure in the upper chamber is brought back to its set value.

Flow Controllers: A constant pressure applied to a column does not ensure a constant flow of mobile phase though the chromatographic system, particularly if the column is being temperature programmed. Raising the temperature of a gas causes the viscosity to increase, and at a constant inlet pressure, the flow rate will fall.

The reduction in flow rate will be related to the temperature program limits and to a certain extent on the temperature gradient. To obviate the flow rate change, mass controllers are used which ensure a constant mass of mobile passes through the column in unit time irrespective of the system temperature. A diagram of a mass flow controller is shown.



Mass Flow Controller

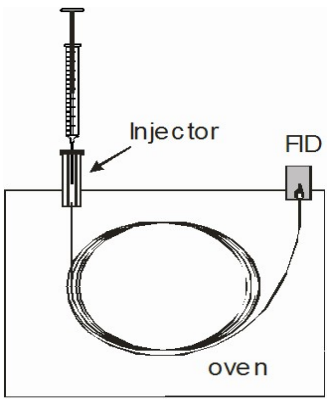
The sensing system consists of a bypass tube with a heater situated at the center. Precision temperature sensors are placed equidistant upstream and downstream of the heater. A proprietary set of baffles situated in the main conduit creates a pressure drop that causes a fixed proportion of the flow to be diverted through the sensor tube. At zero flow rate both sensors are at the same temperature. At a finite flow rate, the downstream sensor is heated, producing a differential temperature across the sensors.

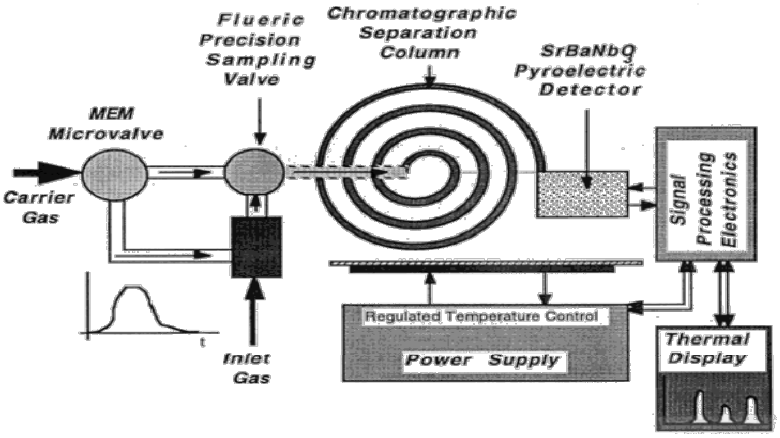
The temperature of the gas will be proportional to product of mass flowing and its specific heat and so the differential temperature that will be proportional to mass flow rate. The differential voltage from the two sensors is compared to a set voltage and the difference used to generate a signal that actuates a valve controlling the flow.

Thus, a closed loop control system is formed that maintains the mass flow rate set by the reference voltage. The device can be made extremely compact, is highly reliable and affords accurate control of the carrier gas flow rate irrespective of gas viscosity changes due to temperature programming.

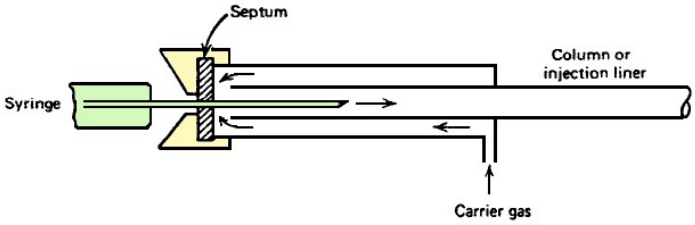
- **Injection Ports and Valves**

The normal sequence of events in a GC injection is as follows. We will assume in this explanation that some analytes are dissolved in a (liquid) solvent although much of this process also holds for gas GC injections too. A small amount of liquid (micro liters) is injected through a silicon rubber septum (using a special micro liter syringe) into the hot (usually +200° C) GC injector that is lined with an inert glass tube.

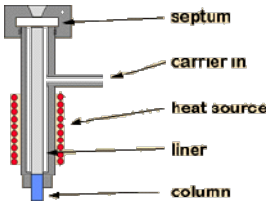




The injector is kept hot by a relatively large, metal heater block that is thermostatically controlled. The sample is immediately vaporized and a pressurized, inert, carrier gas-which is continually flowing from a gas regulator through the injector and into the GC column-sweeps the gaseous sample, solvent, analyte and all, onto the column.

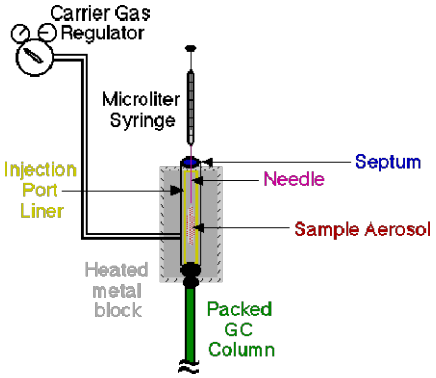


Simplified injection port design.

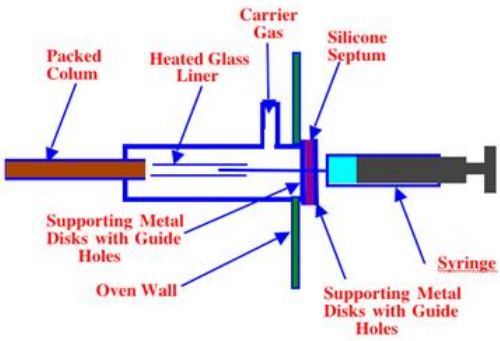


Injection port

In gas chromatography two basic types of sampling system are used, those suitable for packed columns and those designed for open tubular columns. In addition, different sample injectors are necessary that will be appropriate for alternative column configurations.

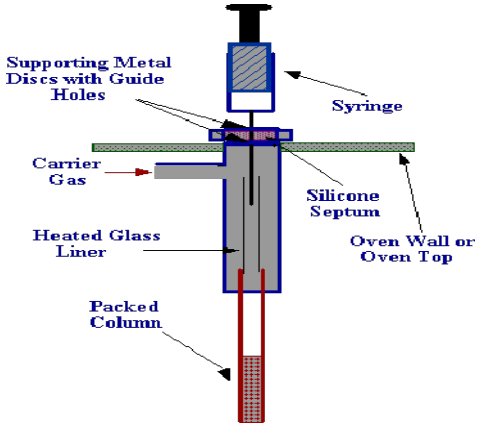


It must be stressed, however, that irrespective of the design of the associated equipment, the precision and accuracy of a GC analysis will only be as good as that provided by the sample injector. The sample injector is a very critical part of the chromatographic equipment and needs to be well designed and well maintained.



Packed Column Injectors

In general, the sample injected onto a packed GC column ranges in volume from 0.5ml to 5ml and usually contains the materials of interest at concentrations ranging from 5%v/v to 10%w/v. The sample is injected by a hypodermic syringe, through a silicone rubber septum directly into the column packing or into a flash heater. Although the latter tends to produce broader peaks it also disperses the sample radially across the column.

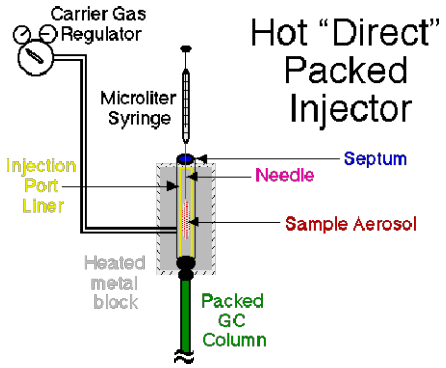


A Packed Column Injector

Direct injection into the packing constrains the sample into a small volume, but can cool the front of the packing. An example of a septum injection system used for packed columns is shown in figure. Silicone septum is compressed between metal surfaces in such a manner that a hypodermic needle can pierce it, but when it is withdrawn the hole is closed as a result of the septum compression and there is no gas leak. Glass liner prevents the sample coming in contact with heated metal wall and thus, reduces the chance of thermal decomposition.

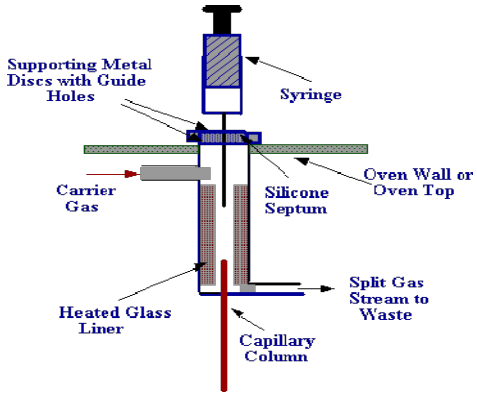
The glass liner can be fitted with a separate heater and the volatilization temperature can, thus, be controlled. This "flash heater" system is available in most chromatographs. By using a syringe with a long needle, the tip can be made to penetrate past the liner and discharge its contents directly into the column packing. This procedure is called 'on-column injection' and, as it reduces peak dispersion on injection and thus, provides higher column efficiencies, is often the preferred procedure.

There are two injection configurations for packed-column instruments. The important criterion is the position of the column packing relative to the end of the needle of the injection syringe. If the packing is close to the end of the needle, the sample will be deposited on the column and is called *on-column injection*. Most of the sample will be sorbed on the column without requiring prior vaporization.



If the packing is a few centimeters from the end of the needle, a liquid sample will be vaporized in the volume of the injection port, which is heated to facilitate that process; this is called *flash vaporization*, analogous to the process in *split/splitless vaporization* for OT columns. When purchasing a commercial column, it is necessary to specify the instrument being used and the mode desired, so the column supplied will have the proper geometry.

Unlike the split/splitless injector for OT, the process for packed columns can be carried out at constant carrier gas flow. *On-column injection* is used most often because it is more efficient and is usually operated at a lower inlet temperature that does not require the sample to come in contact with a hot surface, which might act catalytically to cause decomposition.

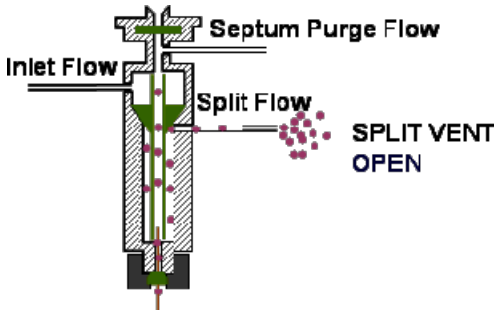


Split Injection System

Open Tubular Column Injectors

Now remember that the size of the capillary column limits the amount of analyte that can be injected, otherwise, chromatographic overloading occurs. Therefore, this packed column injector design, if used with a capillary column, would require that samples with high concentrations of analytes be diluted.

Unless... what other alternative is there to get the amount of analytes that are injected onto the column smaller without having to dilute concentrated samples? The solution is the split/splitless capillary GC injector. Due to the very small sample size that must be placed on narrow bore capillary columns, a split injection system is necessary, a diagram of which is shown in figure. The basic difference between the two types of injection systems is that the capillary column now projects into the glass liner and a portion of the carrier gas sweeps past the column inlet to waste. As the sample passes the column opening, a small fraction is split off and flows directly into the capillary column, this device is called a split injector.



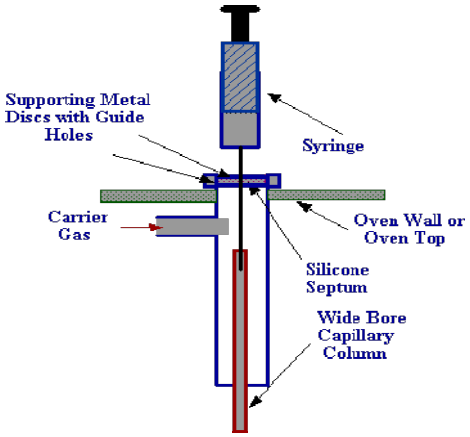
Split Injection

The split ratio is changed by regulating the portion of the carrier gas that flows to waste which is achieved by an adjustable flow resistance in the waste flow line. This device is only used for small diameter capillary columns where the charge size is critical.

The device has certain disadvantages due to component differentiation and the sample placed on the column may not be truly representative. The solutes with the higher diffusivities (low molecular weight) are lost preferentially to those with lower diffusivities (higher molecular weights).

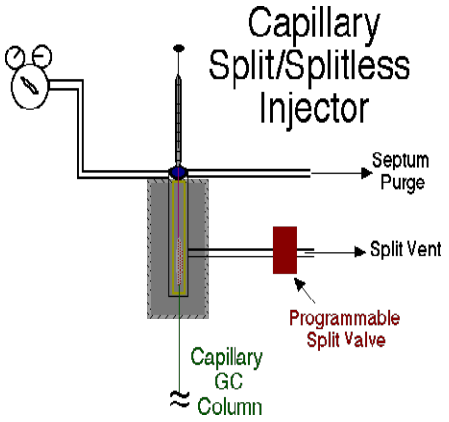
Consequently, quantitative analyses carried out using the high efficiency small diameter capillary columns may have limited accuracy and precision, depending on the nature of the sample. This problem was partially solved by using larger diameter columns that would permit on-column injection.

The columns are constructed to have an I.D. of about 0.056 in; which is slightly greater than diameter of a certain hypodermic needles. This injection system is depicted in figure.



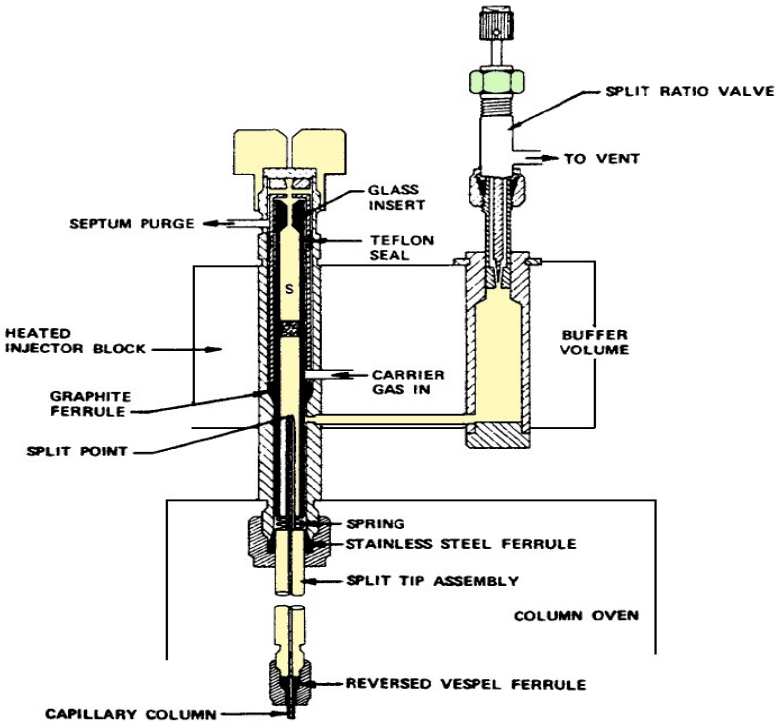
On-Column Injector for Large Bore Open Tubular Columns

However, there are also difficulties associated with this type of injector. On injection, the sample breaks up into separate portions, and bubbles form at the beginning of the column causing the sample to be deposited at different positions along the open tube as the solvent evaporates. On starting to develop the separation, each local concentration of sample acts as a separate injection. As a consequence, a chromatogram containing very wide or multiple peaks is produced. Procedures have been introduced in an attempt to eliminate sample splitting in this manner.



Sample introduction is most often accomplished with a micro syringe through a self-sealing rubber septum. Gas sample valves are more reproducible and are preferred for gases. In all cases, the objective is to get the sample into the column rapidly and in as small a volume as possible. This is usually accomplished by flash vaporization of the sample in a heated liner that also has the capability for splitting the sample into two parts.

Split injection is the oldest, simplest, and easiest injection technique. The procedure involves injecting about 1 µL of the sample by a standard syringe into a heated injection port that contains a deactivated glass liner.

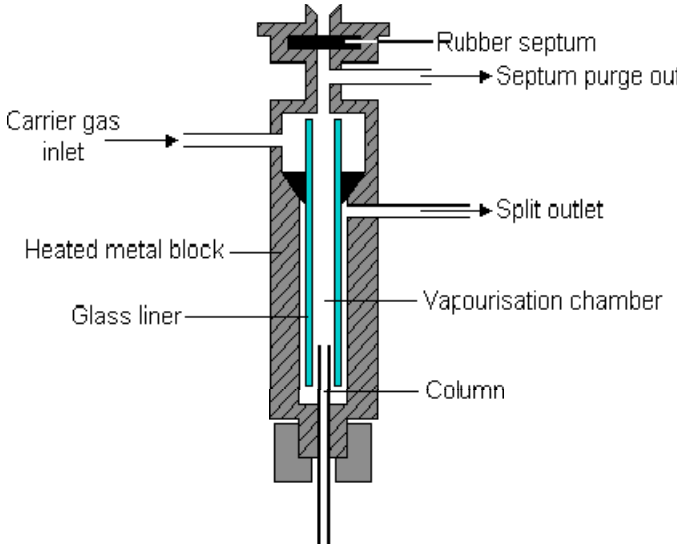


Inlet designed for OT columns and sample splitting.

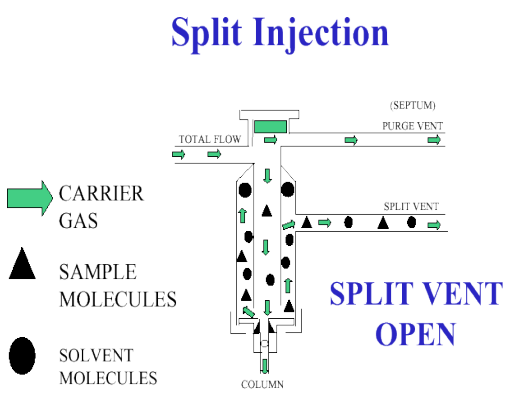
A plug of glass wool or other inert material in the vaporization region can be used to promote complete volatilization. The sample is rapidly vaporized, and only a fraction, usually 0.1-10%, of the vapor enters the column. The rest of the vaporized sample and a large flow of carrier gas passes out through a split or purge valve. The buffer volume allows the sample to pass the point of column splitting before reaching the needle valve, which could change the split ratio.

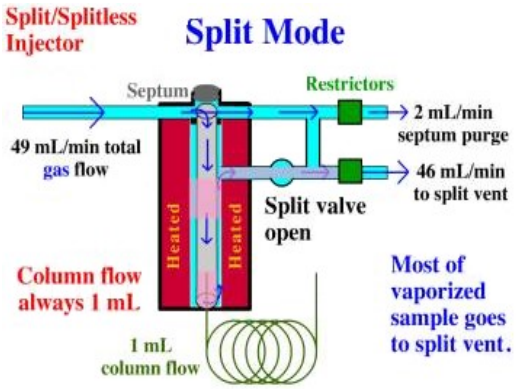
There are several advantages to ***split injections***. The technique is simple because the operator has only to control the split ratio by opening or closing the split (purge) valve. The amount of sample introduced to the column is very small (and easily controlled) as required by capillary columns that have small amounts of stationary phase and therefore can accommodate only small sample sizes. Also, the flow rate up to the split point is fast (the sum of both column and vent flow rates), and the result is high resolution separations.

The split / splitless injector



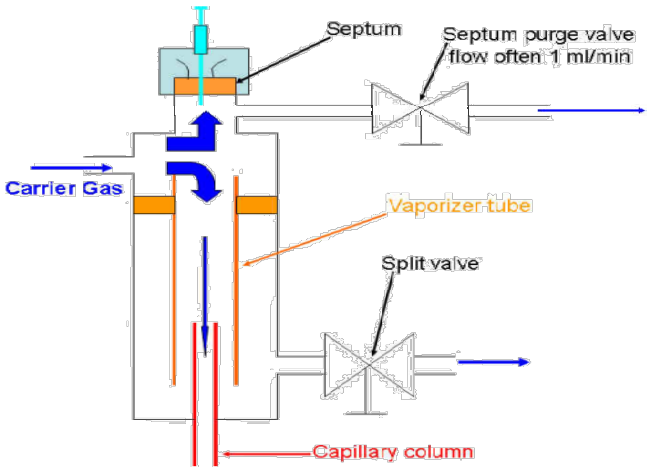
Another advantage is that "neat" samples can be introduced, usually by using a larger split ratio, so diluting the sample is not required. A final advantage is that "dirty" samples can be introduced by putting a plug of deactivated glass wool in the inlet liner to trap nonvolatile compounds.





One disadvantage is that trace analysis is limited since only a fraction of sample enters column. Consequently, splitless or on-column injection techniques are recommended for trace analysis. A second disadvantage is that the splitting process sometimes discriminates against high-molecular weight solutes in the sample so that the sample entering the column is not representative of the sample injected. For these reasons, another vaporization mode, splitless injection, is sometimes used.

Splitless injection uses the same hardware as split injection, but the split valve is initially closed. The sample is diluted in a volatile solvent (such as hexane or methanol) and 1-5 µL is injected in the heated injection port. The sample is vaporized and slowly (flow rate of about 1 mL/min) carried onto a cold column where both sample and solvent are condensed. After about 45 s the split valve is opened (flow rate of about 50mL/min), and any residual vapors left in the injection port are rapidly swept out of the system.



Representation of split/splitless injector

Septum purge, a very small flow of carrier gas sweeping the septum and then being vented, is essential with splitless injections. The column is then temperature programmed, and initially only the volatile solvent is vaporized and carried through the column. While this is happening, the sample analytes are being refocused into a narrow band in residual solvent. At some higher temperature, these analytes are vaporized and chromatographed as described later. High resolution of these higher boiling analytes is observed, big advantage of splitless injection is improved sensitivity over the split mode. Typically 20- to 50-fold more samples

enter column and the result is improved trace analysis for environmental, pharmaceutical, or biomedical samples. However, splitless has several disadvantages. It is time consuming; you must start with a cold column; and you must temperature program. You must also dilute the sample with a volatile solvent and optimize both the initial column temperature and the time of opening split valve. Finally, splitless injection is not well-suited for volatile compounds. For good chromatography the first peaks of interest must have boiling points 30°C higher than the solvent. A comparison of the split and splitless methods is given in Table.

Comparison of Split and Splitless Injection Modes

	Split Mode	Splitless Mode (Grob)
Liner	May contain frit or glass wool	Open
Liner diameter	4 mm	2 mm
Injection method	Simple injection	Several steps (see text)
Sample size	0.1 10 μL	1–5 μL
Sample/solvent ratio	1 : 1–1:1000	$1 : 10^{-3}$–$1 : 10^{5}$

Three other types of capillary inlets are ***direct injection***, ***on-column***, and ***cold on-column***. *On-column* means inserting the precisely aligned needle into the capillary column, usually 0.53-mm id. *Direct injection* involves injecting a small sample (usually 1 μL or smaller) into a glass liner where the vapors are carried directly to the column. Both of these techniques require thick-film capillaries and wide diameter columns with faster than normal flow rates (about 10 mL/min).

Even with these precautions the resolution is not as good as with split or splitless injection. The advantages can be better trace analysis and good quantitation. Both high resolution and good quantitation result from cold on-column injections. A liquid sample is injected into either a cold inlet liner or a cold column. The cold injector is rapidly heated and the sample vaporized and carried through the column. Minimal sample decomposition is observed. For thermolabile compounds, cold on-column is best injection technique. Syringe injection readily automated with commercially available equipment, procedure is analogous to manual injection and has better precision.

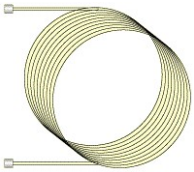
- **Columns**

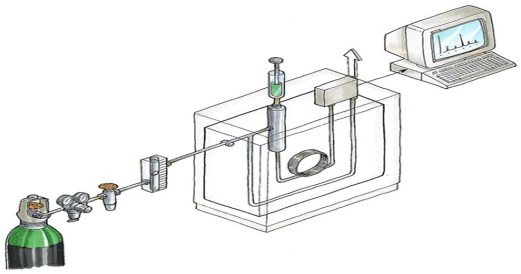
There are two types of columns in common use in GC and they are the packed column and the open tubular column. The former are usually 2 to 4 mm I.D. and 1 to 4 meters long and, packed with a suitable adsorbent, are mostly used for gas analysis. As a result of the simpler injection procedure and the more precise sampling method, the packed column tends to give greater quantitative accuracy and precision.

However, despite its problems with sample injection, the open tubular column is the most popular column system in general use. The length of open tubular columns range from about 10 m to 100 m and can have internal diameters from 100 µm to 500 µm. The stationary phase is coated on the internal wall of the column as a film 0.2 µm to 1 µm thick.

- ***Open Tubular - Capillary GC Column***

Gas chromatography columns are of two designs: packed or capillary. Packed columns are typically a glass or stainless steel coil (typically 1-5 m total length and 5 mm inner diameter) that is filled with the stationary phase.





Capillary columns are a thin fused-silica (purified silicate glass) capillary (typically 10-100 m in length and 250 µm inner diameter) that has the stationary phase coated on the inner surface. Capillary columns provide much higher separation efficiency than packed columns but are more easily overloaded by too much sample. Open tubular columns are usually made of fused silica since inertness is of prime importance. The column is either kept at a constant temperature (isothermal) or programmed during run (PTGC). It is a common misconception that the column temperature should exceed the boiling point of the sample in order to keep the analytes in the vapor phase.

Actually column will produce better separations if the temperature is below the samples boiling point, thereby increasing its interaction with the stationary phase. The situation desired inside column can be described in comparison to water vapor in our environment; there is plenty of vapor well below the boiling point (as we know from those days with high humidity), but we must be above the "dew point" of the analyte or else it will "rain" in the column. The smaller the amount of stationary phase, the lower the temperature at which we can operate, so ***OT columns are usually run at lower temperatures than packed columns.***

In their original and most simple form, OT columns contain a thin film of stationary liquid on their inside walls. Hence they are referred to as wall coated open tubular, or WCOT, columns. Compared to packed columns, they have low pressure drops and small amounts of stationary phase. Originally they were made of stainless steel, and the early technology has been described by Ettre.

A revolution occurred in the 1960s and 1970s when the column material changed first to glass and then to fused silica. Because of the superior performance (inertness) and flexibility of fused silica columns, they have become the most popular type.

For a period of time, OT columns were available having characteristics intermediate between those of WCOT and packed columns. There were two types, but they were similar. Support-coated open tubular (SCOT) columns had a thin layer of solid support coated on the inside wall of a capillary tube of larger diameter than that used for WCOT columns. This layer was coated with stationary liquid similar to packed columns. Porous layer open tubular (PLOT) columns were similar but made differently; for example, the solid support was added while the capillary tube was being drawn.

SCOT columns are no longer popular because larger diameter WCOT fused-silica columns (often referred to as wide-boror) are as good, more stable (no layer to flake off), and easier to use. A few PLOT columns are commercially available and are increasing in popularity, mainly for GSC applications.

Coating the inside of a capillary tube requires pretreatment of the silica so that the liquid will wet the surface uniformly and stick to it. Stable stationary phases are attained by bonding the liquid to the silica and/or cross-linking it.

The cross-linked bonded phase OT columns are very stable and can even be washed with solvents for cleaning. Outside of fused-silica columns must be coated with a high-temperature polyimide to extend their life and keep them flexible.

Thickness of film of liquid can be controlled, and a variety of thicknesses are commercially available. Thicker films are desirable for volatile samples and larger samples, but they are somewhat less efficient.

Comparison of Fused-Silica WCOT Columns

	Conventional	Wide Bore
Inside diameter	0.25 mm	0.53 mm
Film thickness (d_f)	0.25 μm	1–5 μm
Phase volume ratio (β)	250–1000	25–200
Column length	15–60 m	15–30 m
Flow	1 mL/min	5–15 mL/min
H_{min}	0.3 mm	0.6 mm
N_{eff}	3000/m	1200/m
Typical sample size	50 ng	15 μg

To increase the capacity of WCOT columns, wider diameters and heavier loadings are used. These columns have replaced the SCOT columns and are known as wide-bore or mega-bore columns. The two most common sizes of WCOT columns are compared in Table but several other sizes are commercially available, and an excellent discussion of the various types has been given by Duffy.

The wide-bore columns are best for low-boiling mixtures and will accommodate larger samples; the conventional columns give the highest efficiencies and may permit the use of shorter lengths and thus shorter analysis times. To protect the analytical OT column from deterioration and contamination from dirty samples, a pre-column is often inserted between injection port and the analytical column.

This column, also called the retention gap, is not intended to retain analytes, so it is usually uncoated, but deactivated, fused silica. It should not cause appreciable zone broadening but rather should help focus the analytes as they enter the analytical column. It is especially helpful for large solvent injections, and if it is of wide-bore size (0.53 mm), it can facilitate on-column injection.

Metal capillary columns:

Capillary columns are fabricated from stainless steel or quartz. *Metal capillary columns* must be carefully cleaned to remove traces of extrusion lubricants before they can be coated, usually by washing with methylene dichloride, methanol and then water. After removing oil and grease, the columns are washed with dilute acid to remove metal oxides or other corrosion products that may remain adhering to the walls, washed with water and the again washed with methanol and methylene dichloride. Finally the column is dried in a stream of hot nitrogen.

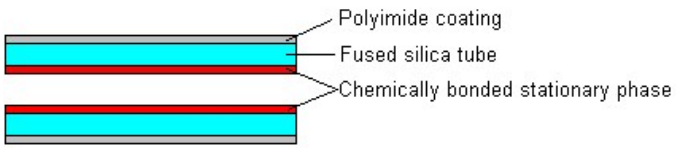
Metal columns provide the high efficiencies expected from open tubular columns and were used for the analysis of petroleum and fuel oils, etc. Metal columns, however, have some disadvantages as although easily coated with dispersive stationary phases (e.g., squalane, Apiezon grease etc.) they are not so easily coated with the more polar stationary phases such as CARBOWAX.

In addition, hot metal surfaces can cause decomposition or molecular rearrangement of many thermally labile materials such as the terpenes contained in essential oils. Metal can also react directly with some materials by chelation and adsorb polar material which results in asymmetric and tailing peaks.

Nevertheless, metal columns are rugged, easy to handle and easy to remove and replace in the chromatograph consequently, their use has persisted in many application areas despite the introduction of fused silica columns.

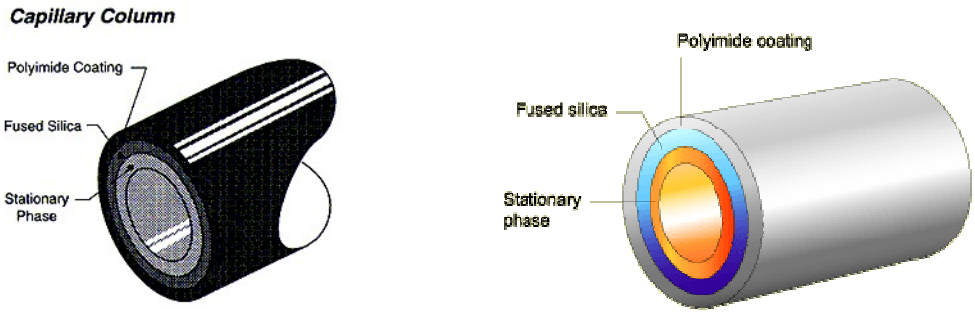
Fused Silica capillary columns:

Desty tried to eliminate the activity of the open tubular column surface by developing the first silica-based columns and invented an extremely clever device for drawing soft glass capillaries. Desty produced both circular rigid soft glass and circular rigid Pyrex capillary columns, but their permanent circular shape, made them difficult to fit to unions connecting columns to injector and column to detector. By careful surface treatment the rigid glass tubes could be coated with polar stationary phases such as *CARBOWAX*.



Cross section of a Fused Silica Open Tubular Column

Dandenau introduced flexible fused silica capillary columns using the quartz fiber drawing technique. The solid quartz rod used in quartz fiber drawing was replaced by quartz tube and the drawing rates adjusted appropriately. The quartz tubes had to be coated on the outside with polyimide to prevent moisture attacking the surface and producing stress corrosion. Coating the capillary tube with a polyimide polymer immediately after drawing prevents moisture coming in contact with the surface and thus stabilizes the tube.



Soft glass capillaries can be produced by the same technique at much lower temperatures but the tubes are not as mechanically strong or as inert as quartz capillaries. Surface treatment is still necessary with fused quartz columns to reduce adsorption and catalytic activity and also make the surface sufficiently wettable to coat with the selected stationary

phase. The treatment may involve washing with acid, silanization and other types of chemical treatment, including the use of surfactants.

Deactivation procedures used for commercial columns are kept highly proprietary. However, a deactivation program for silica and soft glass columns that is suitable for most applications would first entail an acid wash. The column is filled with 10% w/w hydrochloric acid, the ends sealed and the column is then heated to 100˚C for 1 hour. It is then washed free of acid with distilled water and dried.

This procedure is believed to remove traces of heavy metal ions that can cause adsorption effects, column then filled with solution of hexa-methyl-di-silazane contained in a suitable solvent, sealed, and again heated to the boiling point of the solvent for 1 hour.

This procedure blocks any hydroxyl groups that were formed on the surface during the acid wash. If the column is to be coated with a polar stationary phase, it may be advantageous to employ a polar or semi-polar reagent as opposed to the dispersive silicone to facilitate coating. The column is then washed with the pure solvent, dried at an elevated temperature in a stream of pure nitrogen and is then ready for coating.

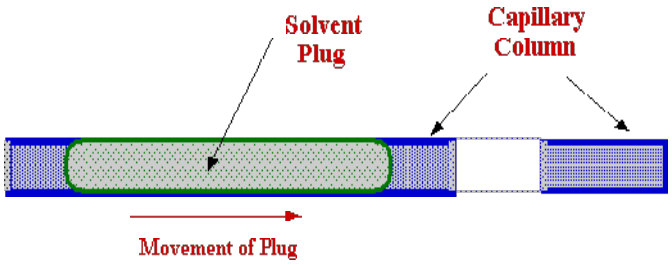
Internally Coating of capillary columns:

Open tubular columns can be coated internally with a liquid stationary phase or with polymeric materials that can be polymerized to form a relatively rigid, internal polymer coating. There are two methods for coating a capillary column the dynamic method and the static method.

- *Dynamic Coating*

A plug of solvent containing the stationary phase is placed at the beginning of the column. The strength of the solution, among other factors, determines the thickness of the stationary phase film. In general the film thickness of an open tubular column ranges from 0.25 mm to about 1.5 mm.

In practice, a 5% w/w of stationary phase in the solvent will produce a film thickness of about 0.5 mm. However, this is only approximate, as the film thickness is also determined by the physical properties of the surface, the solvent and the stationary phase.



Dynamic Coating Procedure for an Open Tubular Column

After the plug has been run into the front of the column (sufficient to fill about 10% of the column length), pressure is applied to the front of the column to force the plug through the column at 2-4 mm per second (it will take about 5.5 hours for the plug to pass through a 60 m column).

When the plug has passed through the column, the gas flow is continued for about an hour. The gas flow must not be increased too soon, or the stationary phase solution on the walls of the tube is displaced forward in the form of ripples, which produces a very uneven film. After an hour the flow rate can be increased and the column stripped of solvent.

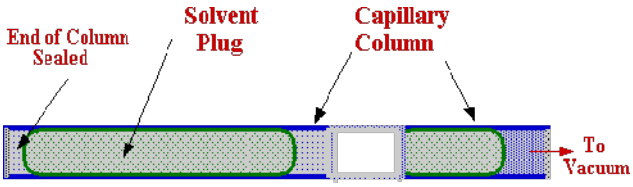
The last traces of the solvent are removed by heating the column above the boiling point of the solvent at an increased gas flow rate. Complete solvent removal can be identified by connecting the column to a detector and observing the baseline drift of the detector.

- *Static Coating*

The entire column is filled with a solution of the stationary phase and one end is connected to a vacuum pump. As the solvent evaporates, the front retreats back down the tube leaving a coating on the walls. A diagram of the static coating procedure is shown in figure. The column is filled with a solution of stationary phase having a concentration appropriate for the deposition of a film of the desired thickness.

Again the required concentration will depend on the stationary phase, the solvent, the temperature and the condition of the wall surface. Unfortunately, the optimum solvent concentration is not theoretically predictable and requires some preliminary experiments to be carried out to determine the best coating conditions.

After filling, one end of the column is sealed, and the other end is connected to a high vacuum pump and placed in an oven and the solvent slowly evaporates and the front retreats leaving a film of solution on the walls.



Static Method for Coating Open Tubular Columns

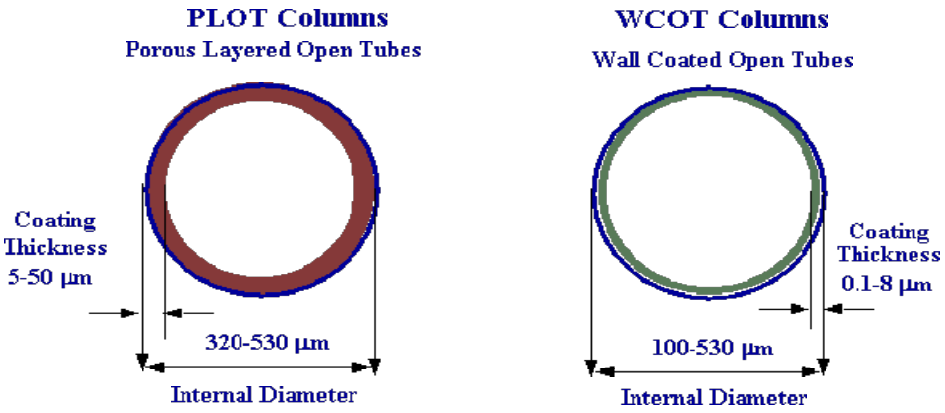
The solvent then evaporates from this film and the stationary phase remains as a thin coating on the wall. The procedure is continued until all the solvent has evaporated and, except for the stationary phase, the column is empty. This process may take hours to complete. The procedure needs no attention and thus, can be carried out overnight. This procedure is more repeatable than the dynamic method of coating but, produces columns having similar performance to those dynamically coated.

Irrespective of the coating method, column stability depends on the stability of the stationary phase film which depends on the constant nature of the surface tension forces that hold it to the column wall. These surface tension forces can be reduced with an increase in temperature or by the solutes passing through the column.

Open Tubular Column Types

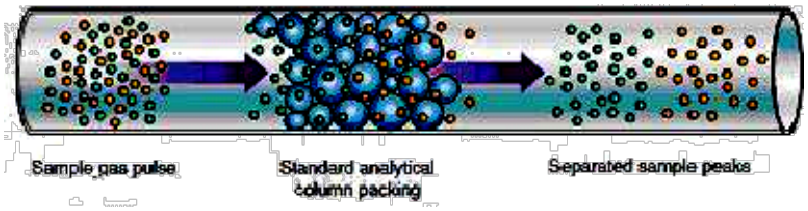
Open Tubular columns are broadly split into two classes, *the wall coated open tubular* columns or ***WCOT*** Columns and *the porous layer open tubes* or ***PLOT*** Columns. The two types of column are shown diagrammatically in figure:

The PLOT columns are largely used for gas analysis and the separation of low molecular weight hydrocarbons. The external diameter of PLOT columns range from 320 to 530 µm with a porous layer that can be 5 to 50 µm thick. The technique of coating the walls with solid particles is again largely proprietary but stable and reproducible columns can be prepared and are commercially available.



- ***The Packed GC Column***

Packed columns are usually constructed from stainless steel or Pyrex glass. Pyrex glass is favored when thermally labile materials are being separated such as essential oils and flavor components. However, glass has pressure limitations and for long packed columns, stainless steel columns are used as they can easily tolerate the necessary elevated pressures. The sample must, of course, be amenable to contact with hot metal surfaces.

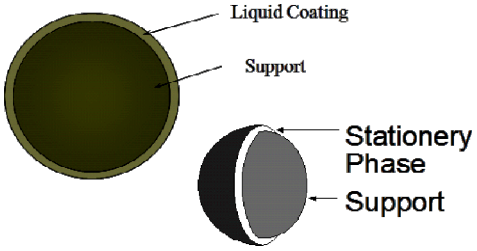


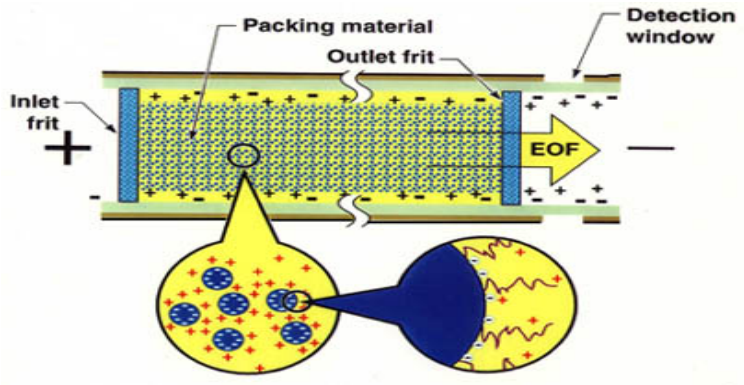
Short columns can be straight, and installed vertically in the chromatograph. Longer columns can be U-shaped but columns more than a meter long are usually coiled. Such columns can be constructed of any practical length and relatively easily installed.

Pyrex glass columns formed to desired shape by coiling at 700˚C and metal columns by bending at room temperature. Glass columns are sometimes treated with an appropriate silanizing reagent to eliminate the surface hydroxyl groups which can be catalytically active or produce asymmetric peaks. Stainless steel columns are usually washed with dilute hydrochloric acid, then extensively with water followed by methanol, acetone, methylene dichloride and n-hexane. This washing procedure removes any corrosion products and traces of lubricating agents used in the tube drawing process, columns are then ready for packing.

Most column packing are siliceous solids but there are many different types and manufacturing processes. Other packing are organic polymers. The characteristics of the packing that are important for chromatographic use include: particle size and distribution, pore size, surface area and shape, surface energy, pH compatibility, and rigidity under pressure. To begin this discussion, let us consider some of the properties of silica packing and compare them with non-siliceous materials.

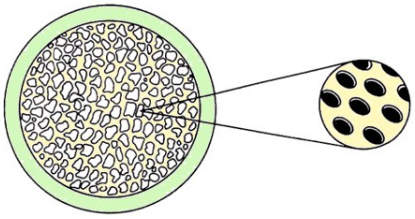
1. Packing can be spherical or irregular in shape. In general, the spherical ones seem best for HPLC because they pack more tightly and uniformly.

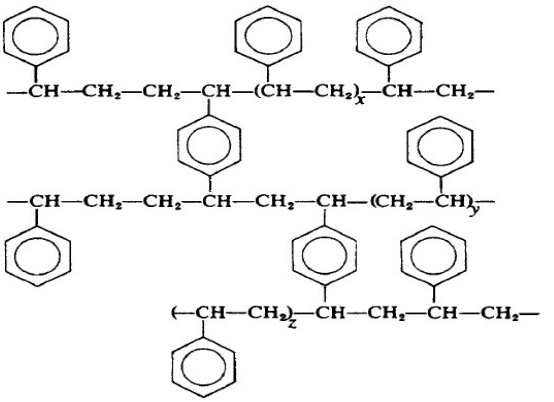




2. Packing can be porous (like some silica gels) or nonporous (like glass beads). Because the porous ones have larger surface areas, they are usually preferred. Larger surface area, the more sites for sorption and the likelihood of improved performance. pores can be of various sizes, and usually a uniform pore size is best. Pore sizes become critical when they are of molecular magnitude because small sizes can exclude large molecules from interior pores.

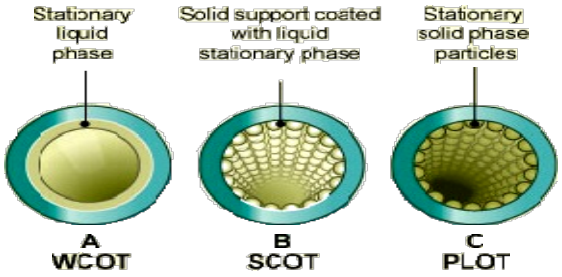
3. Packings can be naturally occurring minerals or synthetically manufactured. The packing is manufactured from a mineral called diatomaceous earth. Typical silica packing for HPLC are manufactured by gelation of sodium silicate, yielding a material with an empirical formula of $SiO_2.H_2O$.

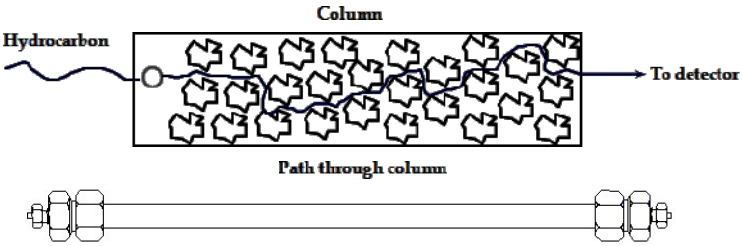




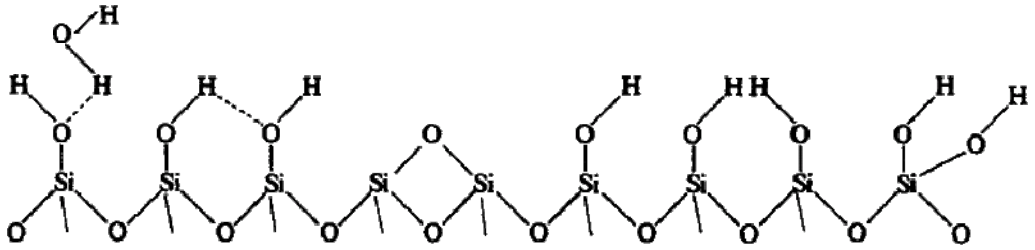
Representation of cross-linked polystyrene–divinylbenzene polymer structure.

4. Packing can be made from materials other than silica. Many chemicals, both inorganic and organic, have been in use for many years; examples are alumina, magnesium silicate, porous graphitic carbon, and more recently, zirconia, ZrO_2. Synthetic porous organic polymers such as poly-methyl-meth-acrylate or copolymers made from di-vinyl-benzene and styrene are very popular and find use in GC and LC.





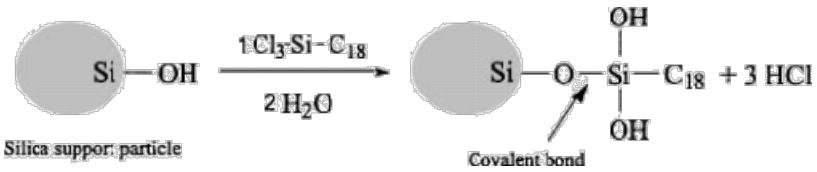
5. Packing usually micro particulates, but new ones are monolithic, a term taken from Greek for "consisting of a single piece." idea of forming a single continuous monolith or rod inside a column was suggested as far back as 1970, and first HPLC columns were reported in late 1990s, first successful commercial columns were introduced by Merck (Darmstadt, Germany) in 2001. Manufactured by a sol-gel process into a single stable rod, monolithic columns so produced are enclosed in a plastic PEEK (poly ether ether ketone) sheath.



Representation of some possible functional groups on the surface of silica.

6. The surfaces of these solid packings can be modified to produce different chemical properties. The styrene-di-vinyl-benzene polymers can be reacted to produce active functional groups on their surfaces for use in ion exchange chromatography (IEC), For example, when reacted with sulfuric acid, sulfonic acid groups are formed that can act as cation exchangers. The surfaces of the silica packings contain hydroxy groups that give them a heterogeneous energy surface that is usually undesirable for chromatographic use.

To make the surfaces more homogeneous for chromatographic use, several treatments have been devised. The natural surface has become known as type A silica; purifying it to remove inorganic metals produces a silica known as type B silica.



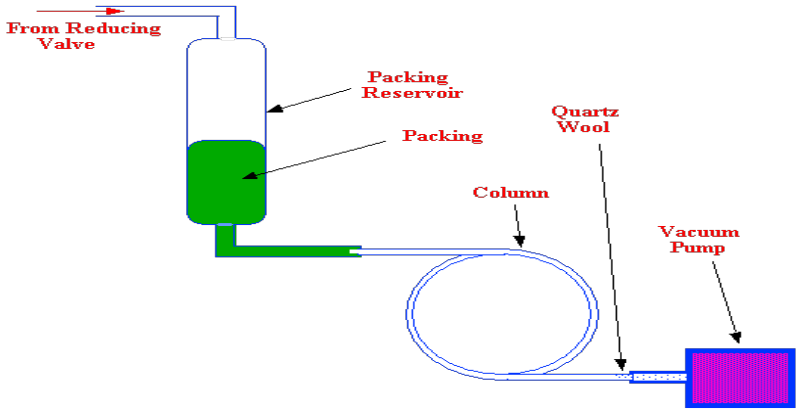
Typical silanizing reactions used to produce bonded phases

Other, proprietary treatments produce surface Si-H groups rather than Si-OH groups, and it is known as type C silica."' The unbonded type C silica does not retain water like type A, so it is a good choice for normal-phase HPLC. Several bonded-phase materials are commercially available for the more popular form of reversed-phase HPLC.

Column Packing

Short columns are usually straight and can be packed vertically. The packing is added, about 0.5 ml at a time, and the column tapped until the packing had settled. Another portion of packing is then added and the process repeated until the column is full. U-shaped columns are packed in the same manner. Columns up to 50 ft long can be packed in a series of U's and then each U column joined with a low dead volume connection. If the columns were glass they were usually filled through an opening at the top of each U which was terminated in a plug of quartz wool and sealed-off in a blow-pipe flame. These long packed columns could be operated at a maximum of 200 psi, and could provide efficiencies of up to 50,00 theoretical plates. Such columns could tolerate charges of several microliters.

However, straight columns are clumsy to use and occupy a large amount of space which is often difficult to thermostat. The coiled column although more difficult to pack has been readily accepted due to the compact nature of their design. To obtain adequate efficiencies, however, a special packing procedure had to be developed.



Example of a column packing apparatus

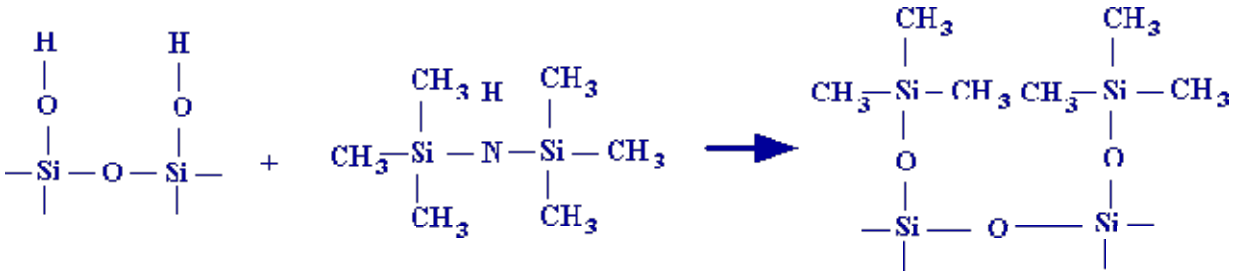
Packing is placed in a reservoir attached to a gas supply that forces the packing through the column. The column exit is connected to a vacuum pump. A wad of quartz wool is placed at the end of the column, constrained by a small restriction that prevents the wad from being sucked into the pump, vacuum and gas flow are turned on simultaneously and the packing is swept rapidly through column. This causes material to be slightly compacted

along total length of the column and has been shown to produce well-packed columns. The procedure is a little tedious and success rate is sometimes less than 90%. In addition, process does not lend itself to automation. The difficulties involved preparing packed columns have also contributed to preferential popularity of open tubular columns. Production of capillary columns can be largely automated and several columns can be prepared simultaneously.

Supports for GLC:

There have been a number of materials used as supports for packed GC columns including, Celite (a proprietary form of a diatomaceous earth), fire-brick (calcined Celite), fire-brick coated with metallic silver or gold, glass beads, Teflon chips and polymer beads. Today however, the vast majority of contemporary packed GLC columns are filled with materials that are either based on of Celtic or polystyrene beads as a support.

The process involves mixing the Celite with sodium carbonate and fluxing the material at 900˚C. This causes the structure of the Celite to be disrupted and the fragments adhere to one another by means of glass formed from the silica and the sodium carbonate. The support is treated with hexamethyldisilazane which replaces the hydrogen of the silanol group with a trimethylsilyl radical. The reaction proceeds as follows,



In this way the strongly polar silanol groups are methylated and assume dispersive characteristics that do not produce peak tailing. Although the major contributors to adsorption by the support are the silanol groups, residual adsorption results from the presence of trace quantities of heavy metals such as iron which can be largely removed by acid washing prior to silanization.

To try to completely eliminate adsorption effects from the support, Teflon was explored as a possible alternative to a diatomaceous earth. Teflon powder proved to have little adsorption, but also proved to be extremely difficult to pack into a column. So difficult, that it is very rarely used in general GLC analyses. Its inert character makes it useful for the separation of certain highly corrosive materials. It has a temperature limit of about 250˚C.

Glass beads have also been used as supports for packed GC columns and, if silanized, have little adsorption properties. Being non-porous, all the stationery phase must reside on the surface of the beads which gives them limited loading capacity. If the loading is increased, the stationary phase collects at the contact points of the spheres and form relatively thick accumulations, producing a high resistance to mass transfer and consequently low column efficiency.

Glass beads appears to be the worst compromise between a column packed with modified Celite and a wall coated glass ,or fused silica, capillary column. The macro porous polymer beads are used as supports as well as adsorbents. They exhibit significant adsorption as the support itself acts as a stationary phase and makes a substantial contribution to retention. However, with normal sample loads, the adsorption isotherm is linear and so the eluted peaks are symmetrical. Only stationary phases that do not affect the polymer in any way can be used with such beads, which is a distinct disadvantage. They also have relatively poor temperature stability.

Coating the Supports

It is important to have an accurate measure of the amount of stationary phase that has been placed on a support to ensure retention time reproducibility and qualitative accuracy. The reproducibility of the coating procedure may have particular significance when the analytical results are to be used for forensic purposes. The material can be coated by the direct addition of the stationary phase to the support, by the filtration method or by the slurry method. The slurry method of coating is the one that is recommended.

Coating by direct addition would appear to be the ideal quantitative method of preparing the column packings. A weighed amount of stationary phase is added directly to a known mass of support contained in a glass flask. The material is well mixed by rotating the flask for several hours, but even with extensive mixing, the stationary phase being is still irregularly distributed throughout the packing.

As a result, efficiency of column slowly increases with use, as stationary phase distributes itself more evenly throughout the packing. It may take several weeks of use for column to give a constant maximum efficiency.

The filtration method gives a packing with the stationary well distributed over the support but the loading can not be accurately calculated. A known mass of stationary phase is dissolved in sufficient solvent to provide excess liquid when mixed with a weighed amount of the support. The mixture is filtered under vacuum and the volume of the filtrate measured. From the volume of filtrate, the amount of solvent remaining on the support can be calculated and hence this stationary phase loading can be accessed.

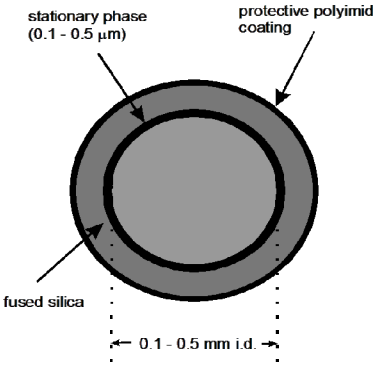
The bed is then sucked dry, the solvent evaporated and the coated support packed into the column. The amount of stationary phase on the support is not determined accurately by this method due to solvent losses by evaporation. In the slurry method of coating, a weighed amount of the support is placed in the flask of a rotary evaporator and the required mass of stationary phase added.

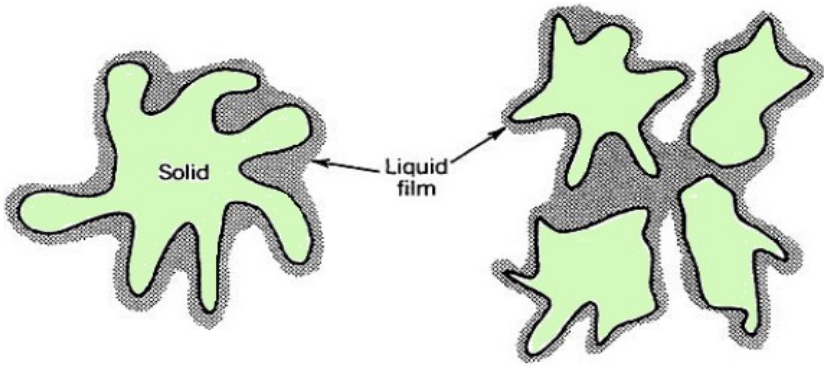
An appropriate volatile solvent is then added in sufficient quantity to produce a free flowing slurry. The flask is then rotated at room temperature for ten minutes to ensure complete mixing. The rotating flask is then heated and the solvent removed by evaporation.

When the packing appears dry, the material is then heated to about 150˚C in and oven to remove the final traces of solvent. This method of coating gives an extremely homogeneous surface distribution of stationary phase throughout the support and an accurate value for the stationary phase loading.

Column volume characteristics

When liquids are coated on solid supports, pools of stationary liquid phase can be formed in the pores of the particles or between the particles. Such non uniform films are undesirable, which is the main reason packed GLC columns have been largely replaced by wall-coated open tubular (WCOT) columns with thin, uniform films.





Nonuniform distribution of liquid on solid support.

The total volume in the column, V_T is made up of three parts:

$$V_T = V_{SS} + V_S + V_M$$

V_{SS} = volume occupied by the solid support

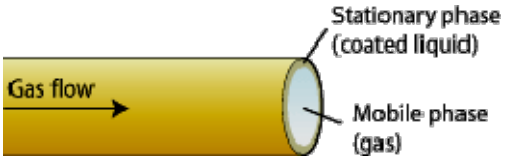
V_S = volume occupied by the stationary phase

V_M = volume occupied by the mobile phase

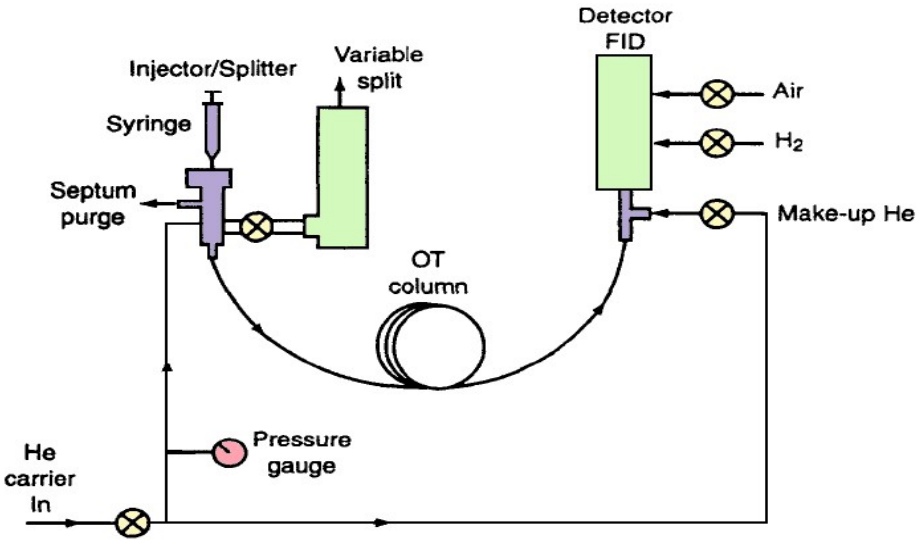
If the solid is nonporous, V_M is the space between the particles (the inter particle volume), but, if it is porous, V_M includes both the inter particle volume and the internal volume of the particles. (In some instances, the size of the pores in the solid may be too small to admit the analyte molecules, and thus the internal portions of the solid may not be accessible to the sample, but that is a complication we will ignore at present.)

Methods for Selecting Stationary Phases

Choosing a stationary phase based on McReynolds' constants has not proven to be effective, and suppliers of columns no longer give these constants in their catalogs, as they once did. Modern approaches to GC stationary phase classifications are based on the cavity model of solvation, but it has little practical value.



In fact, whole process of finding the best SP for a given sample is not as important as it was when packed columns were only ones in use. Today's OT columns are so efficient and auxiliary techniques such as multidimensional operation and mass spectral (MS) detection so effective, that they have removed the necessity to spend time finding the best SP.



Schematic of a typical gas chromatograph.

Configurations of the stationary phase

The stationary phase can be a liquid or a solid. If it is a liquid, it can be coated directly on the inside walls of a capillary tube (column), or it can be coated on an inert solid support and be handled like a solid. In effect, then, there are three stationary-phase configurations: In the first type, a solid (with or without stationary liquid) is packed into a column; in the second type, a solid (with or without stationary liquid) is coated on the surface of a flat, plane material such as glass [thin-layer chromatography (TLC)], and in the third type, a liquid (or a solid) is coated on the inside wall of an open tube (OT).

Packed Columns and Stationary Phases

Packed columns are typically 2-20 ft in length and 0.125 - 0.25 inch in outside diameter. Stainless steel is most common, but glass is also used. The packing is either a solid adsorbent (for gas-solid chromatography, GSC) or a solid support on which a liquid (the stationary phase) is coated (for gas-liquid chromatography, GLC). In either case, the particles should be small and uniform in size as indicated by their mesh range. Typical ranges are 80-100 or 100-120 mesh. The latter range includes particles with diameters from 125 to 149 μm. next table summarizes the differences between packed and OT columns.

Comparison of Packed and WCOT Columns

	$\frac{1}{8}$ inch packed	WCOT
Outside diameter	3.2 mm	0.40 mm
Inside diameter	2.2 mm	0.25 mm
d_f	5 μm	0.25 μm
β	15–30	250–1000
Column length	1–2 m	15–60 m
Flow	20 mL/min	1 mL/min
N_{tot}	6000	180,000
N_{eff}	2000/m	3000/m
H_{min}	0.5 mm	0.3 mm
Advantages	Lower cost	Higher efficiency
	Easier to make	Faster
	Easier to use	More inert
	Larger samples	Fewer columns needed
	Better for fixed gases	Better for complex mixtures

The active solids used for GSC include classic materials such as silica gel, alumina, and charcoal. The zeolite molecular sieves are famous for their ability to separate oxygen, argon, and nitrogen as well as some other fixed gases. Similar materials have been produced commercially especially for GC.

Packed-Column Applications It is generally agreed that OT columns are superior for most GC separations, but there are some applications where packed columns are used. Some of them are Separations that were developed in the early days of GC before OT columns became so widely available.

Separations of large sample sizes as would be required for preparative uses. Packed columns have much more stationary phase in them than do OT columns, so they would be the columns chosen for preparative work.

Separations requiring high selectivity. Packed columns have the flexibility to tailor-make the stationary phase so that it will have the capability to separate a specific pair of difficult-to-separate compounds. One common example is the xylene isomers, which were first successfully separated on a packed column containing the clay Bentone 34. Similarly, when silver salts are incorporated into a liquid phase such as a glycol, special selectivity for olefins is obtained.

Laub and Purnel have described the techniques for combining several liquid phases in same column (or coupled columns) to get best ratio for a given separation. Separations that are best effected by GSC such as the separation of oxygen and nitrogen. Some OT columns, the so-called PLOT columns, are commercially available, but they are few in number. Formerly, packed columns were thought to be more rugged than their OT counterparts, but that is probably no longer true since OT columns are now bonded and cross-linked.

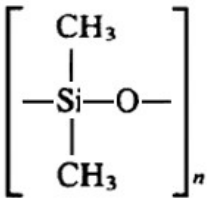
Stationary phase

The selection of a stationary phase (SP) is one of the most important decisions in setting up a method. Because GC is so easy to perform, the process of selection has often been made on a trial-and-error basis and hundreds of liquids have been employed. The theoretical approach, while very complex, has been successful in aiding in the choice of liquid and has permitted the reduction in number of necessary liquids.

The stationary liquid phase is coated on a solid support and packed into a column (packed-column GC) or it is coated on the wall of an open tube (WCOT), as has been discussed. The higher efficiency of OT columns has reduced the necessity for many selective liquids, and the number of OT columns necessary to analyze for "all" types of analytes is smaller than for packed columns.

As GC is used at higher and higher temperatures in an attempt to extend its usefulness to higher and higher boiling analytes, the stationary-phase vapor pressure gets higher and higher and is evidenced as column bleed, an upward sloping baseline. Thus, a major objective has been to find liquids with increasing boiling points-polymers of high molecular weight, special new polymers, and bonded phases.

Liquid phases also have lower temperature limits, represented by their melting points or glass transition temperatures. In most cases these temperatures are low enough so that they are well below the normal working GC temperatures. However, there are some exceptions, so the minimum should also be checked before use. Also, some special applications use subambient temperatures at the beginning of a programmed temperature run, and these are often below the recommended minimum temperature of the stationary phase.



Typical liquids

Silicone polymers, differing in the extent to which they contain polar functional groups, have become the most popular class of liquid phases. The silicone polymers have the backbone structure in which all the alkyl groups are shown as methyl.

$$OH—(CH_2—CH_2—O)_n—H$$

The structure of this polymer is and the approximate average weight is given as a numerical value in the naming of the particular polymer.

Characteristics of Silicone Polymers by OV Number

Name	Type	Solvent	Temp Min. (°C)	Temp. Limit (°C)
OV-1	Dimethylsilicone gum	Toluene	100	325–375
OV-101	Dimethylsilicone	Toluene	20	325–375
OV-3	Phenylmethyldimethylsilicone, 10% phenyl	Acetone	20	325–375
OV-7	Phenylmethyldimethylsilicone, 20% phenyl	Acetone	20	325–375
OV-11	Phenylmethyldimethylsilicone, 35% phenyl	Acteone	0	325–375
OV-17	Phenylmethylsilicone, 50% phenyl	Acetone	20	350–375
OV-61	Diphenyldimethylsilicone	Acetone	20	325–375
OV-73	Diphenyldimethylsilicone gum	Toluene	20	325–350
OV-22	Phenylmethyldiphenylsilicone	Acetone	20	350–375
OV-25	Phenylmethyldiphenylsilicone	Acetone	20	350–375
OV-105	Cyanopropylmethyldimethyl-silicone	Acetone	20	275–300
OV-202	Trifluoropropylmethylsilicone	Chloroform	0	250–275
OV-210	Trifluoropropylmethylsilicone	Chloroform	20	275–350
OV-215	Trifluoropropylmethylsilicone	Chloroform	20	250–275
OV-225	Cyanopropylmethylphenyl-methylsilicone	Acetone	20	250–300
OV-275	Dicyanoallylsilicone	Acetone	20	250–275
OV-330	Silicone carbowax copolymer	Acetone	30	250–275
OV-351	Polyglycolnitroterephthalic	Chloroform	50	250–270
OV-1701	Dimethylphenylcyano substituted polymer	Acetone	20	300–325

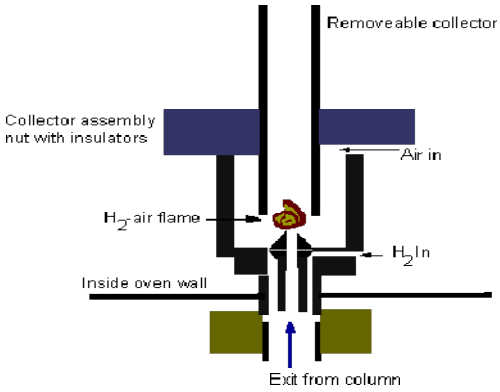
Detectors

After the components of a mixture are separated using gas chromatography, they must be detected as they exit the GC column. The links listed below provide the details of some specific GC detectors. The thermal-conductivity (TCD) and flame-ionization (FID) detectors are the two most common detectors on commercial gas chromatographs. The requirements of a GC detector depends on the separation application. For example, one analysis might require a detector that is selective for chlorine-containing molecules; another analysis might require a detector that is non-destructive so that the analytes can be recovered for further spectroscopic analysis, the FID is usually required for OT chromatographs because of its greater sensitivity. Both are classified and compared in Table.

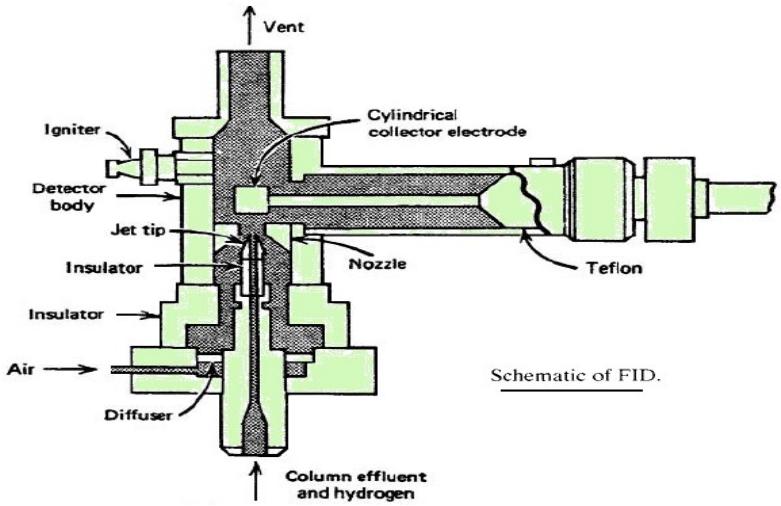
Comparison of TCD and FID

	TCD	FID
Classifications	Concentration	Mass flow rate
	Bulk property	Specific property
	Universal	Slightly selective; only organics
	Nondestructive	Destructive
Characteristics		
Sensitivity	5000 mV·mL/mg	10^{-2} C/g
Detectivity (MDQ)	10^{-10} g/mL	10^{-12} g/s
Minimum sample size	10^{-8} g	10^{-10} g
Linearity	10^{4}	10^{6}

Flame ionization Detector the FID is a small oxygen-hydrogen flame in which the sample is burned, producing some ions in the process. These ions are then collected, constituting a small current, which is amplified and sent to a data system. A typical FID design, shows the column effluent mixed with hydrogen and led to a small burner tip that is swept by a high flow of air for complete combustion.



An igniter is necessary for remote lighting of the flame. The collector electrode is biased about + 300 V relative to the flame tip, and the signal current is amplified. The exact mechanism of flame ionization is still not known, but the ionization efficiency, while low, is one of the highest available in a GC detector and sufficient to give a good sensitivity and linearity. The flow rates of hydrogen and air must be optimized for a particular detector design (and to a lesser extent, analyte).



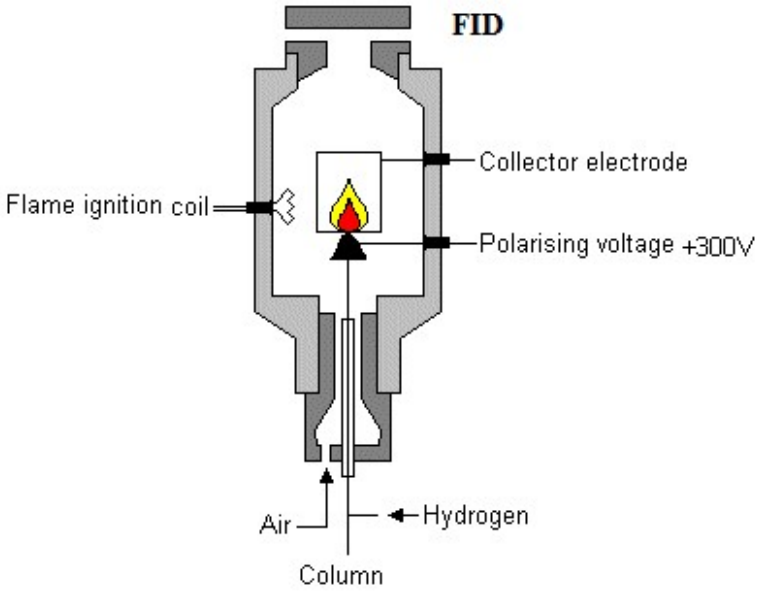
Schematic of FID.

Typical values, as shown in Figures are a hydrogen flow of 30-40 mL/min and an air flow of 300-400 mL/min. For OT columns, a makeup flow of carrier gas is necessary to bring the total up to about 30 mL/min, since OT flow rates are of the order of only 1 mL/min. As noted earlier, hydrogen is becoming increasingly popular as the carrier gas of choice for OT columns; this requires changes in gas flows and has prompted new FID design FID is nearly universal, detecting all organic compounds, but not those listed in Table. Water is a compound that often produces badly tailed peaks on GC columns, so it may be advantageous that the FID does not detect it. However, the FID cannot be used for analysis of fixed gases. Response factors must be determined for good quantitative analysis.

Some Compounds Not Detected by Flame Ionization Detector

He	O_2	NO	CS_2
Ar	N_2	NO_2	COS
Kr	CO	N_2O	$SiCl_4$
Ne	CO_2	NH_3	$SiHCl_3$
Xe	H_2O	SO_2	SiF_4

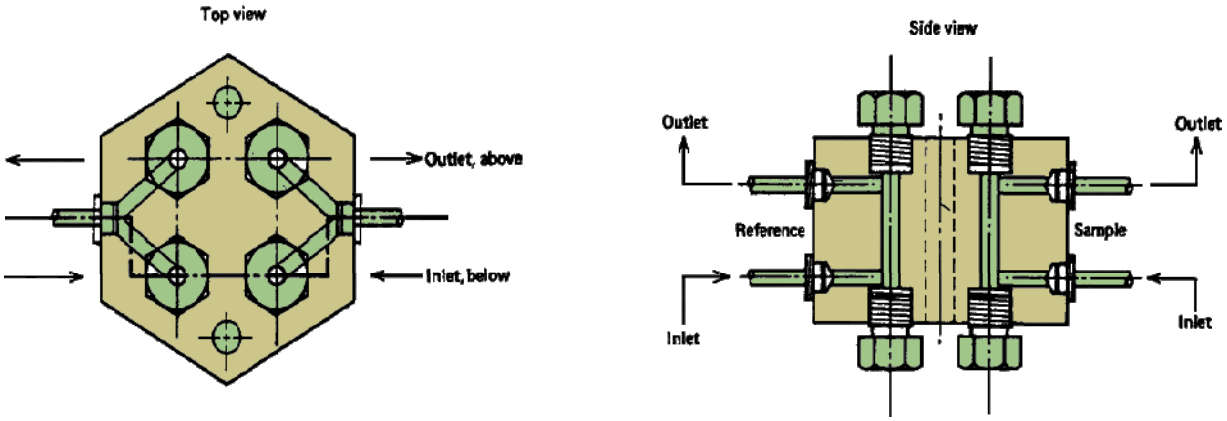
In summary, the FID is the detector of choice for organic analysis because of its sensitivity, which allows it to be used with OT columns. It has a good stability and linearity, but it requires additional gases for its operation. While it is not totally universal, it does detect all organic compounds.



Thermal Conductivity Detector TCD is not as sensitive as other detectors but its non-specific and non-destructive. TCD consists of an electrically-heated wire or thermistor. temperature of sensing element depends on thermal conductivity of gas flowing around it.

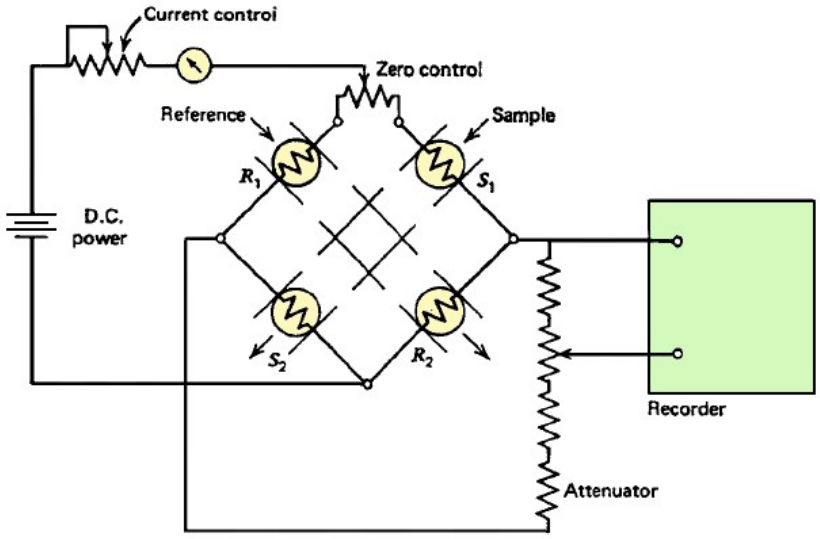
Changes in thermal conductivity, such as when organic molecules displace some of the carrier gas, cause a temperature rise in the element which is sensed as a change in resistance.

TCD cell is metal block in which cavities have been drilled to accommodate transducers, which can be either thermistors or resistance wires (so-called hot wires). Thermistors are most sensitive at low temperatures and find limited use. Wire filaments can be supported on holders or be mounted concentrically in a cylindrical cavity. The latter arrangement permits the volume of the cavity to be minimized, which is highly desirable.



Typical TCD, designed for four concentric hot wires.

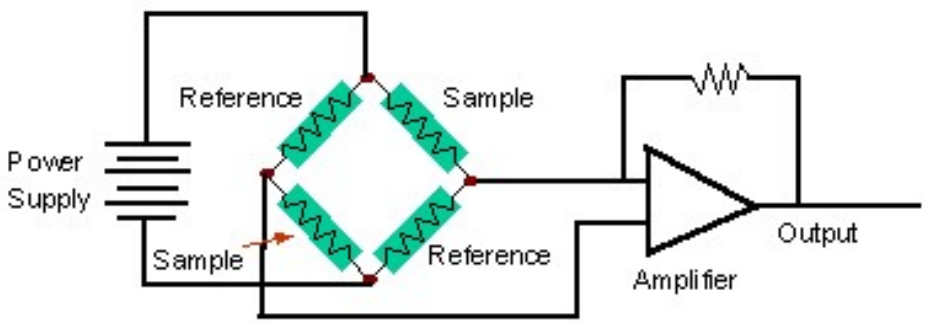
A typical design has four cavities; two is the minimum-one each for the reference and the sample flows. A special low-volume thermal conductivity cell has been produced by Agilent (formerly Hewlett-Packard); to keep the volume small, only one cavity is used and the two gas streams (reference and sample) are passed through it alternately.



Simplified Wheatstone bridge circuit for TCD. Four elements: R_1 and R_2 for reference, and S_1 and S_2 for example.

Two pairs of TCD are used in gas chromatographs. One pair is placed in the column effluent to detect the separated components as they leave the column, and another pair is placed before the injector or in a separate reference column. The resistances of the two sets of pairs are then arranged in a bridge circuit.

The bridge circuit allows amplification of resistance changes due to analytes passing over the sample thermo conductors and does not amplify changes in resistance that both sets of detectors produce due to flow rate fluctuations, etc.



The resistance wires, made of tungsten or a tungsten-rhenium alloy, are heated with a direct current (DC) source to a temperature above the block temperature and lose heat to it at a rate dependent upon the thermal conductivity of the gas in the cavity.

Thus, the temperature, and hence the resistance, of the hot wire depends upon the thermal conductivity of the gas in the cavity. The wires are incorporated into a Wheatstone bridge circuit and produce a voltage imbalance when an analyte passes through one side of the cell. The wires can be heated at constant voltage or constant current or be maintained at a constant temperature by varying the current or voltage.

Keeping the filament temperature constant requires a more complex circuit, and the output signal is derived from the electrical changes that are necessary to bring the bridge back to null, rather than from direct voltage imbalance.

Sensitivity is increased by heating the filament to a higher temperature with the power supply, and it is a function of the difference in temperature (A T) between the filament and the block. If a TCD is operated in air, the filaments are quickly oxidized and burn out. Even small leaks in the chromatographic system will result in gradual destruction of the hot wires.

Thermal Conductivities and TCD Response Values for Selected Compounds

Compound	Thermal Conductivity[a]	RMR[b]
	Carrier Gases	
Argon	12.5	—
Carbon dioxide	12.7	—
Helium	100.0	—
Hydrogen	128.0	—
Nitrogen	18.0	—
	Samples	
Ethane	17.5	51
n-Butane	13.5	85
n-Nonane	10.8	177
i-Butane	14.0	82
Cyclohexane	10.1	114
Benzene	9.9	100
Acetone	9.6	86
Ethanol	12.7	72
Chloroform	6.0	108
Methyl iodide	4.6	96
Ethyl acetate	9.9	111

The carrier gas must have a thermal conductivity (TC) that is very different from the analytes to be detected, so that the most commonly used gases are helium or hydrogen, when a sample enters the sample cavity, that filament gets hotter, its resistance goes up, and a signal is produced.

Other mechanisms contribute to the loss of heat from the filament, and response values cannot be calculated from thermal conductivities alone. For quantitative analysis, response values must be determined; they are included in table remember that the TCD is a concentration detector and the peak areas it produces are flow dependent. Low flow rates have higher analyte concentrations and therefore better sensitivities. Although the TCD is only moderately sensitive, it is universal, simple, rugged, and inexpensive.

Other Detectors

As capillary column based gas chromatography takes its place as the major, highest resolution separation technique available for volatile, thermally stable compounds, the requirements for sensitive and selective detection of these compounds increases.

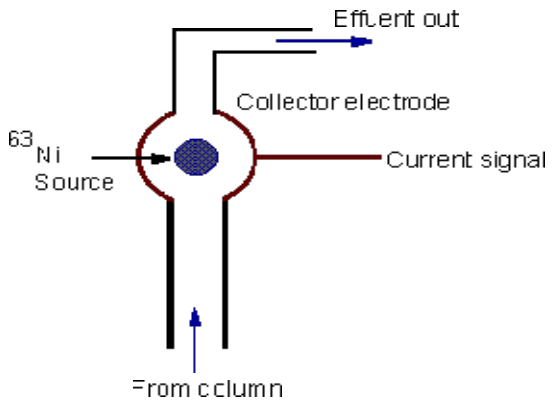
Since more and more complex mixtures can be successfully separated, subsequent chromatograms (output of a chromatographic separation) are increasingly more complex. Therefore, need to differentiate between sample components *using GC* detector as a means of compounds discriminating is more and more common. In addition, each detector has its own characteristics (selectivity, sensitivit, stability, cost, etc.) that helps in a decision about which detector to use. The characteristics of some other detectors are summarized in Table.

Name	Operating Principle	Selective for[a]
	Ionization-Type Detectors	
Alkali flame ionization (AFID) or nitrogen/phos (NPD)	Original alkali salt vapors cause chemical ionization in flame; now rubidium silicate heated electrically	P, N, X
Photoionization (PID)	UV lamp causes photoionization	Aromatics
Discharge ionization (DID)	High-voltage discharge	Universal but used for gases
Helium ionization (HID)	He carrier gas; ionization by tritium	Universal, but used for gases
	Emission Type Detectors	
Flame photometric (FPD)	Flame excitation causes emission	S, P
Atomic plasma emission	Plasma excitation causes emission	Metals; S. P, X
	Other Detectors	
Hall electrolytic conductivity (HECD)	Catalytic reaction to form HX, H_2S, NH_3; measure conductance	S, N, X
Chemiluminescence: Thermal energy analyzer (TEA)	Catalytic pyrolysis	Nitro- and nitroso-
Sulfur chemiluminescence	Flameless thermal fragmentation	S
Radioactivity (RAD)	β or γ detectors	Radioactive

[a] X = halogen.

Electron-capture detector (ECD): The ECD is as sensitive as the FID but has a limited dynamic range and finds its greatest application in analysis organic molecules that contain electronegative functional groups, such as halogens, phosphorous, and nitro groups.

The ECD uses a radioactive Beta emitter (electrons) to ionize some of the carrier gas and produce a current between a biased pair of electrodes. When organic molecules that contain electronegative functional groups, such as halogens, phosphorous, and nitro groups pass by the detector, they capture some of the electrons and reduce the current measured between the electrodes. The ECD is as sensitive as the FID but has a limited dynamic range and finds its greatest application in analysis of halogenated compounds.



Atomic-Emission Detector (AED): One of newest additions to the gas chromatographer's arsenal is the atomic emission detector (AED). This detector, while quite expensive compared to other commercially available GC detectors, is an extremely powerful alternative. Instead of measuring simple gas phase (carbon containing) ions created in a flame as with the flame ionization detector, or the change in background current because of electronegative element capture of thermal electrons as with the electron capture detector, the AED has wider applicability because it is based on detection of atomic emissions.

The strength of the AED lies in the detector's ability to simultaneously determine the atomic emissions of many of the elements in analytes that elute from a GC capillary column (called eluants or solutes in some books). As eluants come off the capillary column they are fed into a microwave powered plasma (or discharge) cavity where the compounds are

destroyed and their atoms are excited by the energy of the plasma. The light that is emitted by the excited particles is separated into individual lines via a photodiode array. The associated computer then sorts out the individual emission lines and can produce chromatograms made up of peaks from eluants that contain only a specific element. The components of the AED include:

- Interface for incoming capillary GC column to microwave induced plasma chamber,
- Microwave chamber itself, a cooling system for that chamber,
- A diffraction grating and associated optics to focus then disperse spectral atomic lines,
- A position adjustable photodiode array interfaced to a computer. Microwave cavity cooling is required because much of energy focused into cavity is converted to heat.

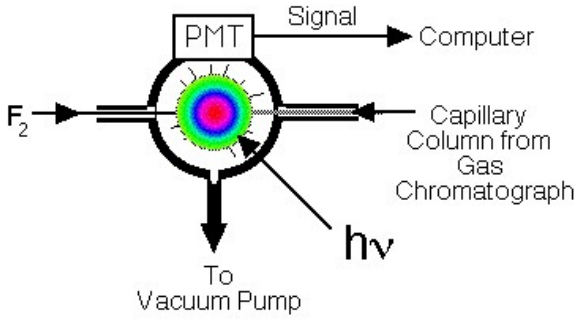
Chemiluminescence's detector : it can take place in either the solution or gas phase. Though liquid phase chemiluminescence plays a significant role in laboratories using this analytical technique (often in conjunction with liquid chromatography), we will concentrate on gas phase chemiluminescence reactions since the instrumental components are somewhat simpler. These detectors are also often used as detectors for gas chromatography.

The major limitation to the detection limits achievable by chemiluminescence involves the dark current of the photomultiplier (PMT) necessary to detect the analyte light emissions. If the excitation energy for analytes in chemiluminescence doesn't come from a source lamp or laser, where does it come from? The energy is produced by a chemical reaction of the analyte and a reagent. An example of a reaction of this sort is shown below:

$$CH_3{-}S{-}CH_3 + F_2 \longrightarrow \text{products} + \underset{\text{(light)}}{h\nu}$$

In gas phase chemiluminescence, light emission is produced by reaction of an analyte (dimethyl sulfide in the above example) and a strongly oxidizing reagent gas such as fluorine (in the example above) or ozone, for instance. The reaction occurs on a time scale such that production of light is essentially instantaneous; therefore, most analytical systems simply mix analytes and the reagent in a small volume chamber directly in front of PMT.

If the analytes are eluting from a gas chromatographic column then the end of the column is often fed directly into the reaction chamber itself. Here is a schematic of the components necessary for a gas phase chemiluminescence detector interfaced to a capillary gas chromatograph.



Flame-photometric detector (FPD): The reason to use more than one kind of detector for gas chromatography is to achieve selective or highly sensitive detection of specific compounds encountered in particular chromatographic analyses. The determination of sulfur or phosphorus containing compounds is the job of flame photometric detector (FPD).

This device uses the chemiluminescent reactions of these compounds in a hydrogen/air flame as a source of analytical information that is relatively specific for substances containing these two kinds of atoms. The emitting species for sulfur compounds is excited S_2. The lambda max for emission of excited S_2 is approximately 394 nm. The emitter for phosphorus compounds in the flame is excited.

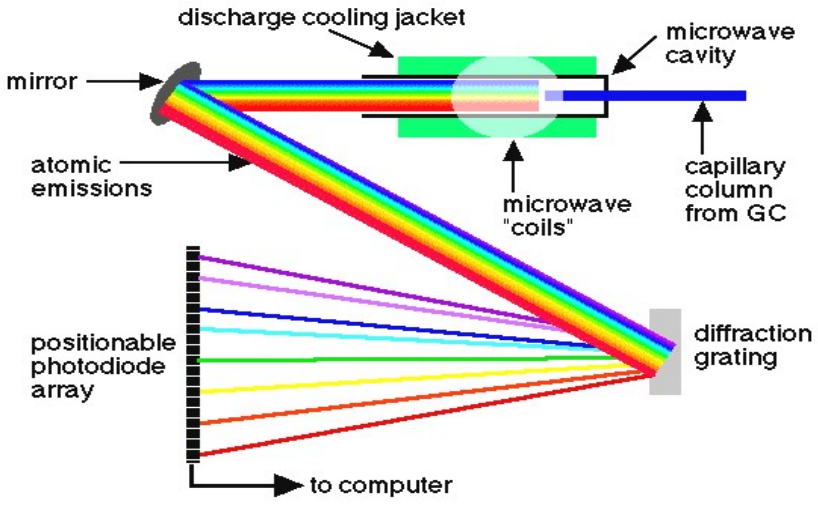


Photo ionization detector (PID): The reason to use more than one kind of detector for gas chromatography is to achieve selective and highly sensitive detection of specific compounds encountered in particular chromatographic analyses. The selective determination of aromatic hydrocarbons or organo-heteroatom species is the job of the Photo ionization detector (PID).

This device uses ultraviolet light as a means of ionizing an analyte exiting from a GC column. The ions produced by this process are collected by electrodes. The current generated is therefore a measure of the analyte concentration.

If the energy of an incoming photon is high enough (and the molecule is quantum mechanically "allowed" to absorb the photon) photo-excitation can occur to such an extent that an electron is completely removed from its molecular orbital, i.e. ionization.

$$R + h\nu \longrightarrow R^{+} + e^{-}$$

If the amount of ionization is reproducible for a given compound, pressure, and light source then the current collected at the PID's reaction cell electrodes is reproducibly proportional to the amount of that compound entering the cell.

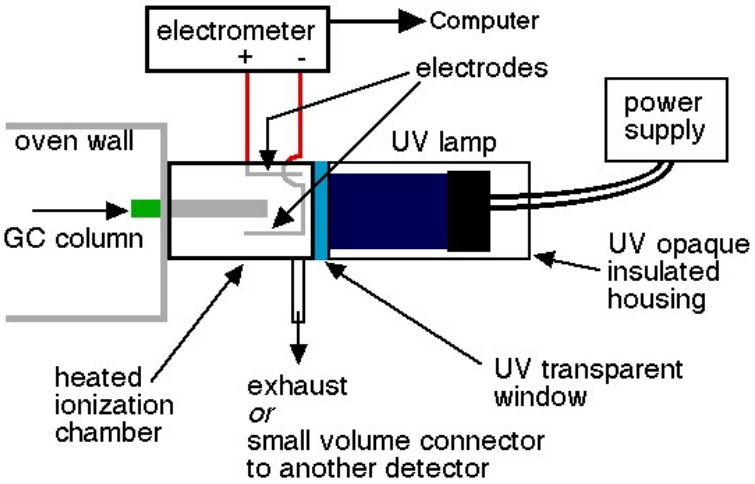
The reason why the compounds that are routinely analyzed are either aromatic hydrocarbons or heteroatom containing compounds (like organo sulfur or organo phosphorus species) is because these species have ionization potentials (IP) that are within reach of commercially available UV lamps.

The available lamp energies range from 8.3 to 11.7 ev, that is, lambda max ranging from 150 nm to 106 nm. Although most PIDs have only one lamp, lamps in the PID are exchanged depending on the compound selectivity required in the analysis.

Selective detection using a PID: Here is an example of selective PID detection: Benzene's boiling point is 80.1. (This compound would respond in a PID with a UV lamp of 9.5 ev (commercially available) because this energy is higher than benzene's IP (9.24). Isopropyl alcohol has a similar boiling point (82.5 °C) and these two compounds might elute

relatively close together in normal temperature programmed gas chromatography, especially if a fast temperature ramp were used.

However, since isopropyl alcohol's IP is 10.15 ev this compound would be invisible or show very poor response in that PID, and therefore the detector would respond to one compound but not the other.

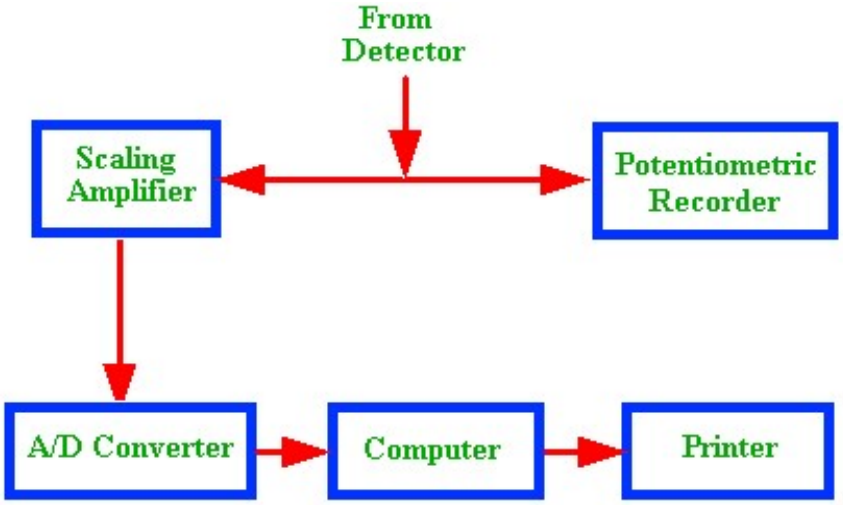


Since only a small (but basically unknown) fraction of the analyte molecules are actually ionized in the PID chamber, this is considered to be a nondestructive GC detector. Therefore, the exhaust port of the PID can be connected to another detector in series with the PID. In this way data from two different detectors can be taken simultaneously and selective detection of PID responsive compounds augmented by response from, say, an FID or ECD.

Major challenge here is to make design of ionization chamber and downstream connections to the second detector as low volume as possible (read small diameter) so that peaks that have been separated by the GC column do not broaden out before detection.

Data Acquisition and Processing

Originally, analytical results were calculated from measurements made directly on the chromatogram provided by the chart recorder. This is still true for many chromatographs in use today, but analyses obtained from contemporary instruments commonly process the results using a computer. The output from the detector (which is only rarely the direct output from the detector sensor) is usually in millivolts and is suitable for direct connection to a potentiometric recorder.



Data Acquisition and Processing System

This output represents a voltage that is linearly related to solute concentration being measured by the detector sensor and as the sensor response is often nonlinear, the signal usually requires nonlinear processing to provide the required output.

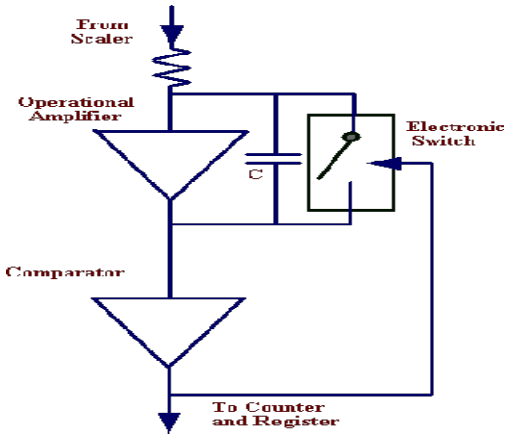
This is carried out by the detector electronics. The FID is an exception to this, as the ion current from the flame itself happens to be linearly related to the mass of carbon passing through it per unit time. A block diagram showing the essential elements of a data acquisition and processing system is given in figure.

Scaling Amplifier

The output from the detector usually passes directly to a scaling amplifier that modifies the signal to a range that is appropriate for the analog-to-digital (A/D) converter. The output can alternatively pass to a potentiometric recorder and produce the chromatogram in real time. The computer system can also produce a real time chromatogram but, to do so, the data must be processed and the chromatogram presented on the printer. The output from most detectors ranges from 0 to 10 mV? whereas the input required by most A/D converters is considerably greater e.g. 0 to 1.0 V. For example, if the FSD of the signal is 10 mv, the instantaneous measurement of 2 mV (assumed from the detector) must be scaled up to 0.2 volt, which is carried out by a simple linear scaling amplifier having a gain of 100.

A/D Converter

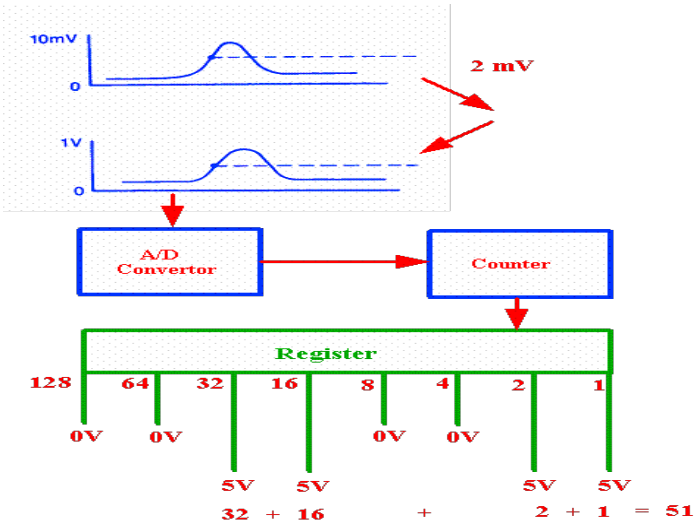
After scaling, the signal must be converted to digital form. There are a number of ways to digitize and only one, the simplest will be described. A diagram showing the operating principle of an voltage/frequenc V/F type A/D converter is shown in figure 32.



Basic V/F Analog–to–Digital Converter.

The converter consists of an integrator that can be constructed from an operational amplifier with a feedback capacitor. The capacitor is charged by the voltage from the scaling amplifier through the operational amplifier. The output from the integrator is sensed by a comparator which activates the electronic switch when the potential across the capacitor reaches a preset voltage.

The activation of the comparator also causes a pulse to be passed to a counter and at the same time the capacitor is discharged by the electronic switch. The process then starts again. The time taken to charge the capacitor to the prescribed voltage will be inversely proportional to the applied voltage and consequently the frequency of the pulses from the comparator will be directly proportional to applied voltage, frequency of pulses generated by the voltage controlled oscillator is sampled at regular intervals by a counter which then transfers the count in binary form to a register.



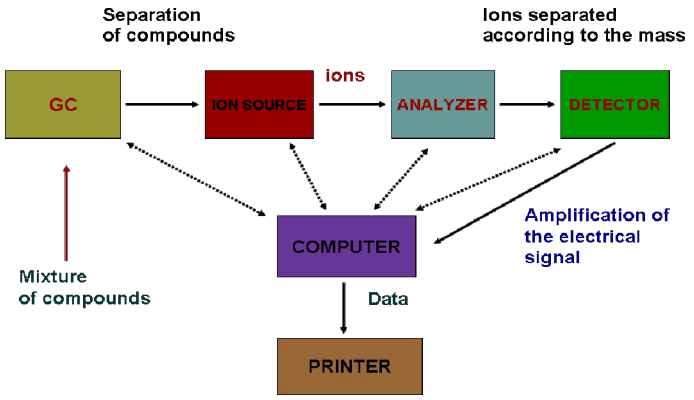
Stages of Data Acquisition

The output from most detectors ranges from zero to ten millivolts and the input range of many A/D converters is from zero to one volt. Thus, the instantaneous measurement of 0.2 mV from the detector must be scaled up by a factor of 100 to 0.2 volts, which is carried out by the scaling amplifier in the manner shown.

The A/D converter changes the analog voltage to a digital number, the magnitude of which is determined by the number of "bits" that the computer employs in its calculations. If, for example, eight bits are used, the largest decimal number will be 255. The output from the A/D converter is sampled regularly by the computer and the curve relating this data to time will reconstitute the chromatogram. The precision of the chromatogram and any calculations made with the data will obviously depend on the frequency of sampling which is normally user selected.

Data Processing

In the early days of gas chromatography, the associated computers used core storage which was bulky, expensive and had a very limited capacity (e.g., 8 kilobytes was a large memory). The limited memory meant that the programming was limited and had to be written extremely economically (i.e. employing the minimum of memory) and much of the data processing was done 'on-the-fly'. This meant that after each peak was eluted, it retention time and height was noted and its area calculated and then the raw data was discarded and only the retention time, peak height and peak area were stored. This economic processing package could not recalculate the data after the separation was complete, it could not reconstitute the chromatogram and it could not employ an alternative algorithm for area measurement if the one used was not appropriate. These restrictions were entirely a result of the cost and size limitation of computer memory at that time.



With the introduction of cheap, compact solid state memory and the high capacity disk memory, situation has completely changed. 8 megabytes is now a small memory and disk capacities are now measured in gigabytes. All chromatography data can now be stored and reprocessed after separation as many times as required, chromatograms can be reconstituted (with modified axes if necessary) and quantitative data manipulated as necessary. In addition, because the computer speeds have also increased greatly, on the fly processing can be carried out in parallel with normal data processing if required. The processing can include a variety of fairly sophisticated mathamatical procedures such as base-line correction, peak skimming, and multi-peak deconvolution.

Programmed Temperature GC

The column oven should operate over a fairly wide temperature range (e.g. from 5˚C to 400˚C). In practice, however, the maximum oven temperature needed is usually less than 250˚C, particularly when synthetic stationary phases are being used, as many of them tend to be unstable and either decompose or volatilize at higher temperatures. Similarly, initial temperatures below 50˚C are also rarely needed.

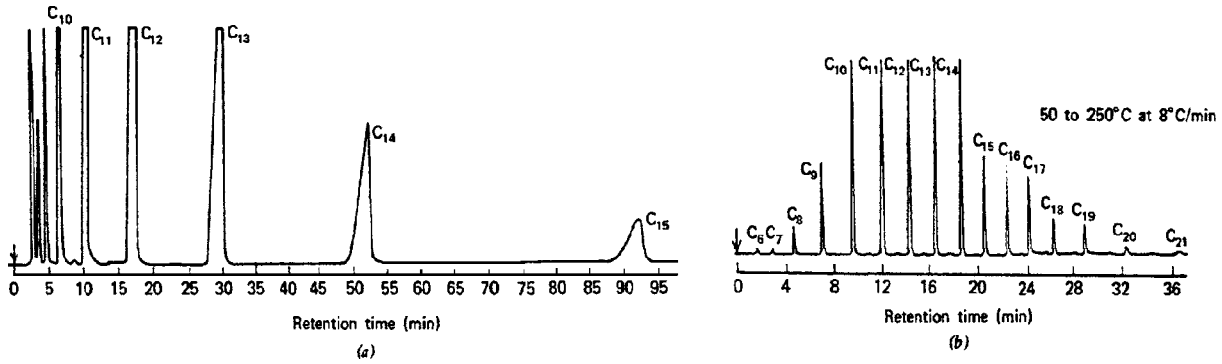
The oven usually has air circulation driven by a powerful fan to ensure an even temperature throughout the oven. The temperature in any part of the oven should be stable to ± 0.5 ˚C and when operating isothermally the column temperature should be constant to ± 0.2 ˚C. The oven should have a capacity of 1-2 cu. ft. and is supplied with fittings to accept more than one column and some switching valves if so desired. Such equipment is needed for multidimensional chromatography.

The temperature programmer (hardware and software) usually has a range of linear gradients from 0.5˚C/min. to about 20˚C/min. Some programmers include nonlinear programs such as logarithmic and exponential, but most GC analyses can be effectively accomplished using linear programs only. The program rate can be changed at any time in the chromatographic development or intermittent isothermal periods can be inserted where necessary in the program.

The temperature programming limits are usually the same as those of the oven (viz. 5˚C to 400˚C). All connections between the column and the detector, that pass though the column oven wall to the detector oven, are supplied with their own heaters so that no part of the conduit can fall below the column oven temperature. A cool spot in the conduit will cause condensation which can result in broad and distorted peaks.

Programmed temperature operation is good for screening new samples. A maximum amount of information about the sample composition is obtained in minimum time. Usually one can tell when the entire sample has been eluted, which is often a difficult judgment to make with isothermal operation.

Temperature is one of the two most important variables in GC. Retention times decrease as temperature increases. Programmed temperature GC (PTGC) is the process of increasing the column temperature during a run. As we have just seen, the increasing temperature will cause the partition coefficients of the analytes still on the column to decrease, and they will move faster through the column, yielding decreasing retention times.



A comparison of (*a*) isothermal and (*b*) temperature-programmed separation of *n*-paraffins.

Some major differences between the two runs can be seen and are typical of PTGC. For a homologous series, the adjusted retention times are logarithmic under isothermal conditions, as we saw earlier, but they are linear when temperature programmed. The programmed run was begun at a lower temperature (50°C) than the one used for the isothermal run (150°C), which facilitated the separation of the low-boiling paraffins.

It ended at a higher temperature (250°C), which increased the number of paraffins detected and extended the range to C_{12} versus C_{15} for the isothermal run. The peak widths are about equal in PTGC, while some fronting is evidenced in the higher boilers in the isothermal run. Since the peak widths do not increase in PTGC, the heights of the late-eluting analytes will be increased (peak areas are constant), providing better detectivity. The advantages and disadvantages of PTGC are summarized in Table.

Advantages and Disadvantages of PTGC

Advantages	Disadvantages
1. Better separation for wide boiling mixtures	1. Has additional instrument requirements
2. Constant peak width and shape	2 Ghost peaks may occur
3. Decreases time of analysis	3. Extra time required for cool down
4. Sample introduction less critical	4 Fewer stationary phases can be used
5 No loss of quantitative accuracy	5 Subject to baseline drift and noise due to column bleeding
6. Lower limits of detection	

Fast GC

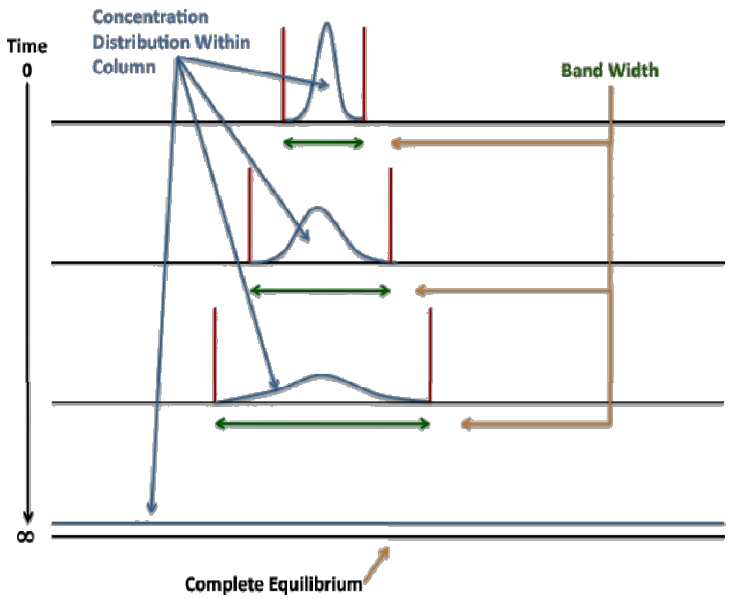
Compared to other methods of analysis, GC is relatively fast, but analysts are always interested in making a process as fast as possible in order to save time. However, since fused-silica OT columns have become popular and GC has achieved the status of a mature technique, chromatographers have made a special effort to minimize the time needed for an analysis. Their efforts have been summarized in two reviews.

Briefly, they are:

- Using OT capillaries of very small inner diameter such as 100 µm.
- Using very thin films of stationary phase.
- Using very fast program rates, often optimized by method translation.

Instruments capable of achieving these rates have become commercially available to support this requirement.

- Using hydrogen as the carrier gas, often, but not always at high flow rates.
- Using a detector with a fast time constant and operated at a vacuum.
- Again, this requires a suitable instrument. Fortunately, the MS detector is available.



Programmed Temperature Vaporizers and Large-Volume Injections: The injection of large samples into a GC is another procedure with an obvious advantage-better detectability. However, the popular OT columns are not capable of handling large samples, so some special techniques are necessary to make large-volume injections (LVI) possible. The most important one is:

- Cold on-column (COC)
- Programmed temperature vaporization (PTV)
- Loop-type injection
- Vapor-overflow
- A combination of the latter two, called AT-column injection

Cold on-column and PTV are the two most widely used techniques. PTV requires a special injection port that has the capability to heat up and cool down very rapidly

Quantitative Analysis

There are three important stages in a GC analysis,

- The preparation of the sample.
- The development of the separation and the production of the chromatogram
- The processing of the data and the presentation of the results.

Each stage is equally important and if not carried out correctly the results will be neither precise nor accurate. Sample preparation can be very simple involving no more that diluting a known weight of sample with mobile phase or be much more complex including an extraction procedure followed by derivatization and then dilution.

For some samples the preparation can be the most time consuming and difficult part of the whole analysis. Liquid extraction is a clumsy procedure, particularly when used on the micro scale which is often necessary in sample preparation.

An alternative procedure is solid phase extraction. The procedure is relatively simple and involves the use of a short tube packed with an appropriate adsorbent such as silica, reversed phase silica or, for some applications, macro porous polymer beads. The adsorbent must be capable of removing the substances of interest from the liquid medium.

Extracting trace materials from water (e.g., pollution analysis) a reversed phase would be appropriate. Then the substances could be displaced into solvents such as n-hexane, methylene dichloride etc.

Evaluation of GC

Advantages

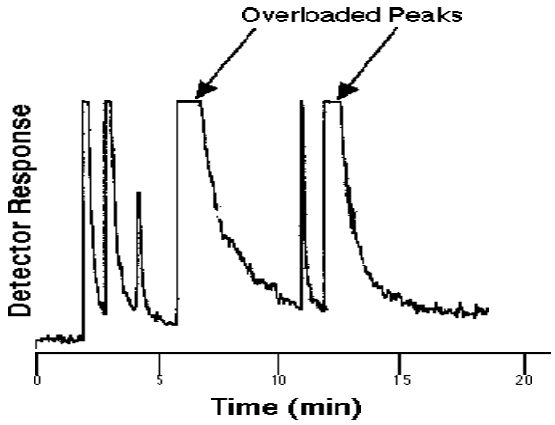
- Efficient, selective, and widely applicable
- Easily combined with MS
- Fast
- Inexpensive and simple
- Easily quantitated and automated
- Reuires only a small sample (milligram range)
- Nondestructive detectors available

Disadvantages

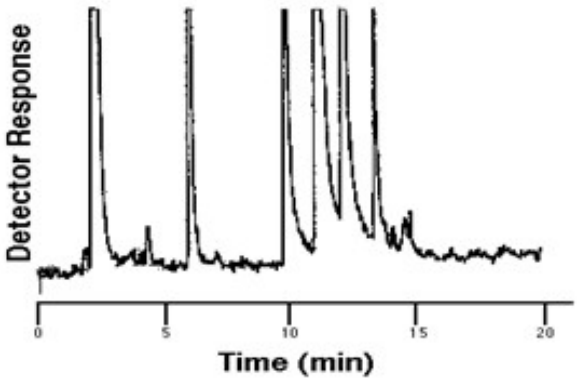
- Samples must be volatile
- Not suitable for thermally labile samples
- Difficult for large samples (preparative scale)
- Only fair for qualitative analysis

Overloaded Chromatography

This sample size requirement initially meant that if samples contained components that were too concentrated for a capillary chromatographic analysis, the sample had to be diluted before it was analyzed. Otherwise the column would be overloaded by those high concentrated components. An example of this appears in the figure below. The clearly overload peaks are indicated, and while some of the other components are in the resolvable (not overloaded) range, having large masses of components can also distort the peak shape of some of the lower mass components.



The following figure shows a little better chromatography with fewer overloaded peaks. The second eluting peak (about 6 minutes) is clearly not overloaded while the group between 10 and 14 minutes still shows overloading characteristics: long drawn-out tailing and much less than baseline separation with peaks that elute nearby (the 11 and 12 minute peaks, for instance).



Applications

Gas chromatography has a very wide field of application but its first and main area of use is in the separation and analysis of multi component mixtures such as essential oils, hydrocarbons and solvents, with use of the flame ionization detector and electron capture detector (which have very high sensitivities) gas chromatography can quantitatively determine materials present at very low concentrations. It follows, that the second most important application area is in pollution studies, forensic work and general trace analysis.

- **Non-volatile samples analysis**

Since non volatiles can be analyzed by LC, those methods are often preferred. But, there are a few alternatives that permit some of them, such as sugars and amino acids, to be run by GC. Other possibilities are pyrolysis and inverse GC. Pyrolysis GC is used mainly for identifying polymers from the pattern of peaks that is obtained. Instead of injecting a sample, polymer is put in a furnace capable of being heated very rapidly to a sufficiently high temperature to cause its thermal decomposition. The decomposition products are chromatographed by PTGC and are reproducible enough to provide peak patterns that can be compared with those from known polymers to provide qualitative identifications.

Inverse GC is used to produce data that are opposite or inverse of normal GC methods. Since its objective is to get information about large nonvolatile molecules that cannot be run by normal GC, sample, composed of large molecules (often polymers or fibers) is used as the stationary phase and is then subjected to investigation with small molecules that serve as probes. In effect, the roles of solute and solvent are reversed.

- **Inorganic GC**

Most inorganic compounds are not volatile enough for analysis by GC, with the exception of some fixed gases such as CO_2 and SO_2 Consequently, inorganic GC is usually treated as a separate topic that is concerned with the formation of volatile derivatives and the use of detectors having elemental specificities.

- **Simulated Distillation**

When GC replaced distillation as a method of analyzing petroleum products, the GC equivalent of distillation was formulated by groups such as ASTM. Appropriately enough, the methods are called simulated distillation.

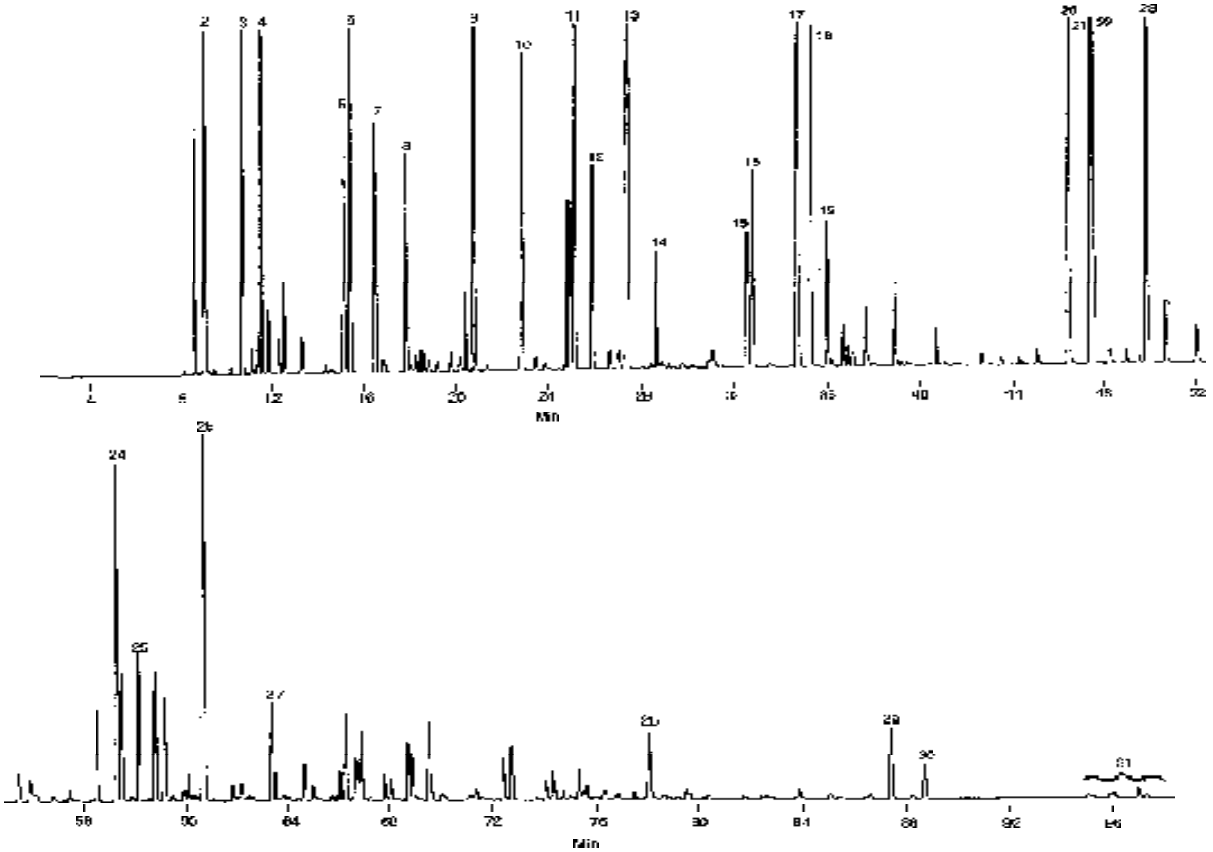
- **Gasoline**

Gasoline is a multi component mixture containing a large number of hydrocarbons, many of which have very similar molecular weights and all are almost exclusively dispersive in interactive character. The structure of many of the hydrocarbons are also very similar and there are many isomers present, due to their interactive similarity the separation factors between individual components is very small. It follows that columns of very high efficiency will be mandatory to achieve an effective separation. It is clear that open tubular columns are ideal for this type of separation problem. In fact, it would be impossible to separate the components of gasoline efficiently with a packed column, even one that is 50 ft long, and even if the inherent long analysis times could be tolerated.

In addition this type of separation demands the maximum number of theoretical plates and therefore not only must open tubes be used but tubes of relatively small diameter to produce the maximum number of theoretical plates. In fact, several hundred thousand theoretical plates will be necessary and so the column must also be very long.

As has already been discussed, it is necessary to use small radius open tubular columns with a split injection system. Furthermore, as a result of the wide range of molecular weight of the components present, gasoline has a relatively wide boiling range and so will require a temperature program that will heat the column to 200 ˚C or more.

A thermally stable stationary phase must be employed. The individual gasoline components are present over a wide concentration range and thus, for accurate quantitative results, the linear dynamic range of the detector must also be large. These latter demands mandate that the detector must be the FID.

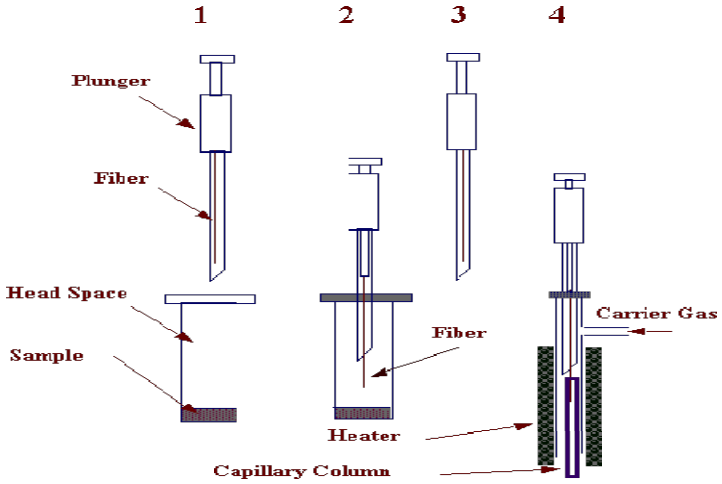


1/ Isobutane
2/ n-Butane
3/ Isopentane
4/ n-Pentane
5/ 2,3-Dimethylbutane
6/ 2-Methylpentane
7/ 3-Methylpentane
8/ n-Hexane
9/ 2,4-Dimethylpentane
10/ Benzene
11/ 2-Methylhexane
12/ 3-Methylhexane
13/2,2,4Trimethylpentane
14/ n-Heptane
15/ 2,5-Dimethylhexane
16/ 2,4-Dimethylhexane
17/2,3,4Trimethylpentane
18/ 2,3-Dimethylhexane
19/ 2,3-Dimethylhexane
20/ethylbenzene
21/ m-Xylene
22/ p-Xylene
23/ o-Xylene
24/ -Me-3-Ethylbenzene
25/ 1,3,5TriMe-benzene
26/ 1,2,4TriMe-benzene
27/ 1,2,3TriMe-benzene
28/ Naphthalene
29/ 2-Methylnaphthalene
30/ 1-Methylnaphthalene
31/ Dimethylnaphthalene

A Chromatogram of Gasoline

- **Tobacco**

Tobacco is a herbaceous plant, the leaves of which are harvested, cured and suitably prepared for smoking, as cigars or cigarettes, or alternatively, chewing or taken as snuff. Its main component, nicotine is habit forming and other compounds produced by pyrolysis during smoking are carcinogenic and can cause a number of other health problems. Tobacco can be flue cured, air cured, fire cured or sun cured, but the quality of the product can often be monitored by analyzing the vapors in the headspace above the tobacco. The headspace over tobacco can be sampled and analyzed using a Solid Phase Micro Extraction (SPME) technique. The apparatus used for SPME is shown in figure.

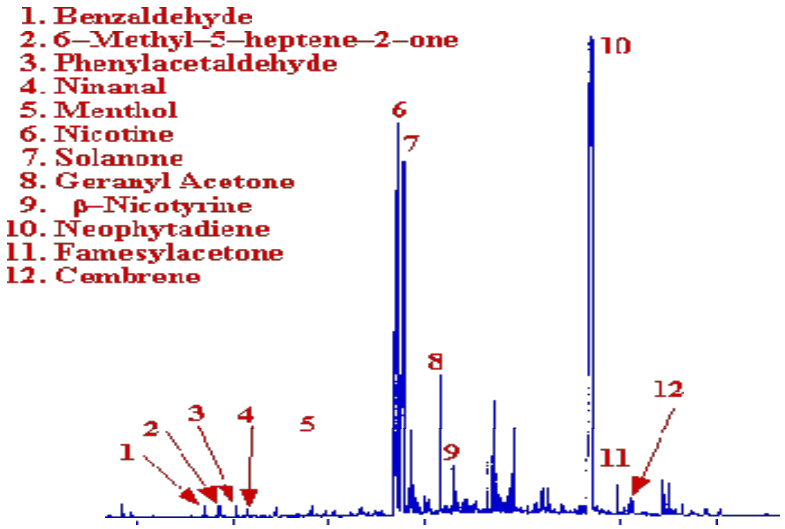


The extraction apparatus consists of a length of fused silica fiber, coated with a suitable polymeric adsorbent, which is attached to the steel plunger contained in a protective holder. The steps that are taken to sample a vapor using the apparatus are represented in figure.

The sample is placed in a small headspace vial and allowed to come to equilibrium with the air (1). The needle of the syringe containing the fiber is the made to piece the cap, and the plunger pressed to expose the fiber to the headspace vapor. The fiber is left in contact with air above the sample for periods that can range from 3 to 60 minutes, depending on the nature of the sample (2). The fiber is then removed from the vials (3) and then passed through the septum of the injection system of the gas chromatograph into the region surrounded by a heater (4). The plunger is again depressed and the fiber, now protruding into

the heater is rapidly heated to desorb sample onto the GC column. It is advisable to arrange for the column is kept cool so the components concentrate on the front of the column.

The procedure is as follows. 1 g of tobacco (12% moisture) was placed in a 20 ml headspace vial and 3.0 ml of 3M potassium chloride solution added. The fiber was coated with polydimethyl siloxane (a highly dispersive adsorbent) as a 100 mm film. The vial was heated to 95˚C and the fiber was left in contact with the headspace for 30 min. The sample was then desorbed from the fiber for one minute at 250˚C.



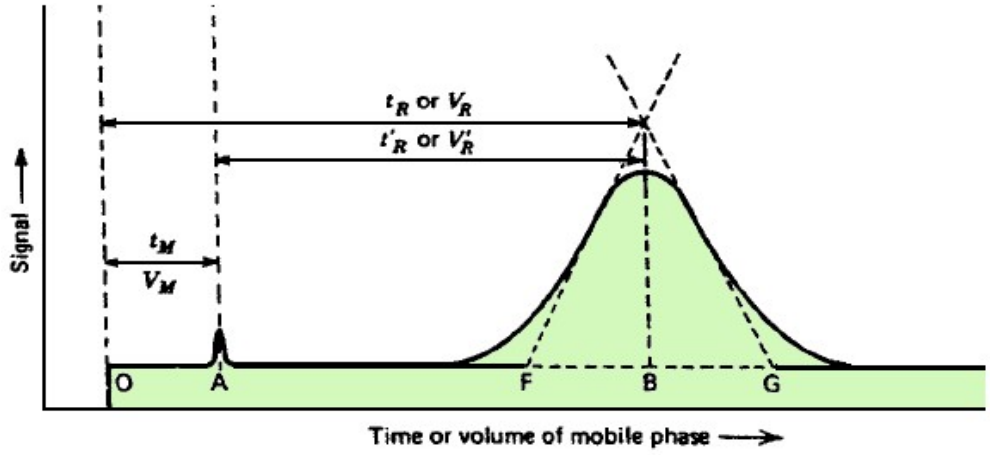
A Chromatogram of Tobacco Headspace

The separation was carried out on a column 30 m long, 250 mm I.D. carrying a 0.25 mm thick film of 5% phenyl-methyl-siloxane. Stationary phase was predominantly dispersive with a slight capability of polar interactions with strong polarizing solute groups by the polarized aromatic nuclei of the phenyl groups.

Helium was used as carrier gas at 30 cm/sec. The column was held isothermally at 40˚C for one minute and programmed to 250˚C at 6˚C/min. and then held at 250˚C for 2 min. It is seen that a clean separation of components of tobacco headspace is obtained and resolution is quite adequate to compare tobaccos from different sources, tobaccos with different histories and tobaccos of different quality.

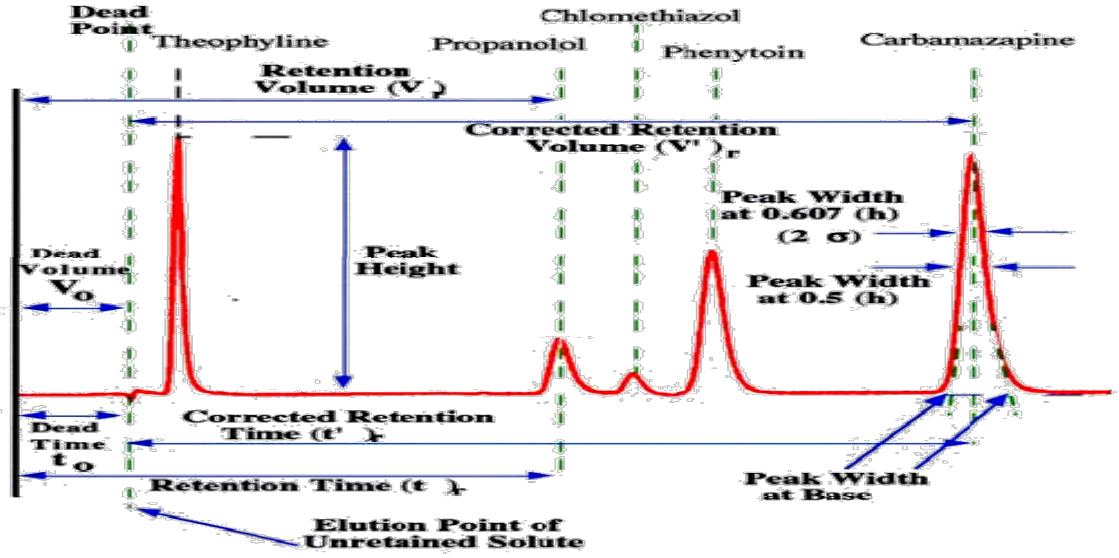
Chromatogram & symbols of Chromatography

In 1993, the IUPAC in cooperation with many other agencies published a codified list of chromatographic terms, symbols, and definitions. Since the IUPAC list has the widest use and greatest acceptance, these terms and symbols will be used.



Typical chromatogram.

Unfortunately, not all international groups have adopted the IUPAC list, thus causing some confusion to remain among pharmaceutical chromatographers.



Baseline: any part of chromatogram where only mobile phase is emerging from column.

Peak maximum is the highest point of the peak.

Injection point is that point in time/position when/where sample is placed on column.

Dead point is the position of the peak-maximum of an unretained solute.

Dead time (t_o) is the time elapsed between the injection point and the dead point.

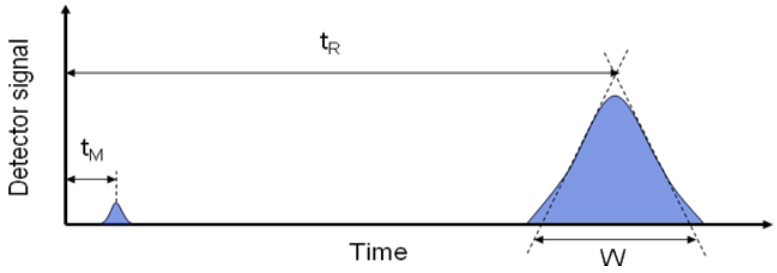
Dead volume (V_o) is the volume of mobile phase passed through the column between the injection point and the dead point. Thus, $V_o = Qt_o$ where (Q) is the flow rate in ml/min.

Retention time (t_r) is the time elapsed between the injection point and the peak maximum. Each solute has a characteristic retention time.

Retention volume (V_r) is the volume of mobile phase passed through the column between the injection point and the peak maximum.

Thus, $V_r = Qt_r$ where Q is the flow rate in ml/min.

Each solute will also have a characteristic retention volume.



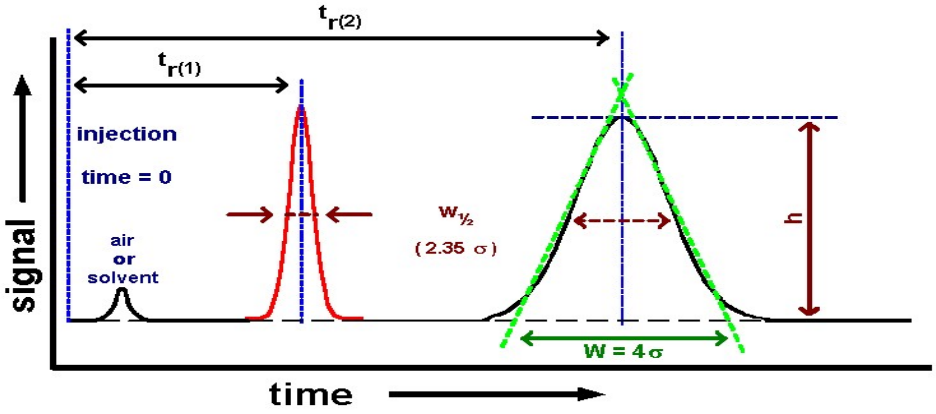
Corrected retention time (t'r) is time elapsed between dead point and peak maximum. The corrected retention volume (V'r) is the volume of mobile phase passed through the column between the dead point and the peak maximum. It will also be retention volume minus the dead volume. Thus, $V'_r = V_r - Vo = Q(t_r - t_o)$ where (Q) is the flow rate in ml/min.

Peak height (h) is the distance between peak maximum and the base line geometrically produced beneath the peak.

Peak width (w) is the distance between each side of a peak measured at 0.6065 of the peak height (*ca* 0.607h). The peak width measured at this height is equivalent to two standard deviations (2σ) of the Gaussian curve and, thus, has significance when dealing with chromatography theory.

Peak width at half height ($w_{0.5}$) is the distance between each side of a peak measured at half the peak height. The peak width measured at half height has no significance with respect to chromatography theory.

Peak width at the base (w_B) is the distance between the intersections of the tangents drawn to the sides of the peak and the peak base geometrically produced. The peak width at the base is equivalent to four standard deviations (4) of the Gaussian curve and thus also has significance when dealing with chromatography theory.



The common way of presenting chromatographic data is called a chromatogram. Two sample components or peaks are shown in Figure, and it is clear that they have been separated, one sample component plus a small peak representing a non retained component (which may not always appear). This figure will be used to illustrate some of the chromatographic definitions and symbols. Most chromatographs are operated with a constant flow (F) of mobile phase unless the flow is being changed or programmed, the x axis of the chromatogram can be labeled as time (t) or as volume (V) since:

$$V = t \times F$$

Thus, V represents the volume of mobile phase that flowed during a specified operating time t. If we wish to designate time required for a component to elute from chromatographic system, retention time, the symbols t and V are designated with a subscript R:

$$V_R = t_R \times F$$

In making an actual measurement of retention time or retention volume from a chromatogram produced by a recording device, one can measure the distance on the chromatogram from the start to the maximum of the peak of interest. Thus, V_R and t_R can be represented as the distance from 0 to B as indicated in Figure.

Distance on a chromatogram can be converted to time by multiplying it by the recording speed and then to volume by multiplying the time by the flow rate. When constant flow is assumed, retention time and volume can be used interchangeably.

Chromatographic Terms and Symbols

Symbol and Name Recommended by the IUPAC		Other Symbols and Names in Use	
K_c	Distribution constant	K_p	Partition coefficient; distribution coefficient
k	Retention factor	k'	Capacity factor; capacity ratio; partition ratio
N	Plate number	n	Theoretical plate number; No. of theoretical plates
H	Plate height	HETP	Height equivalent to one Theoretical plate
R	Retardation factor (in columns)	R_R	Retention ratio
R_s	Peak resolution	R	Theoretical plate
α	Separation factor		Selectivity; solvent efficiency
t_R	Total retention time	t	Elution time
V_R	Total retention volume	—	Elution volume
V_M	Hold-up volume	V_0	Volume of the mobile phase; void volume; dead volume

Part of the time an analyte spends in the chromatographic system is the time required to go through the interstitial space in the column (assuming other volumes in connections and the like are negligible), and part is caused by the time it spends in the stationary phase, not moving downstream. Thus, the total time or volume can be broken down into two parts:

$$V_R = V_M + K_c V_S$$

Where V_M represents the mobile-phase volume, V_S is the stationary-phase volume, and K_C is a partition coefficient called (by IUPAC) the distribution constant. Strictly speaking, this equation is only valid when V_S represents a stationary liquid phase, which, we have already noted, is not often the case in most columns in use today.

Also, in the past, V_M, has sometimes been called the dead volume in the system, the total volume of mobile phase (V_R) required to elute an analyte is composed of two parts: the interspecial volume in the column, which is occupied by mobile phase and through which every analyte must pass (V_M), and the mobile phase which flows while the analyte is held immobile (equal to $K_C V_S$).

The latter contribution (the length of time the analyte is immobile) is in turn determined by the amount (volume, Vs) of stationary phase and the tendency of the analyte to sorb in the stationary phase as measured by its distribution constant Kc. To recap, the equation shows that there are only two things a given analyte molecule can do: move down the column with the mobile phase or sorb in or on the stationary phase.

Before looking at the distribution constant more closely, note the small peak at A in Figure. It represents the time required for an analyte to pass through the system without being sorbed, and thus it measures the mobile phase volume V_M. The IUPAC has selected the name hold-up volume for V_M defined as “the volume of the mobile phase (MP) required to elute the unretained compound from the chromatographic column and reported at column temperature and ambient pressure.

The analogous time parameter is hold-up time, t,, "the time required for the MP to pass through the chromatographic column. Because the original terms were found to be misleading or superfluous, the IUPAC published these more precise, new definitions, and included some new terms.

In GC, air or methane is often used as the unretained component; in HPLC, there is no single simple marker, but a slight shift in the baseline is sometimes observed, depending on the solvent and the detector. The exact measurement of V_M especially in HPLC, can be difficult, but we will not digress to discuss that topic.

The retention volume (or time) that has the mobile-phase volume subtracted out is of interest for theoretical work:

$$V_R - V_M = V'_R = K_c V_S$$

It is called the adjusted retention volume (or time), and it is designated with a superscript prime. It is also shown in Figure. The distribution constant K , is defined as the equilibrium concentration of an analyte (A) in the stationary phase divided by its equilibrium concentration in the mobile phase:

$$K_c = \frac{[A]_S}{[A]_M}$$

It is this ratio that controls the rate of migration of A. That is, as analyte A proceeds through the system at a given temperature, it partitions between the two phases and is retained in the system in proportion to its affinity for the stationary phase.

Analyte A is shown moving faster down the column than analyte B because molecules of A spend less time in the stationary phase, as indicated by the smaller peak in that phase; that is, A has a smaller partition coefficient K, than B.

The use of a classical equilibrium constant, K_C. in chromatographic theory implies that the system can be assumed to operate at equilibrium, even though chromatography is clearly a dynamic system. Ideally, the operating parameters are such that the system is not far from

equilibrium and the use of an equilibrium constant (distribution constant) is valid. The only place true equilibrium exists is near the apex of the chromatographic peak. Appendix B includes a calculation of distribution constants from HPLC data and compares them with true equilibrium values from a similar extraction system. The closeness of the values further serves to substantiate the equilibrium assumption.

Furthermore, the equation assumes that analyte A is present in only one form (one molecular structure or ion). When this is not realized in practice, a more complex equilibrium constant must be used. Another assumption that is usually not stated is that the analytes do not interact with each other; that is, molecules of analyte pass through the chromatographic system as though no other analytes were present. This assumption is reasonable because of the low concentrations at which analytes are present and because they are increasingly separated from each other as they pass through the system.

In making use of the distribution constant in chromatography, it is useful to break it down into its two parts -*B*, the phase volume ratio, and *K*, the retention factor:

$$K_c = \beta k \qquad \beta = \frac{V_M}{V_S} \qquad k = \frac{(\text{mass of A})_S}{(\text{mass of A})_M}$$

The retention factor used to be called the capacity factor or partition ratio, and k' was also used as its symbol. Unfortunately, many authors continue to use the old terms and symbols. By combining Eqs. can be derived. It serves as another definition of *k*, one that can easily be measured:

$$k = \frac{V'_R}{V_M} = \frac{t'_R}{t_M}$$

Another chromatographic parameter is the retardation factor, R. It is the relative average speed u of an analyte through a chromatographic bed compared to the average mobile phase speed or velocity u:

$$R = \frac{v}{u}$$

It will always be equal to or less than 1, and it expresses the fractional rate at which an analyte is moving. It also represents the fraction of molecules of a given analyte in the mobile phase at any given time and, alternatively, the fraction of time an average analyte molecule spends in the mobile phase as it travels through the system.

For planar chromatography (such as TLC), the retardation factor has the same meaning, but it is calculated differently and its symbol is R, rather than R. Each of the velocities in Eq. can be defined and measured according to the length L of the chromatographic system:

$$v = \frac{L}{t_R} \qquad u = \frac{L}{t_M} \qquad R = \frac{t_M}{t_R} = \frac{V_M}{V_R} = \frac{V_M}{V_M + KV_S} = \frac{1}{1+k}$$

Equation which shows the relationship between the two important chromatographic parameters, k and R. Most chromatographers use k to express the extent of retention of an analyte on a given column, but R could also be used. As described above, R has more physical relevance to the behavior of an analyte, and it has the advantage of allowing comparisons between columns and planar forms of chromatography.

Next table contains some relative values of k and R. For example, a retention factor k of 3, which is common in chromatographic practice, corresponds to a retardation factor R of 0.25. Thus, we can say that this analyte spent 25% of its time in the mobile phase while it passed through the column and 75% in the stationary phase.

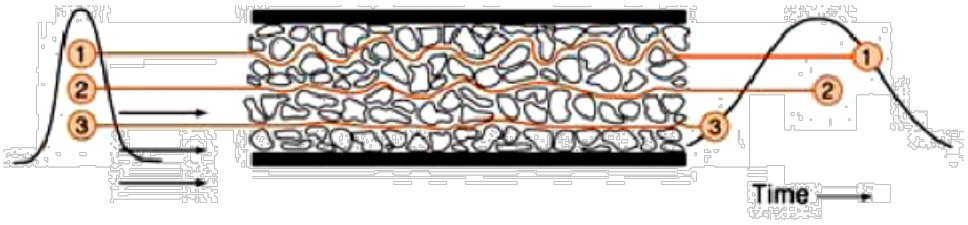
Relationships between *k* and *R*

k	R	$(k/k+1)^2$	$k/(1+k)^2$
1	0.5	0.25	0.25
2	0.33	0.44	0.22
3	0.25	0.56	0.19
4	0.20	0.64	0.16
9	0.10	0.81	0.09
49	0.02	0.96	0.0196

- **Normal Distribution**

The shapes of all the peaks in the chromatograms shown thus far are symmetrical, approximating the normal or Gaussian shape. This is the ideal shape, but it is not always achieved in practice.

Theoretically, the Gaussian shape is closely approached if the analyte has undergone a sufficiently large number of sorptions and desorptions, as is the case for most peaks with retention factors of about 1 or greater. In fact, an asymmetrical peak is usually evidence that some undesirable interaction is taking place in the system and that remedial action should be undertaken to find it and change it.

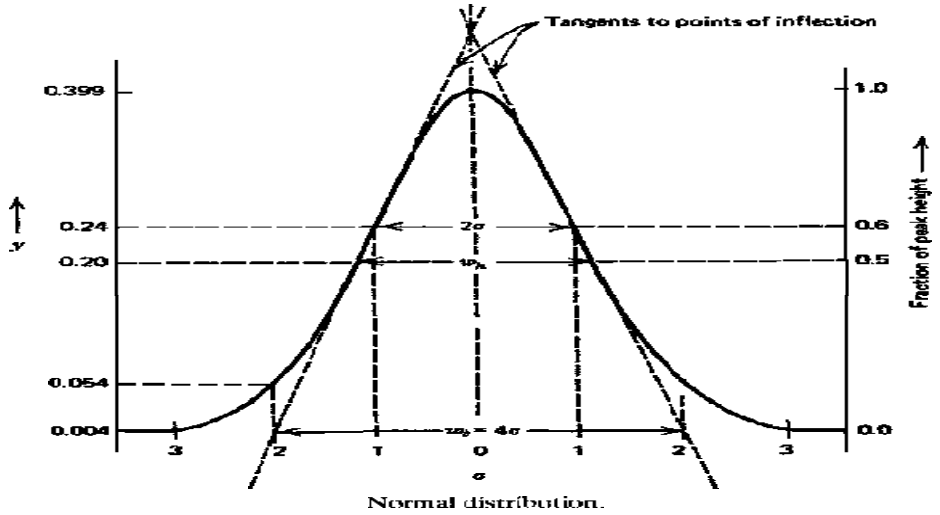


Remember that a peak represents the frequency distribution of all molecules of a particular analyte as they move as a group through the system or as they are detected as they exit from the system.

The "average" molecule is found at the center of the distribution, at the position of the peak maximum, and it is this average position that is used to characterize the particular analyte. The familiar equation for the normal distribution is:

$$y = \frac{1}{\sigma\sqrt{2\pi}} \exp\left[-\frac{1}{2}\left(\frac{x - \bar{x}}{\sigma} \right)^2 \right]$$

Where y is the dependent variable, x is the independent variable, x' is the average of a large number of x, and σ is the standard deviation. When this equation is used to represent a chromatographic zone or band, y represents concentration, x represents a retention parameter like retention volume, and a represents a peak width parameter.



Normal distribution.

In the analysis of peaks resulting from a separation, it will be most useful to express the variable x in units of standard deviation. Hence, for our purposes, Eq. can be written as:

$$y = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{\sigma^2}{2}\right) = 0.3989 \exp\left(-\frac{\sigma^2}{2}\right)$$

This equation is plotted in Figure. Also shown as broken lines are tangents to the points of inflection that occur at 60.7% of the peak height. Where they intersect the baseline, they cut off the distance w_b known as the peak width at the baseline. It can be seen in Figure, that w_b has a value of 4σ(±2σ). Consequently, σ, the standard deviation, is also called the *Quarter peak width* (at the base). Note also that the width at 60.7% of the peak height (the inflection point) is 2σ. At 50% of the peak height the peak width is 2.354σ. he latter is called the peak width at half height, w_h .

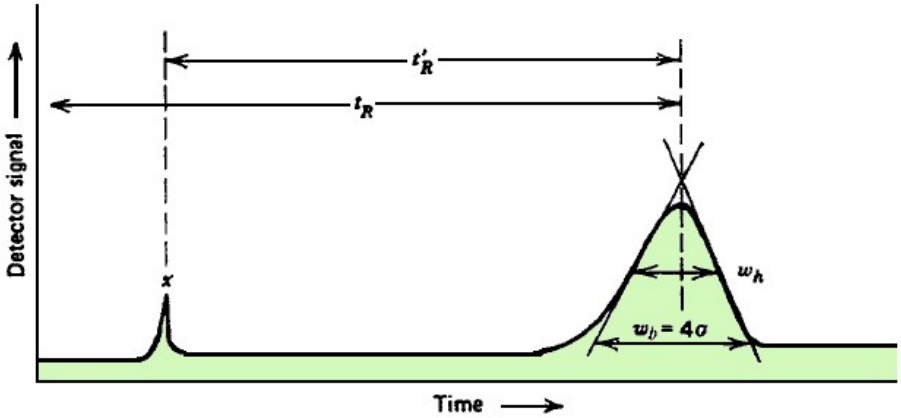
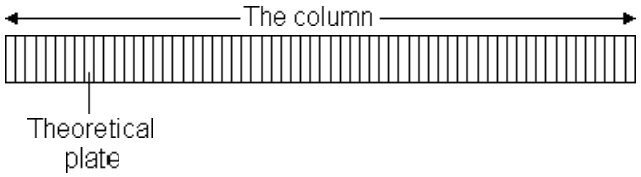


Figure used to define *N*, the plate number (*x* = nonretained component).

- **Other Terms**

Plate Number: The plate model supposes that the chromatographic column is contains a large number of separate layers, called theoretical plates. Separate equilibrations of the sample between the stationary and mobile phase occur in these "plates". The analyte moves down the column by transfer of equilibrated mobile phase from one plate to the next.



The most common measure of the efficiency of a chromatographic system is the plate number N. Because the concept originated from the analogy with distillation, it was originally called the number of theoretical plates contained in the chromatographic column (system). This is not a useful analogy because a chromatographic column does not contain "plates," but the terminology has remained, and the original definition has persisted:

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

The parameters t and σ must be measured in the same units, so the number N is dimensionless. For Gaussian peaks, we can express σ in terms of peak width since we know the relationships as just presented in the last section. For example, the base width w_b is equal to 4 σ, so N becomes

$$N = \left(\frac{4t_R}{w}\right)^2 = 16\left(\frac{t_R}{w}\right)^2$$

Similarly, W_h corresponds to 2.354 σ , and N equals

$$N = 5.54\left(\frac{t_R}{w_h}\right)^2$$

If the peak is not symmetrical, different values will be calculated for N because the width measurements will not follow the predicted Gaussian distribution. In general, for

asymmetrical peaks, N increases the higher up on the peak the width is measured. Most computers will make the computation at any of several positions on a peak, so one must be very cautious in assigning an N value to an asymmetric peak. Another method of calculating the plate number uses statistical moments. A summary of moment analysis is:

- Zeroth moment measures peak area.
- First moment measures peak mean and hence t_R
- Second moment measures peak variance (and σ) and hence peak width.
- Third moment measures peak asymmetry (skew).

Higher moments can also be calculated, but these four are the most important. From them, one can also calculate skew and excess. The only practical way to make these measurements is with a personal computer (PC) and appropriate data collection software. Further details are provided in several works.

The plate number is a measure of the relative peak broadening (w) that has occurred while the analyte passed through the system (in time t_R). As we will see, peaks broaden (w increases) as the retention time increases, which is the reason peak width alone is not sufficient to specify the efficiency of the system.

Furthermore, according to theory, N should increase slightly as k increases, but effect is usually not large; consequently, column efficiencies (N) are usually quoted for a given column, without regard to particular analyte and its k value, even though this practice is really not accurate. A related measure of system efficiency is effective plate number, N_{eff}.

$$N_{\text{eff}} = 16\left(\frac{t'_R}{w}\right)^2$$

In which the adjusted retention time is used instead of the retention time. We have already noted that the adjusted retention time has more theoretical significance than the retention time, and consequently the effective plate number is often a better parameter for use in comparing chromatographic columns, particularly comparing packed columns to open tubular columns.

By combining equations already presented, it can be shown that the effective plate number is related to N by

$$N_{eff} = N\left(\frac{k}{k+1}\right)^2$$

The effective plate number will increase as k increases (see Table 2.3 for some values), and it will approach N at high k values where V_M is no longer of significant size compared to V_R As already mentioned, the number of theoretical plates is a term also used in distillation, but it is important to note that comparisons of efficiencies of the two techniques cannot be made by comparing plate numbers. It takes more chromatographic plates to achieve a given separation by GC than it does to achieve the same separation by distillation.

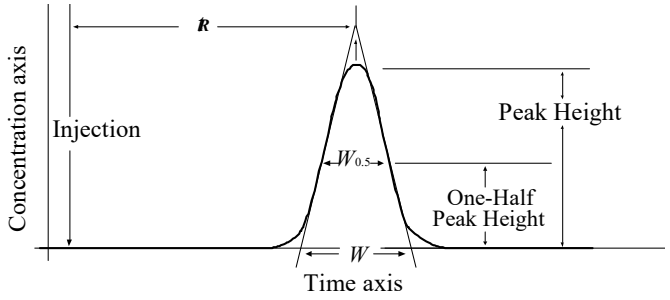


Plate Height: The plate number depends on the length of the column, making comparisons among columns difficult unless all are of the same length. Another related parameter that removes this dependence is the plate height H:

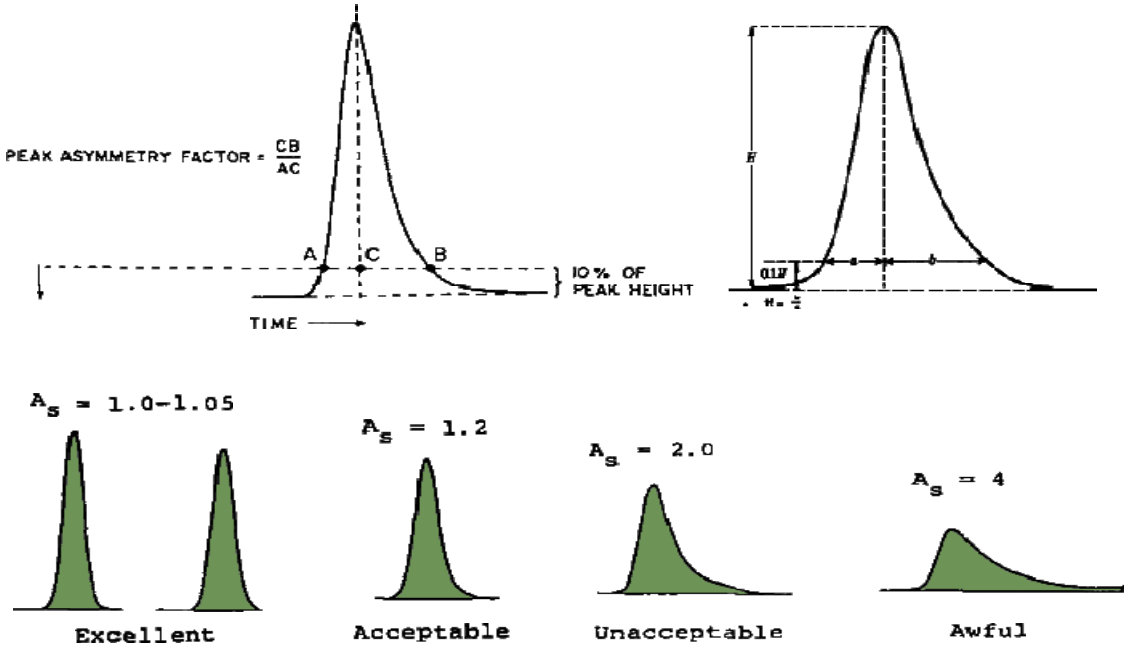
$$H = \frac{L}{N}$$

Where L is the total column length, so H can be thought of as the length of column that contains one plate. Clearly, N and H are inverse to each other, and H is a measure of efficiency that is independent of total length. It has the units of length, usually centimeters or millimeters, and, like N, it originated in distillation. In fact, it is sometimes referred to by the full name used in distillation-HETP, or height equivalent to a theoretical plate. But, again, it should be emphasized that the column does not contain plates, and the use of these two terms that resulted from their historical development should be discontinued.

Asymmetry & Tailing: Several measures of asymmetry devised by chromatographers for asymmetric peaks. One is called asymmetric ratio (tailing factor (TF));

$$TF = \frac{b}{a}$$

Where a and b are measured at 10% of the height of the peak as shown. A symmetrical peak will have a value of 1 and tailed peaks a value of greater than 1. A fronted peak (one with a leading edge and a > b) will have a tailing factor of less than 1. Since it is not common practice to calculate a fronting factor, it is recommended that TF be used to cover both situations and that the limits on TF include both upper and lower values.



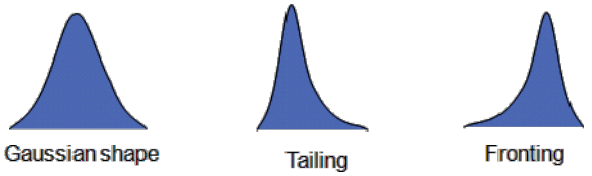
Since this definition is not included in the IUPAC list of terms and symbols, some confusion has arisen because another, different definition is also in common use. It is the one recommended by USP:

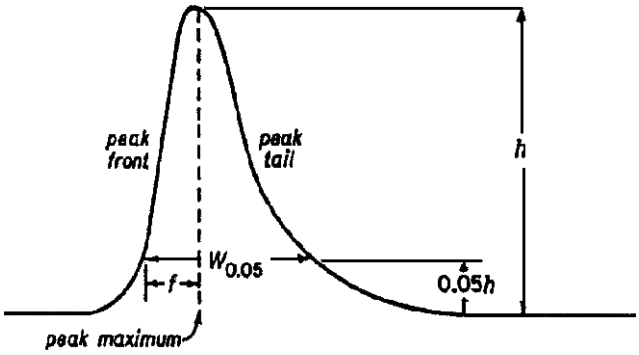
$$T = w_{0.05}/2f$$

Where $w_{0.05}$ is the width of the peak at 5% of the height and 2f is twice the front part of the peak as shown in Figure. The measurements of peak widths or partial peak widths for Eqs. are made to the actual peak lines, not to tangents drawn to the points of inflection as was done for the definition of N in Eq.

Note that the two definitions differ and the position of measurement on the peak differs. The two values always differ (the 10% value being greater). Most computer software used with chromatographic systems is capable of making both of these calculations.

Therefore, one must be careful to designate which one is to be used in a given laboratory. Failure to do so could result in the wrong definition being used for the calculation, and an erroneous value being used for a system suitability test resulting in an incorrect conclusion about the test.





• $T = \frac{W_{0.05}}{2f}$ Figure defining USP tailing factor.

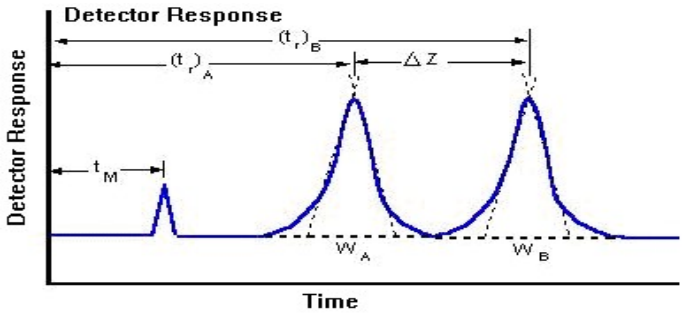
Several authors have expressed the opinion that the measurement at 10% of the peak height is better that the measurement at 5%, but no agreement has yet been arrived at. An IUPAC task force has the issue under consideration, and a recent study has proposed a new method for determining peak asymmetry. Calculation of peak number, N, for asymmetric peaks is also a concern. Foley and Dorsey discussed the various figures of merit for ideal and skewed peaks and proposed a new empirical equation:

$$N_{SYS} = \frac{41.7(t_R/w_{0.01})^2}{TF + 1.25}$$

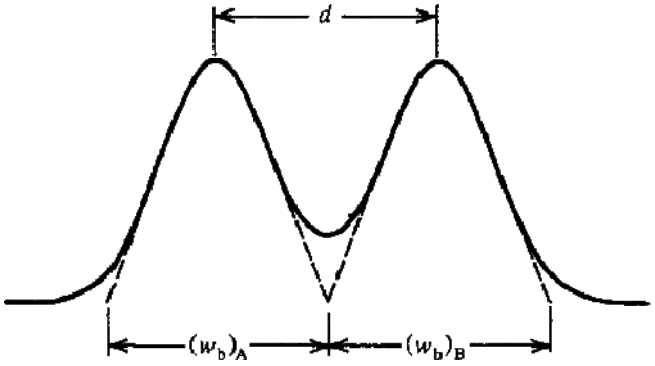
Where TF is the tailing factor at 10% of the peak height, and $W_{0.01}$ is the peak width at 10% of the peak height and equal to b + a. Unfortunately, suppliers of LC columns (in 1984) did not use these superior methods in calculating the plate numbers of columns they sold.

Peak Resolution: (R_s) between two peaks in a chromatogram is given by:

$$R_S = \frac{2\,\Delta Z}{W_A + W_B} = \frac{2[(t_R)_A - (t_R)_B]}{W_A + W_B}$$



Where delta Z is the separation between peaks A and B; and W_a and W_b are the widths at the base of peaks A and B, respectively.



Two nearly resolved peaks illustrating the definition of resolution.

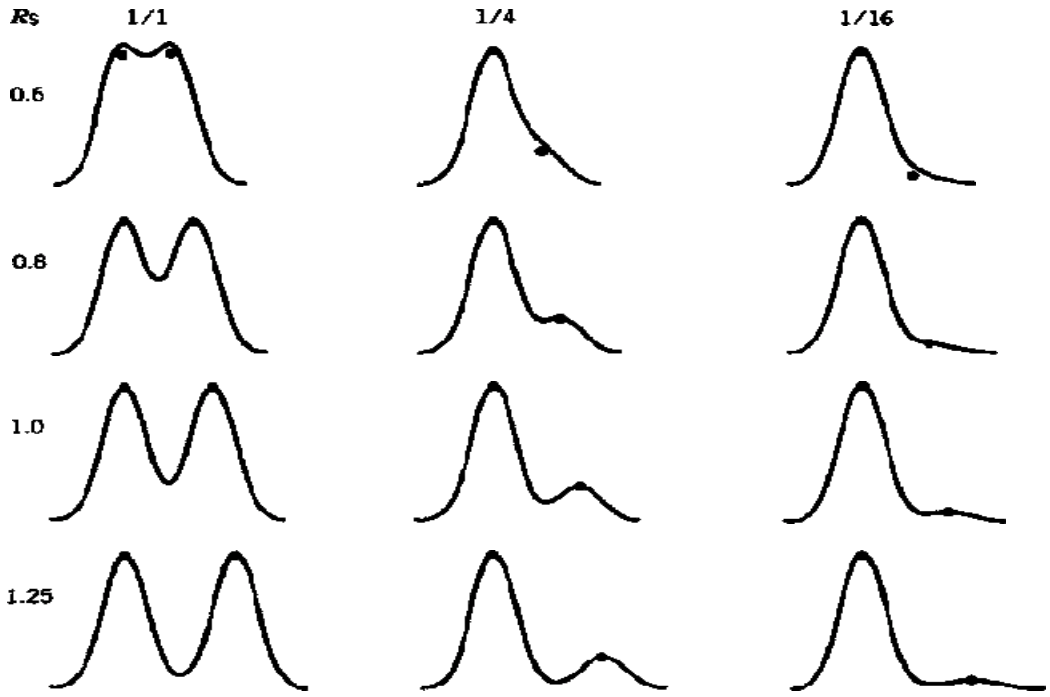
Resolution A better measure of the efficiency of a chromatographic system is resolution, R_S, defines the degree of separation of two analytes or peaks:

$$R_s = \frac{d}{[(w_b)_B + (w_b)_A]/2} = \frac{2d}{[(w_b)_B + (w_b)_A]}$$

Where d is the distance between the peak maxima and W_b is the width of each peak at the base. The larger the resolution, the better the separation; a value of 1.0 is shown in the figure representing about 98% resolution. If one chooses to measure the peak width at half height, W_h Eq. becomes:

$$R_s = \frac{2d}{1.70[(w_h)_B + (w_h)_A]}$$

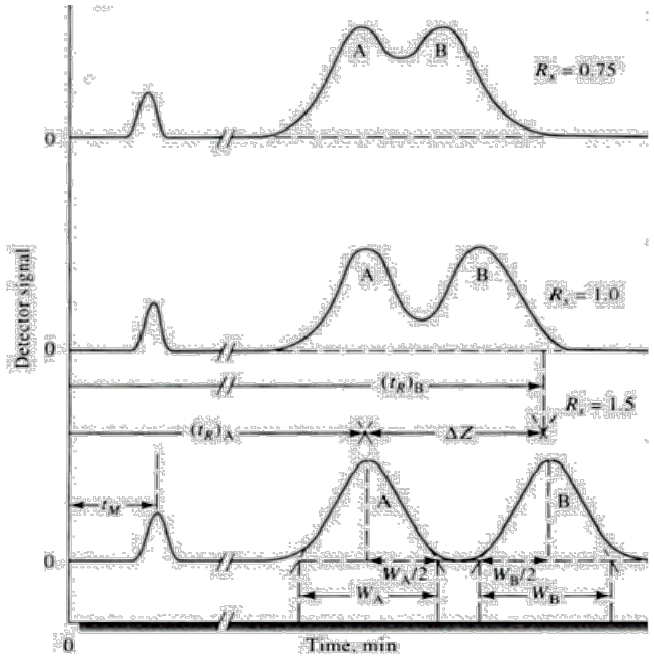
A resolution value of 1.0 can be easily estimated if it is assumed that the widths of the two peaks were equal as expected according to theory. If $W_A = W_B$ $R_S = 2d/2w = d/w$. Since the tangents to the peaks (dotted lines) are just touching, and since $w = 4\sigma$ for each peak, d must also equal 4σ (2σ from A plus 2σ from B) and $R_S = 4\sigma/4\sigma = 1$. Thus, for estimating resolution, Eq. is often satisfactory:

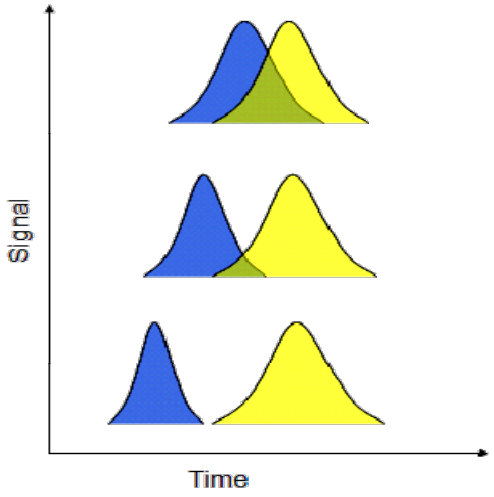


Comparison of resolution values for peaks of equal and nonequal heights.

A practical approach for calculating the resolution of non equal peaks has been provided by Snyder. His work includes computer-drawn pairs of peaks for a variety of resolutions and peak height ratios from 1:1 to 128:1. Snyder's recommendation is to compare a given separation with those he has provided and to choose the best match, assigning that value of resolution to the chromatogram in question.

$$R_s = \frac{d}{(w_h)_B}$$





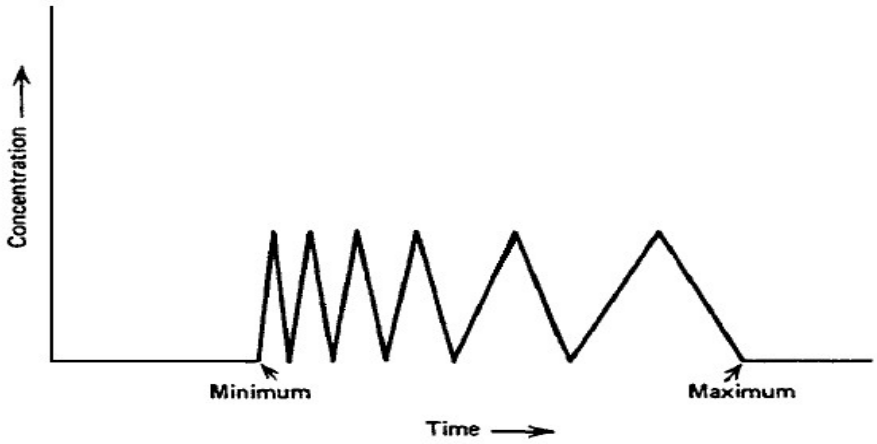
This practice has become accepted by many, although others choose to ignore the effect on resolution by analytes of unequal concentration. Other possibilities have been suggested by Carle, Kargerd (for peaks with different widths), and Bly (for size exclusion chromatograms). Resolution is also affected by peak tailing (asymmetry), and, alternatively, a poorly resolved pair of peaks of unequal height can appear as one tailed peak. Dolan has addressed these problems in his series on LC troubleshooting.

Calculated Peak Capacities for Different Chromatographic Techniques

	Peak Capacity		
Plate No.	GC	LC	SEC
100	11	7	3
400	21	13	5
1000	33	20	7
2500	51	31	11
10000	101	61	21

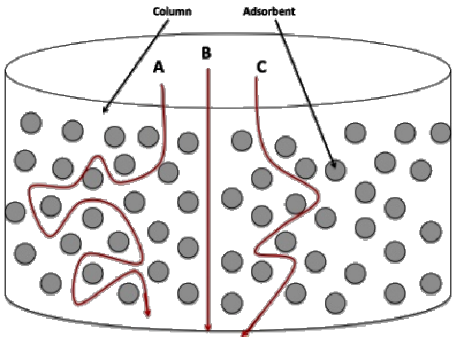
Peak Capacity: A final measure of column efficiency is the peak capacity, or the number of peaks that can be resolved ($R_S = 1$) by a given system in a given time (t_R or V_R). Giddings introduced it in 1967 and used it to compare the potential separating capabilities of the various chromatographic modes.

Figure show a series of analytes represented by triangles and a peak capacity of six, and Table lists the values he obtained for three chromatographic systems-GC, LC, and SEC (size exclusion chromatography.



A hypothetical separation illustrating the concept of peak capacity.

A more recent work reports a comparative study of equivalent capillary columns in the three techniques, GC, LC, and capillary electro chromatography (CEC)." This work contains a good summary of the developments in measuring peak capacity following Giddings' original work.



The conclusions from an empirical study of 10 alkyl benzenes on several capillary columns of about 36,000 plates are summarized in Table. The GC columns were open tubular [L = 10 and 13 m; inner diameter (i.d.) = 200 µm] and the HPLC and CEC columns were packed with 1.5- and 3.0-pm particles.

Comparison of Peak Capacities of GC Capillary Columns and HPLC and CEC Packed Columns

Technique	Peak Capacity	Technique	Peak Capacity	N
—		GC	248	39,000
Columns packed with 1.5-μm particles		Columns packed with 3.0-μm particles		
CEC	139	CEC	137	58,000
HPLC	148	HPLC	102	29,000

As expected, the GC column had the highest peak capacity, and CEC was slightly higher than HPLC using the 3-pm particles; with the smaller particles, the peak capacities were about the same.

For more conclusions regarding ways to increase peak capacity, see the original study. A similar concept was suggested by Kaiser," who calls his parameter a separation number (SN), or Trennzahl (TZ) in German. It is the number of analytes that can be resolved between two consecutive members of the paraffin homologous series x and x + 1. It can be calculated by Eq:

$$TZ = \frac{t_{R(x+1)} - t_{R(x)}}{w_{h(x+1)} + w_{h(x)}} - 1$$

This term is very similar to resolution, which is equal to $\Delta\, t_R/1.7w_h$ when defined with similar symbols. We get:

$$TZ = 0.85R_s - 1$$

Which can be rearranged to:

$$R_s = 1.177TZ + 1.177$$

Thus, baseline resolution of 1.5 is equivalent to a TZ value of 0.275, and a TZ value of 1.0 requires an R_S of 2.35. The TZ numbers can be used for programmed temperature operation in GC and gradient elution in LC, conditions under which plate numbers are not considered to be valid.

Regardless of the operating conditions, a TZ number of 12, for example, means that 12 analytes eluting adjacent to each other (R_S = 1) can be resolved between two consecutive paraffins in that region of the chromatogram. Figure taken from Freeman, compares N, N_{eff} further discussion of peak capacity, see the study by Ettre.

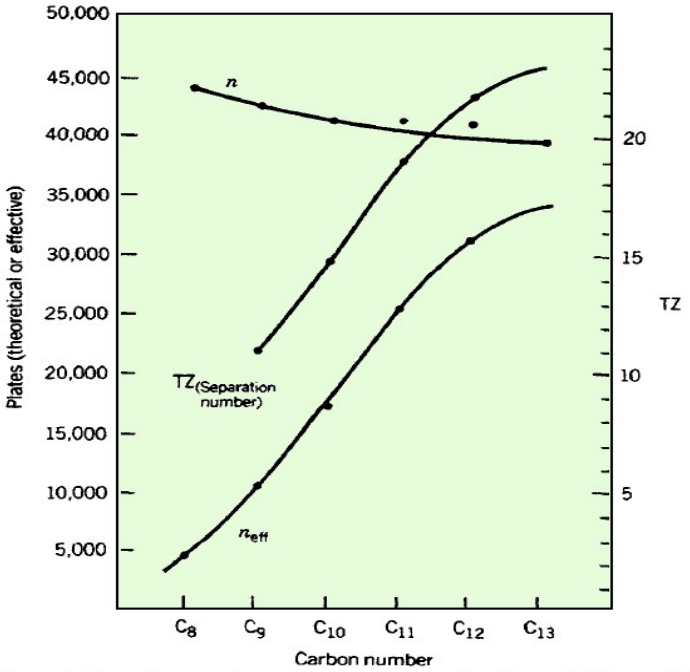
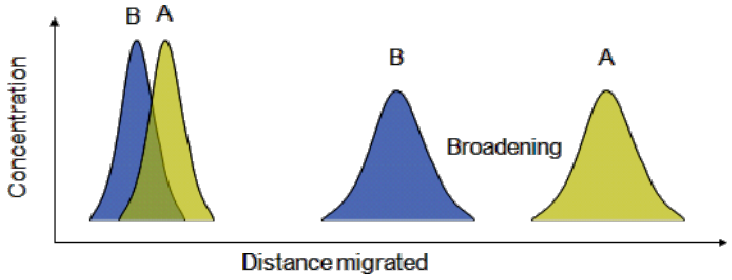


Plate number and separation number vs. carbon number for 10-m × 0.25-mm OT column at 100°C.

Separation Factor: Separations can be effected by differences in partition coefficients as well as by the efficiencies of the columns in which they are run. Thus, another useful thermodynamic measure of the separability of two analytes is the ratio of their distribution constants on a given column. This ratio is called the separation factor, α:



$$\alpha = \frac{K_B}{K_A} = \frac{(V'_R)_B}{(V'_R)_A}$$

It is defined so that its numerical value is > 1.0, which would mean that analyte B is retained longer on the system than analyte A.

Summary of Important Chromatographic Equations and Definitions

1. $(K_c)_A = \frac{[A]_S}{[A]_M}$

2. $K_c = k\beta$

3. $\beta = \frac{V_M}{V_S}$

4. $\alpha = \frac{K_B}{K_A} = \frac{(V'_R)_B}{(V'_R)_A}$

5. $V_R = V_M + K_c V_S$

6. $V_N = K_c V_S$

7. $k = \frac{(W_A)_S}{(W_A)_M} = \frac{V'_R}{V_M} = \left(\frac{V_R}{V_M}\right) - 1 = \frac{1-R}{R} = \left(\frac{1}{R}\right) - 1$

8. $R = \frac{V_M}{V_R} = \frac{\bar{v}}{\bar{u}} = \frac{V_M}{V_M + KV_S} = \frac{1}{1+k}$

9. $R_F = \frac{S_{analyte}}{S_{solvent\ front}}$

10. $V_R = V_M(1+k) = \frac{L}{\bar{u}}(1+k) = N(1+k)\frac{H}{\bar{u}}$

11. $(1-R) = \frac{k}{k+1}$

12. $R(1-R) = \frac{k}{(k+1)^2}$

13. $N = 16\left(\frac{t_R}{w}\right)^2 = \left(\frac{t_R}{\sigma}\right)^2 = 5.54\left(\frac{t_R}{w_h}\right)^2$

14. $H = \frac{L}{N}$

15. $N_{eff} = \left(\frac{k}{k+1}\right)^2 N$

16. $h = \frac{H}{d_p}$

17. $\nu = \upsilon\frac{d_p}{D_M}$

18. $R_S = \frac{2d}{w_A + w_B}$

19. $TZ = \frac{t_{R(x+1)} - t_{R(x)}}{w_{h(x+1)} + w_{h(x)}} - 1$

Method Development & Validation

- **Calibration**

Calibration is operations that establish, under specified conditions, the relationship between values of quantities indicated by a measuring instrument or measuring system, or values represented by a material measure, and corresponding values realized by standards The usual way to perform calibration is to subject known amounts of the quantity (e.g. using a measurement standard or reference material) to the measurement process and monitor the measurement response, overall programmed for calibration in chemical laboratory shall be designed to ensure that all measurements that have a significant effect on test or calibration results traceable to measurement standard, preferably national or international measurement standard such as a reference material.

Where appropriate and where feasible, certified reference materials should be used. Where formally designated measurement standards are not available, material with suitable properties and stability should be selected or prepared by the laboratory and used as laboratory measurement standard, required properties of this material characterized by repeat testing, preferably by more than one laboratory and using a variety of validated methods.

Analytical tests may be sub-divided into general classes depending on the type of calibration required: Some analytical tests depend critically on the measurement of physical properties, such as weight measurement in gravimetry and volume measurement in titrimetry. Since these measurements have a significant effect on the results of the test, a suitable calibration programmed for these quantities is essential. In addition, calibration of measuring devices used to establish the purity or amount concentration of the chemical standards, need to be considered. Where a test is used to measure an empirical property of sample, such as flashpoint, equipment is often defined in a national or international standard method and traceable reference materials should be used for calibration purposes where available. New or newly acquired equipment must be checked by laboratory before use to ensure conformity with specified design, performance and dimension requirements.

Instruments such as chromatographs and spectrometers, which require calibration as part of their normal operation, should be calibrated using reference materials of known composition (probably solutions of pure chemicals). In some cases, calibration of the whole analytical process can be carried out by comparing the measurement output from a sample with the output produced by a suitable reference material that has been subjected to the same full analytical process as the sample.

For example calibration of a gas chromatography method may be carried out using a series of measurement standards which are synthetic solutions of the analyte of interest at various concentrations. Such calibration does not take into account factors such as contamination or losses that occur during the sample preparation and extraction.

It is therefore essential during the method validation process to explore the potential problems of contamination and losses by taking matrix reference materials or spiked samples through the whole measurement process, and design the day to- day calibration procedure and quality control checks accordingly. Individual calibration programmes shall be established depending on the specific requirements of the analysis.

Also, it may be necessary to check instrument calibration after any shutdown, whether deliberate or otherwise, and following service or other substantial maintenance. The level and frequency of calibration should be based on previous experience and should be at least that recommended by the manufacturer.

The calibration of volumetric glassware normally relates to a particular solvent at a particular temperature. The calibration is rarely valid when the glassware is used with other solvents because of different densities, wetting characteristics, surface tension etc. This is particularly pertinent for volumetric glassware calibrated to deliver a certain volume.

Other volumetric equipment may be affected when using solvents with high rates of thermal expansion. In such situations the glassware should be recalibrated using the relevant solvent, at the correct temperature. Alternatively, for the highest accuracy, measurements can often be made by mass rather than by volume.

- **Reference Materials**

The reference material may be either a synthetic mixture prepared in the laboratory from materials of known (and preferably certified) purity, or a purchased certified matrix reference material. However, in such cases, a close match between the test sample and the matrix reference material, in terms of the nature of the matrix, and the concentration of the analyte has to be assured. However, in many cases, calibration is only performed on the final measurement stage.

Reference materials they are used for calibration, method validation, measurement verification, evaluating Measurement Uncertainty and for training purposes. Reference materials may take a variety of forms, including pure substance RMs, matrix RMs and solutions or mixtures. The following are all examples of reference materials:

- 95% pure sodium chloride;
- Aqueous solution 1% (w/v) copper (II) sulphate and 2% (w/v) magnesium chloride;
- Powdered polymer with a particular weight distribution range;
- Crystalline solid melting in the range 150-151°C;
- Dried milk powder containing a known amount of vitamin C

For many types of analysis, calibration may be carried out using reference materials prepared within the laboratory from chemicals of known purity and composition. Some chemicals may be purchased with a manufacturer's certificate stating purity.

Alternatively, chemicals of a stated but uncertified purity may be purchased from reputable suppliers. Whatever the source, it is the users' responsibility to establish that the quality of such materials is satisfactory.

Sometimes additional tests will need to be carried out by the laboratory. Normally a new batch of a chemical should be checked against the previous batch.

Ideally, all chemicals to be used for reference material purposes should be purchased from producers with demonstrated QA systems. However QA system does not automatically guarantee the quality of the producer's products and laboratories should take all reasonable steps to confirm the quality of critical materials.

The control of impurities is important, especially for trace analysis, where they may cause interferences. Due regard should be paid to the manufacturers recommendations on storage and shelf life. In addition, caution is needed, as suppliers do not always provide information about all impurities.

The use of appropriate reference materials can provide essential traceability and enable analysts to demonstrate the accuracy of results, calibrate equipment and methods, monitor laboratory performance and validate methods, and enable comparison of methods by use as transfer (measurement) standards. Their use is strongly encouraged wherever appropriate.

The uncertainty of purity of a pure substance reference material needs to be considered in relation to the uncertainty associated with other aspects of the method. Ideally, the uncertainty associated with a reference material, used for calibration purposes, should not contribute more than one third of the overall measurement uncertainty.

The composition of the certified reference material should be as close as possible to that of the samples. Where matrix interferences exist, ideally a method should be validated using a matched matrix reference material certified in a reliable manner. If such a material is not available it may be acceptable to use sample spiked with a reference material. Reference materials and measurement standards should be handled in order to safeguard against contamination or degradation. Staff training procedures should reflect these requirements.

- **Method of calibration**

Calibration is the process of establishing the relationship between the signal we measure (such as absorbance) and known concentrations of analyte. Once this relationship is established, it is possible to calculate the concentration of the analyte in an unknown sample by measuring its signal. The calibration methods discussed subsequently are applicable to most of the analytical instrumental methods, not just spectroscopic measurements.

In order to use Beer's Law to determine the concentration of analyte in an unknown, it is necessary to establish the relationship between absorbance at a given wavelength and the concentration of the analyte. Solutions containing known concentrations of analyte are called standard solutions or more simply, standards.

For some types of analyses, the standards may be in the form of solids or gases. Standards must be prepared accurately from high purity materials so that the concentration of analyte is known as accurately as possible.

A series of standards covering an appropriate concentration range is prepared. The standards should include one solution with no added analyte; the concentration of analyte in this standard is zero. This solution is called the reagent blank and accounts for absorbance due to impurities in the solvent and other reagents used to prepare the samples.

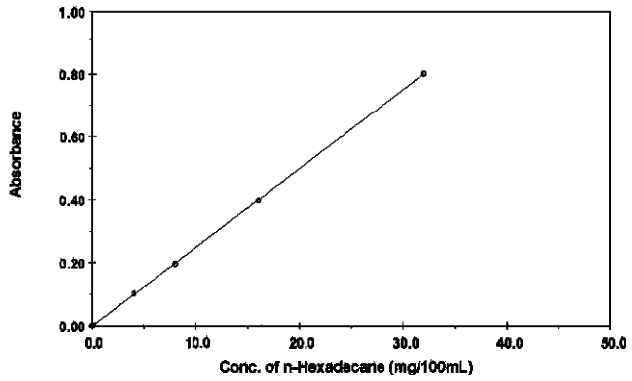
It also accounts for the instrumental baseline. The absorbance of the reagent blank and each standard is measured. The absorbance of the reagent blank is subtracted from the absorbances of the other standards before any calculations are performed.

The absorbances from which the blank absorbance has been subtracted are called "corrected absorbances". A plot is made of corrected absorbance on the y-axis vs. the known concentration of the standard on the x-axis. Such a plot used to be constructed manually on graph paper; now, plots are generated by computer software.

For example calibration curve which show the relationship between the absorbance of n-hexadecane, $CH_3(CH_2)_{14}CH_3$, a hydrocarbon found in petroleum, at 3.41 μm in the IR region and the concentration of solutions of n-hexadecane in tetrachloroethylene, C_2Cl_4. This measurement of the absorbance of solutions of n-hexadecane at 3.41 μm is a method used for determining petroleum contamination in water, soil, and other environmental samples, because most hydrocarbons absorb at this wavelength.

It is an official method developed by the US EPA and relies on Beer's Law to permit the measurement of petroleum hydrocarbons in unknown samples. It is used to measure environmental contamination from oil spills, illegal dumping of oil, and leaking underground oil storage tanks.

Concentration of *n*-hexadecane (mg/100 mL solution)	Absorbance at 3.41 μm	Corrected absorbance
0.0	0.002	0.000
4.0	0.103	0.101
8.0	0.199	0.197
16.0	0.400	0.398
32.0	0.804	0.802



Calibration Data for Measurement of Petroleum Hydrocarbons by IR Absorption Spectrometry

Performing a linear regression on the data in Table provides us with the exact Beer's Law relationship for this method: $A = 0.0250x - 0.001$, where x is the concentration of n-hexadecane (in mg/100 mL). From the equation for the calibration curve, the concentration can be determined for any measured absorbance.

- **Method of Standard Additions**

An alternate method of calibration is the Method of Standard Additions (MSA) calibration. This calibration method requires that known amounts of the analyte be added directly to the sample, which contains an unknown amount of analyte. The increase in signal due to the added analyte (e.g., absorbance, emission intensity) permits us to calculate the amount of analyte in the unknown.

For this method of calibration to work, there must be a linear relationship between the concentration of analyte and the signal. The MSA is often used if no suitable external calibration curve has been prepared. There may be no time to prepare calibration standards—for example, in an emergency situation in a hospital it may be necessary to measure sodium rapidly in a patient's serum.

It may not be possible to prepare a valid set of calibration standards because of the complexity of the sample matrix or due to lack of sufficient information about the sample. MSA calibration is very useful when certain types of interferences are present in the sample matrix. MSA permits us to obtain accurate results without removing the interferences by performing the calibration in the presence of the interferences. It is often used when only one sample must be analyzed, and the preparation of external standards would be inefficient.

- **Internal Standard Calibration**

An internal standard is a known amount of a non analyte element or compound that is added to all samples, blanks, and standard solutions. Calibration with internal standardization is a technique that uses the signal from the internal standard element or compound to correct for interferences in an analysis.

Calibration with internal standardization improves the accuracy and precision of an analysis by compensating for several sources of error.

For determination of analyte A, an internal standard S, which must not be present in the samples, is selected. The same concentration of S is added to all samples, standard solutions and blanks. The signals due to both A and S are measured.

The ratio of the signal due to the analyte A to the signal due to the internal standard S, is calculated. The signal ratio, signal A/signal S , is plotted against the concentration ratio of A/S in the standards. The equation of the calibration curve, which should be linear for best results, is obtained by linear regression.

The equation permits the calculation of the concentration ratio A/S in any unknown samples by measuring the signal of A and S in the sample and calculating the signal ratio for the sample. The relationship between concentration and signal may be expressed as follows:

$$\frac{\text{Concentration ratio (A/S) in sample}}{\text{Concentration ratio (A/S) in standard}} = \frac{\text{signal ratio (A/S) in sample}}{\text{signal ratio (A/S) in standard}}$$

Internal standardization is widely used in spectroscopy, chromatography, MS, and other instrumental methods. The use of internal standards can correct for losses of analyte during sample preparation, for mechanical or electrical “drift” in the instrument during analysis, for volume change due to evaporation and other types of interferences.

The internal standard must be chosen carefully, usually so that the chemical and physical behavior of the internal standard is similar to that of the analyte.

The internal standard must not interact chemically or physically with analyte. Whatever affects the signal from the analyte should affect the signal from the internal standard in the same way. The ratio of the two signals will stay constant, even if the absolute signals change; this provides more accuracy and precision than if no internal standard is used.