Analytical chemistry

Lecture №31

Gas chromatography

One of the most difficult and frustrating problems ever encountered in the domain of pharmaceutical analysis is that of the simultaneous separation, identification and above all the quantitation of more than one compound from a complex mixture in a pharmaceutical product.

A good of sophisticated chromatographic techniques of separation have been put forward since early fifties that may be categorized into the following *four groups, namely :*

(*a) Gas-Solid adsorption Chromatography (GSC),*

(*b) Gas-Liquid partition Chromatography, (GLC),*

(*c) Liquid-Solid adsorption Chromatography (LSC), and*

(*d) Liquid-Liquid partition Chromatography (LLC).*

The first two groups have been collectively termed as **'Gas Chromatography'.**

Gas chromatography - A chromatographic technique in which the mobile phase is a gas.

In **gas-liquid chromatography,** the mobile phase is a gas, and the stationary phase is a liquid that is retained on the surface of an inert solid by adsorption or chemical bonding. In **gas-solid chromatography,** the mobile phase is a gas, and the stationary phase is a solid that retains the analytes by physical adsorption. Gas-solid chromatography permits the separation and determination of low-molecular-mass gases, such as air components, hydrogen sulfide, carbon monoxide, and nitrogen oxides.

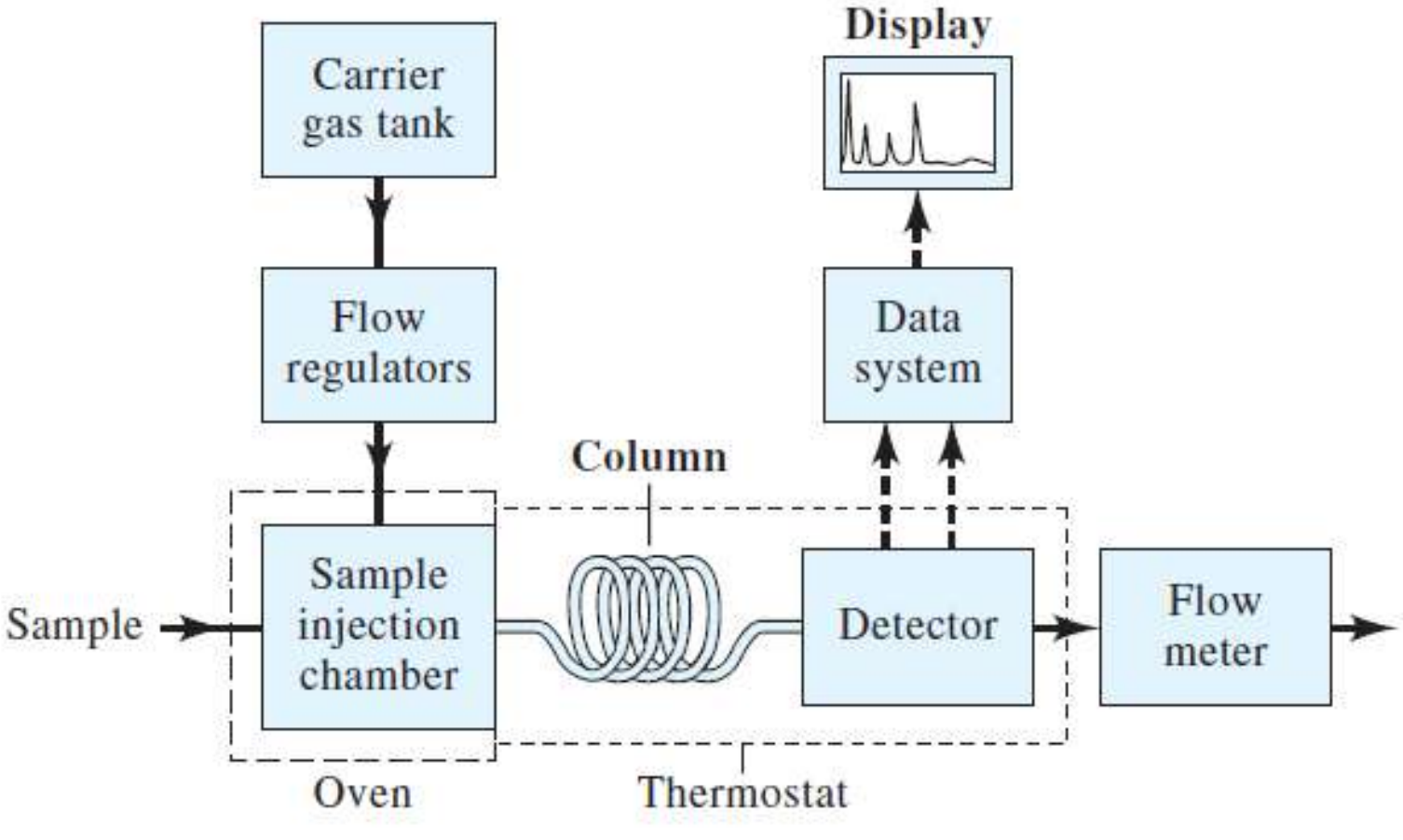
The concept of gas-liquid chromatography was first enunciated in 1941 by Martin and Synge, who were also responsible for the development of liquid-liquid partition chromatography.

More than a decade was to elapse, however, before the value of gas-liquid chromatography was demonstrated experimentally and this technique began to be used as a routine laboratory tool.

In 1955, the first commercial apparatus for gas-liquid chromatography appeared on the market. Since that time, the growth in applications of this technique has been phenomenal. Currently, several hundred thousand gas chromatographs are in use throughout the world.

In gas chromatography (GC) the sample, which may be a gas or liquid, is injected into a stream of an inert gaseous mobile phase (often called the carrier gas). The sample is carried through a packed or capillary column where the sample's components separate based on their ability to distribute themselves between the mobile and stationary phases.

A schematic diagram of a typical gas chromatograph



The primary components to a GC system

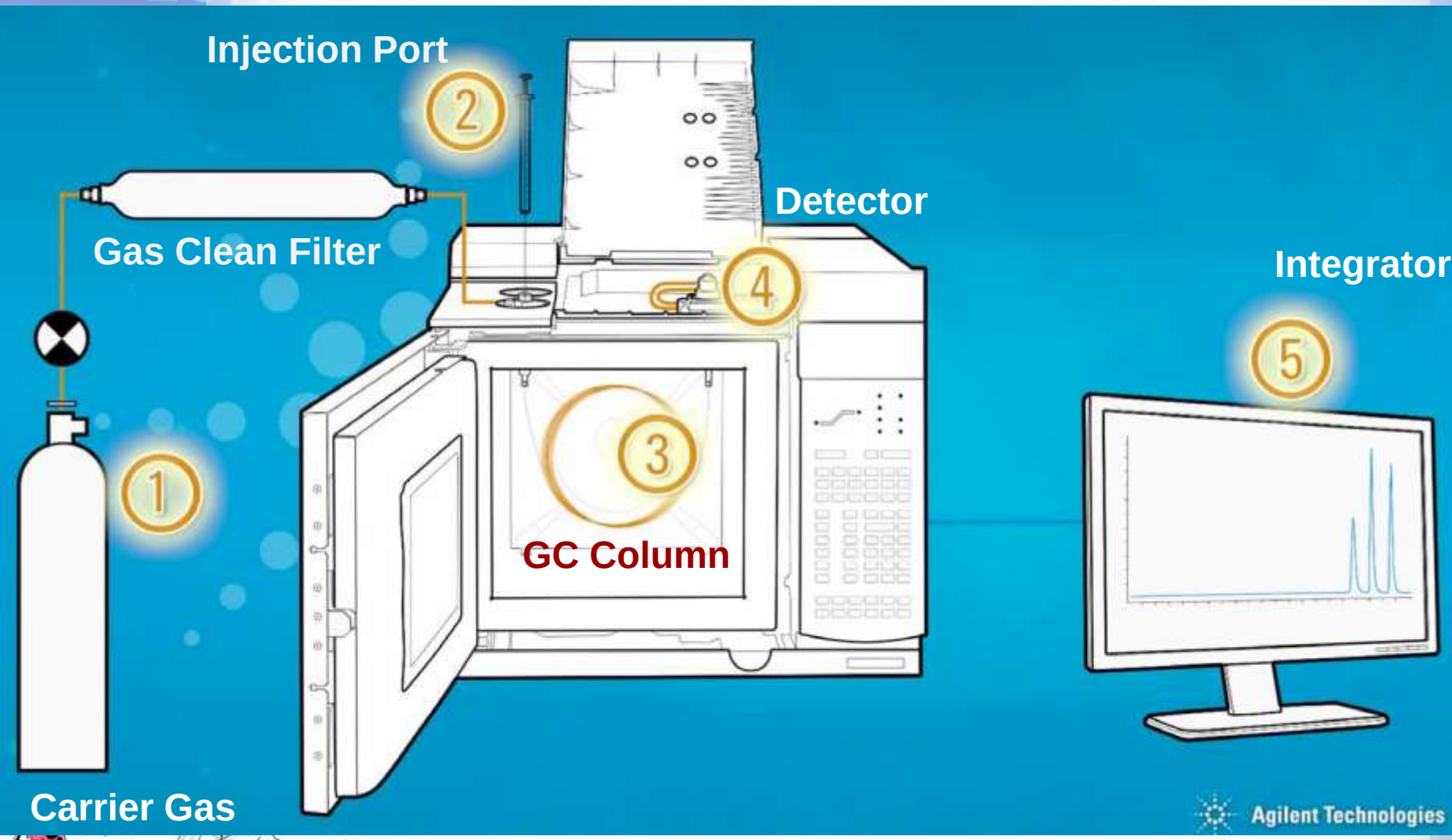
- **Carrier Gas System (including Gas Clean Filters)** The concept of theoretical plates and *van Deemter curves*
  Selection of proper carrier gas

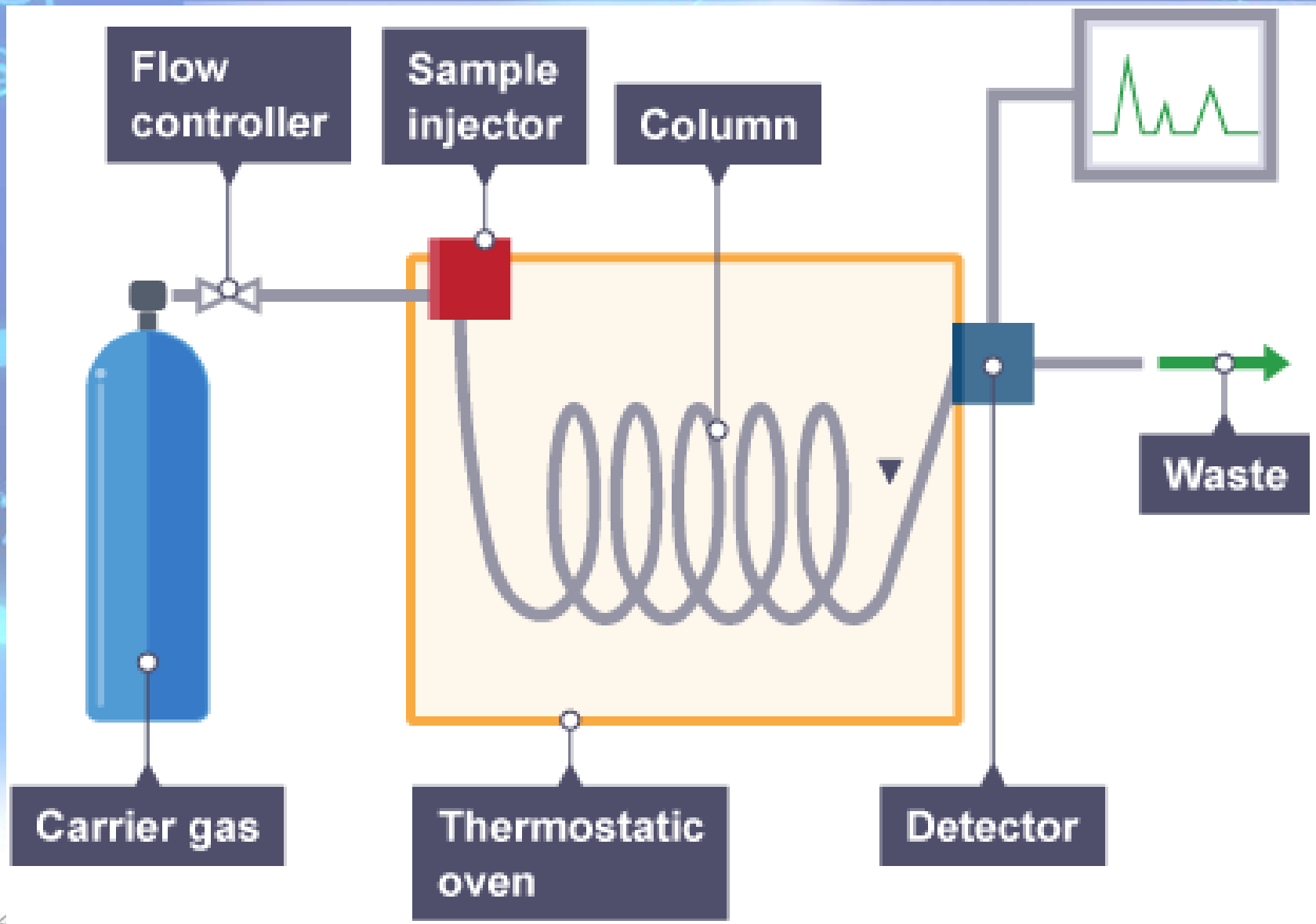
- **Sample Introduction System Split & splitless injection**

- **Column (most critical component)** Column configurations: packed vs. open tubular/capillary
  Stationary phase

- **Detection System/GC Detectors** Types of detectors and their specific applications

- **Computer ChemStation/Integrator**


Injection Port
2
Gas Clean Filter
Detector
4
Integrator
5
1
3
GC Column
Carrier Gas
Agilent Technologies

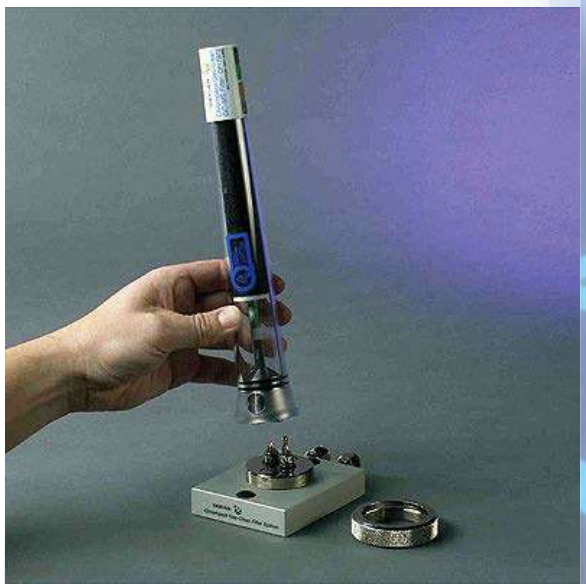

Flow controller
Sample injector
Column
Waste
Carrier gas
Thermostatic oven
Detector

I. Carrier Gas System

The mobile phase gas in gas chromatography is called the **carrier gas** and must be chemically inert. Helium is the most common mobile phase, although argon, nitrogen, and hydrogen are also used. These gases are available in pressurized tanks. Pressure regulators, gauges, and flow meters are required to control the flow rate of the gas. Classically, flow rates in gas chromatographs were regulated by controlling the gas inlet pressure. The choice of which carrier gas to use is often determined by the instrument's detector. With packed columns the mobile-phase velocity is usually within the range of 25–150 mL/min, whereas flow rates for capillary columns are 1–25 mL/min. Actual flow rates are determined with a flow meter placed at the column outlet.

Gas Clean Filter

Significant damages can be done to the column if it is heated above 70℃ with even trace amounts of O_2 in the column. Use carrier gas that meets the 99.9995% specification (UHP grade). Use O_2 & moisture traps.



The various carrier gas used in GC along with their characteristic features are stated below:

H_2: It has a distinctly better thermal conductivity and lower density. Demerits are its reactivity with unsaturated compounds and hazardous explosive nature,

He: It has an excellent thermal conductivity, low density, inertness and it permits greater flow rates. It is highly expensive,

N_2: It offers reduced sensitivity and is inexpensive, and

Air: It is employed only when the atmospheric O_2 is beneficial to the detector separation.

Importantly, the operating efficiency of a chromatograph is directly dependent on the maintenance of a highly constant carrier gas-flow-rate. Carrier gas passes from the tank through a toggle value, a flow meter, a few feet of metal capillary restrictors, and a 0-4 m pressure gauze. The flow rate could be adjusted by means of a needle value mounted on the base of the flow meter and is controlled by the capillary restrictors. On the downstream side of the pressure regulator, a tee (T) may split the flow and direct it to the sample and the reference side of the detector.

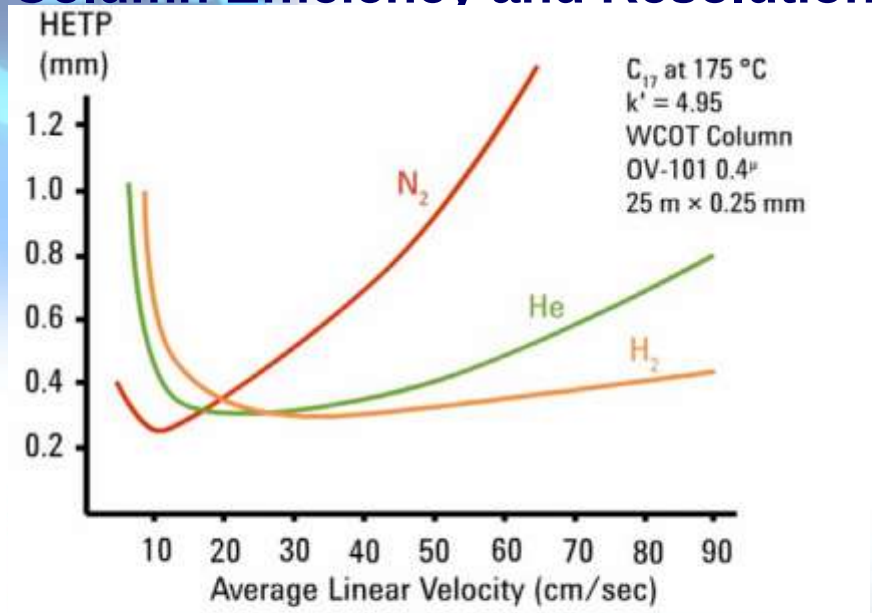
Flow Rate of Carrier Gas

- Flow rates must be precisely controlled
  - Reproducible retention times, minimize detector drift
- Flow rates of carrier gas:
  - Linear flow rate (cm/s): $u = L/tr$
  - Volumetric flow rate (mL/min): $u (\pi r^2)$

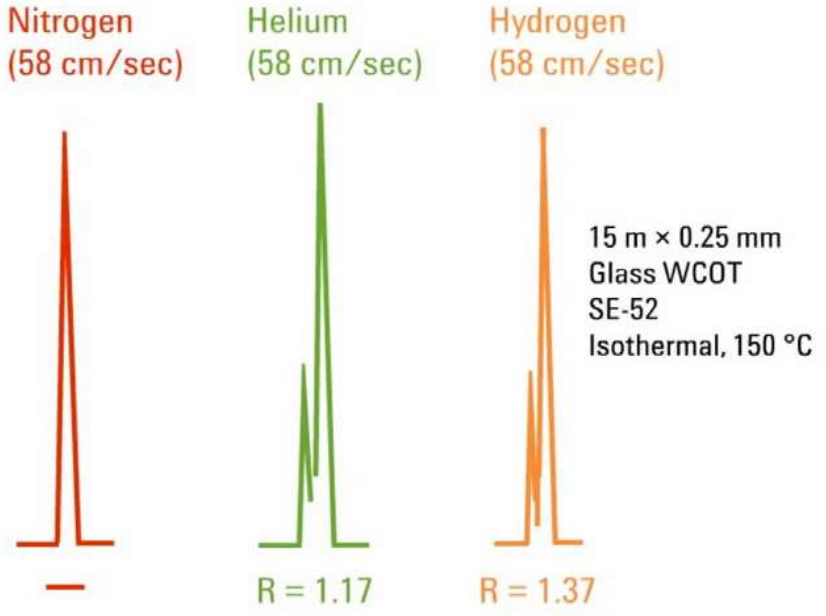
L is length of column, tr is retention time, r is the internal radius of column

- Flow rate depends on type of column
  - Packed column: 25-100 mL/min
  - Capillary column: µL/min to 1 mL/min

Type of Carrier Gas Effect on Column Efficiency and Resolution



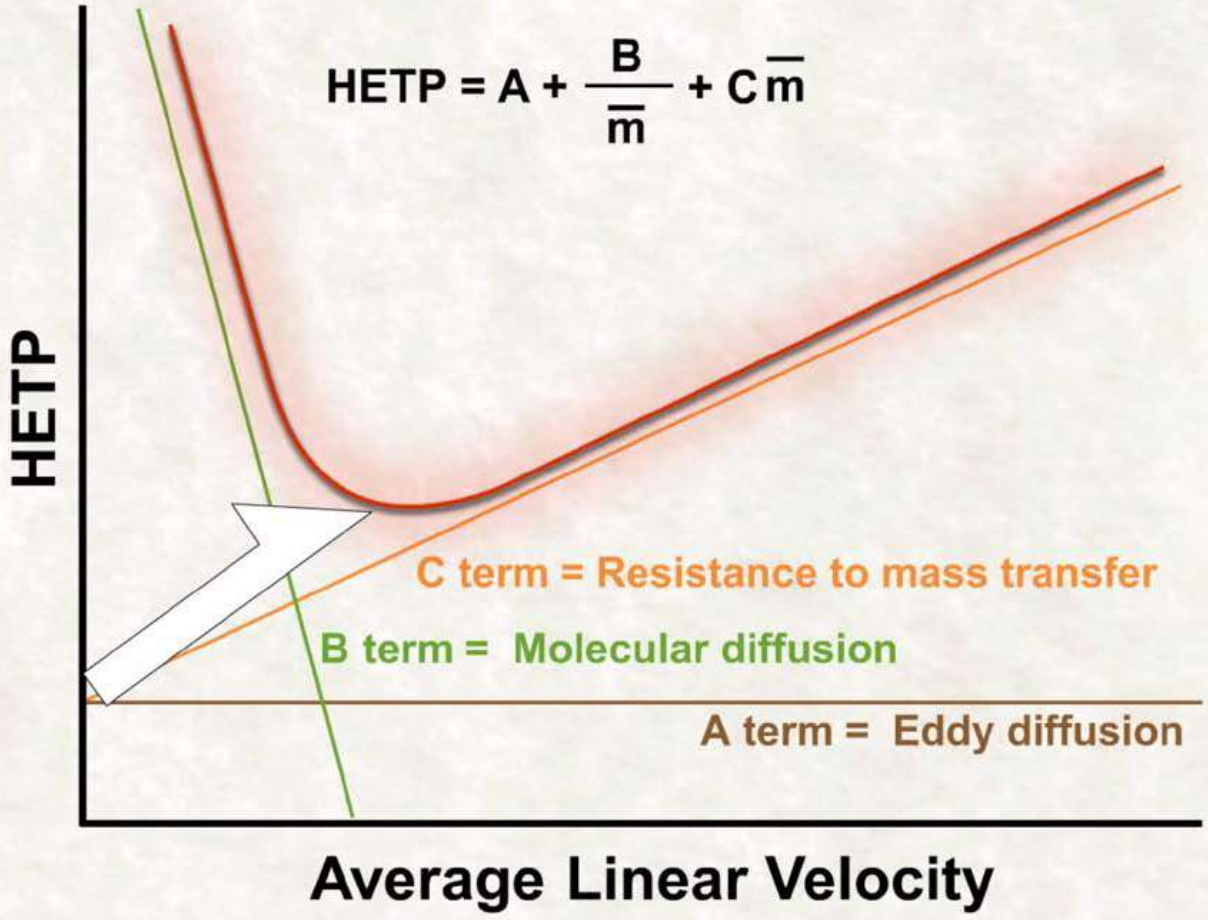
Efficiency curves for a 25 m × 0.25 mm id



Effect of carrier gas on the resolution of n-heptadecane and pristane

Optimizing Linear Velocity/Flow Rate for High Column Efficiency

van Deemter Plot



Efficiency is a function of the carrier gas linear velocity or flow rate.

HETP (height equivalent to theoretical plates) is defined as the length of the column divided by the number of theoretical plates (L/N).

Plot of HETP vs. linear velocity is know as the Van Deemter plot.

The minimum of the curve represents the smallest HETP (or largest plates per meter) and thus the best efficiency.

The linear velocity value at the minimum of the curve is the optimum value for achieving the best efficiency.

II. Sample Introduction

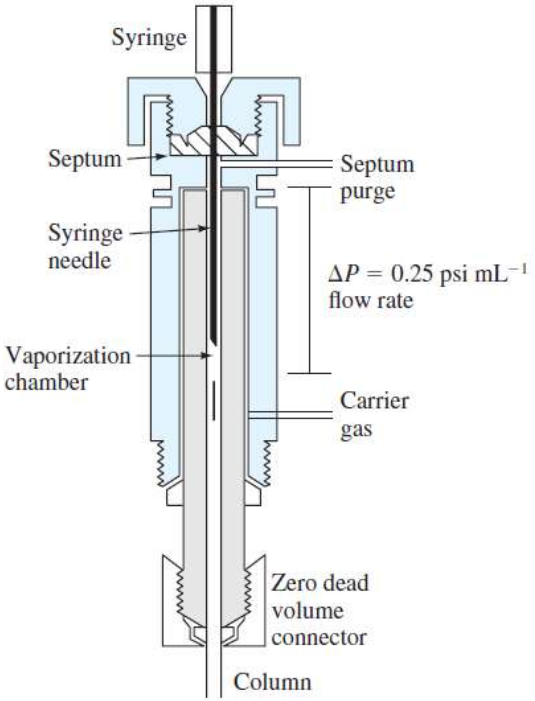
Injection Chamber

- Port where sample is introduced through a septum
- Heated, usually between 30-50º higher than the column temp to insure volatilization of the sample

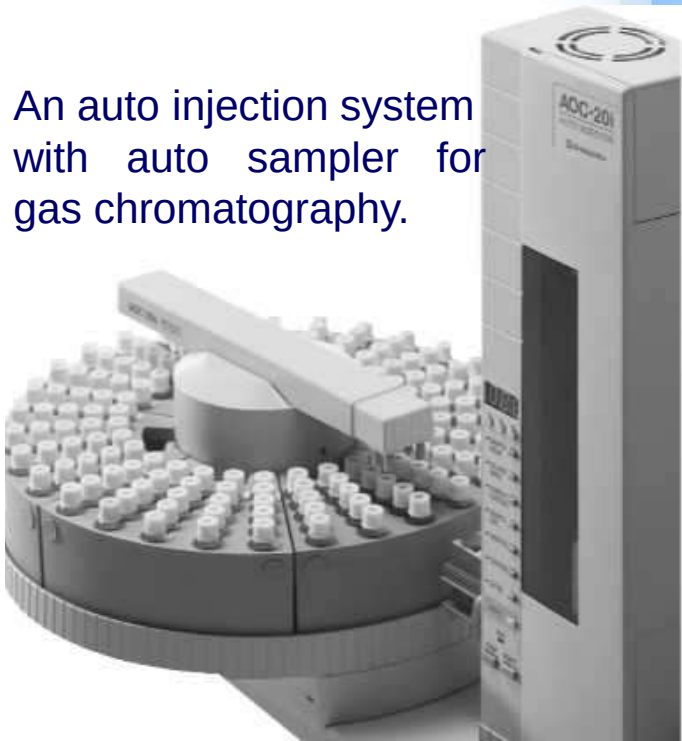
Calibrated microsyringes are used to inject liquid samples through a rubber or silicone diaphragm, or septum, into a heated sample port located at the head of the column.

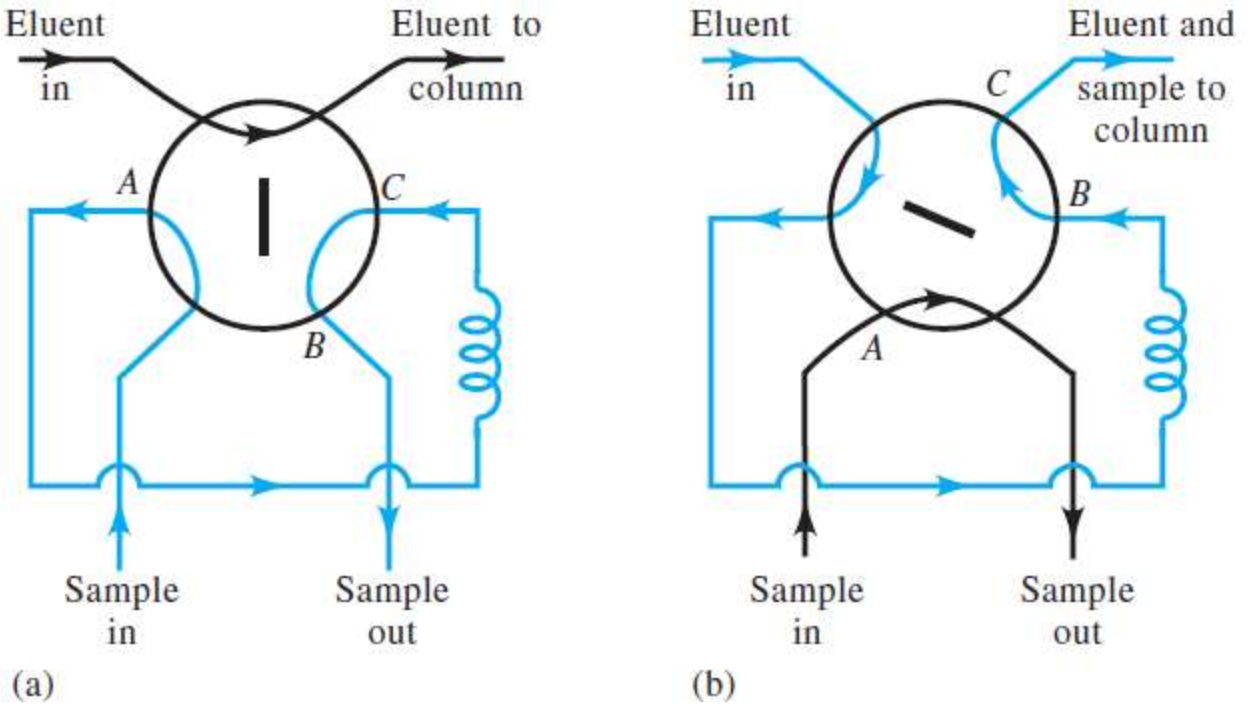
Slow injection/oversize causes peak broadening and poor resolution

Cross-sectional view of a microflash vaporizer direct injector.



An auto injection system with auto sampler for gas chromatography.





A rotary sample valve. Valve position (a) is for filling the sample loop *ACB; position (b) is* for introduction of sample into the column.

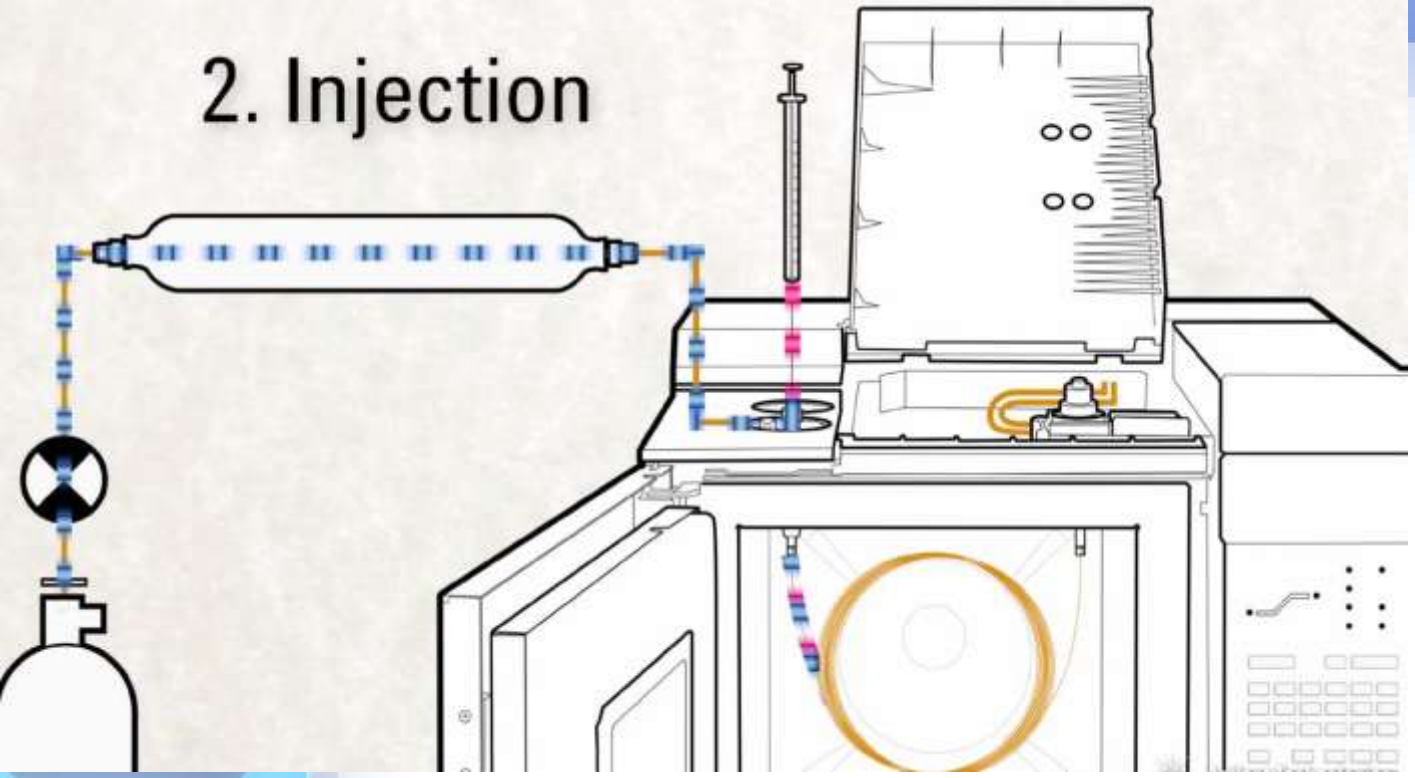
For introducing gases, a sample valve, is often used instead of a syringe. With such devices, sample sizes can be reproduced to better than 0.5% relative. Liquid samples can also be introduced through a sampling valve. Solid samples are introduced as olutions or alternatively are sealed into thin-walled vials that can be inserted at the head of the column and punctured or crushed from the outside.

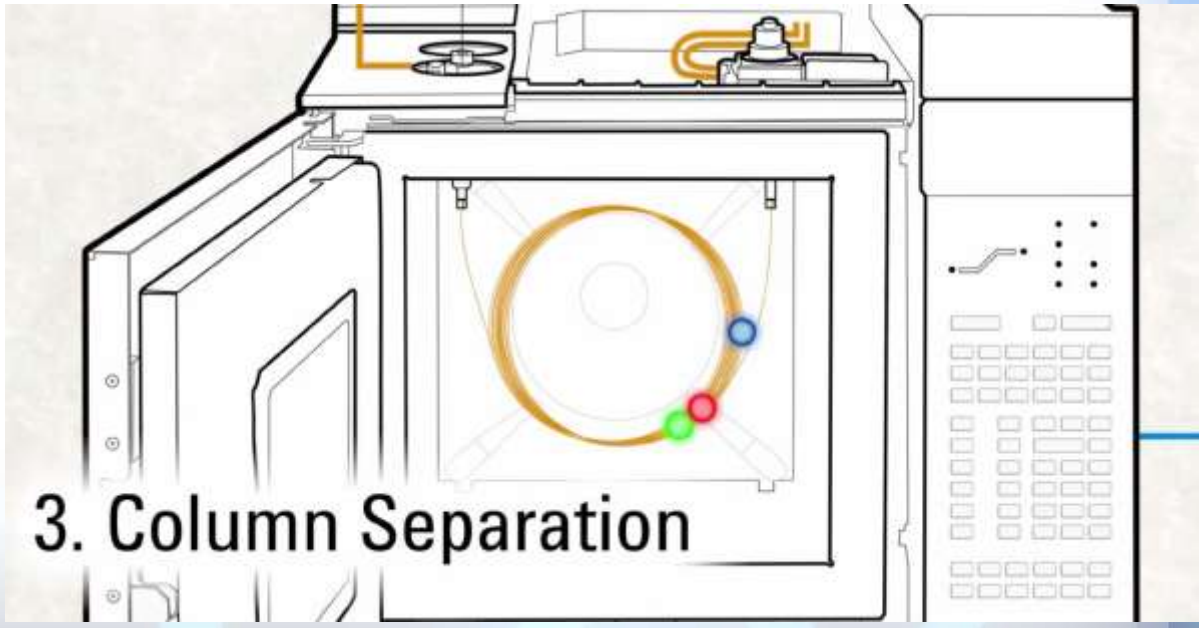
Capillary columns require the use of a special injector to avoid overloading the column with sample. Several capillary injectors are available, the most common of which is a split/splitless injector. When used for a **split injection** only about 0.1–1% of the sample enters the column, with the remainder carried off as waste. In a splitless injection, which is useful for trace analysis, the column temperature is held 20–25 °C below the solvent's boiling point. As the solvent enters the column, it condenses, forming a barrier that traps the solutes. After allowing time for the solutes to concentrate, the column's temperature is increased, and the separation begins. A splitless injection allows a much higher percentage of the solutes to enter the chromatographic column.

For samples that decompose easily, an **on-column injection may be necessary.**

In this method the sample is injected on the column without heating. The column temperature is then increased, volatilizing the sample with as low a temperature as is practical.

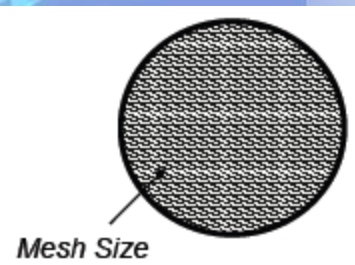
2. Injection





3. Column Separation

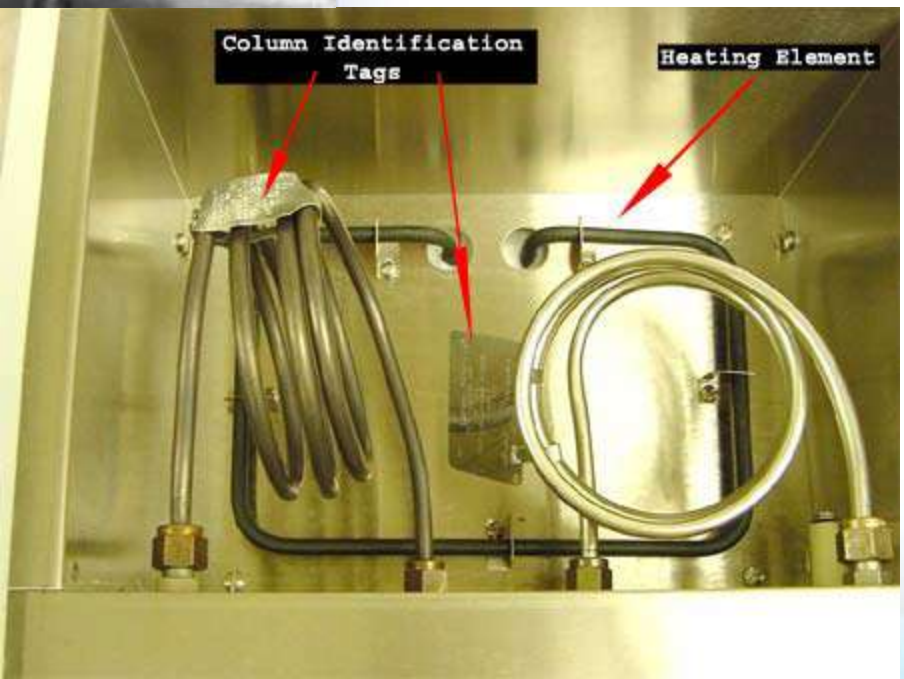
III Gas Chromatography Columns



The two types of columns used in GC are **packed columns and capillary columns.**

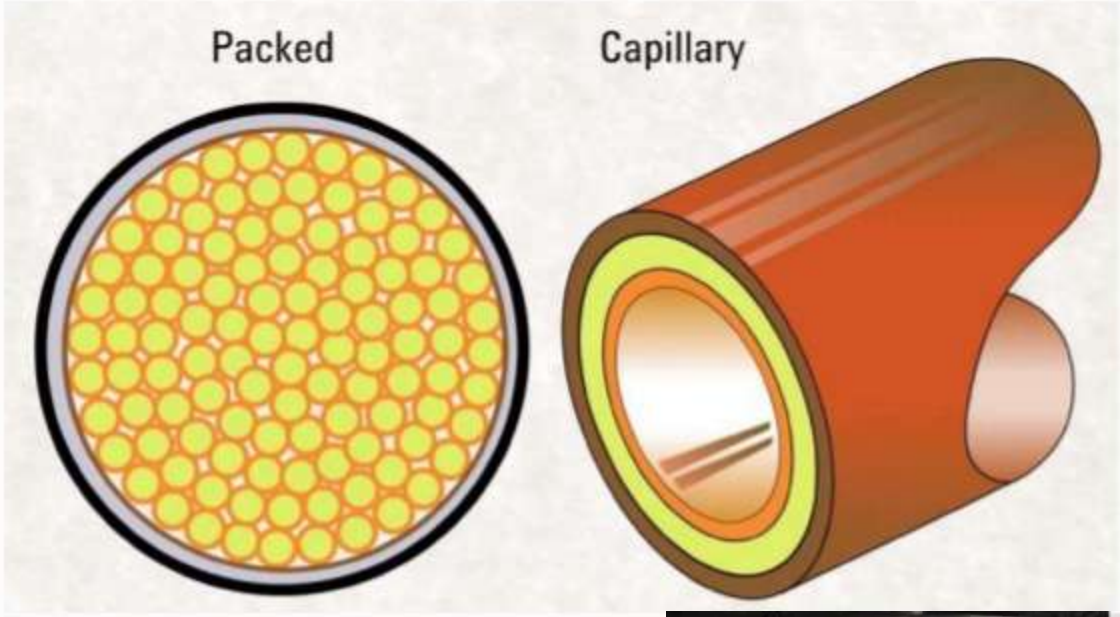
Packed columns came first and were used for many years. Capillary columns are more commonly used today, but packed columns are still used for applications that do not require high resolution or when increased capacity is needed.

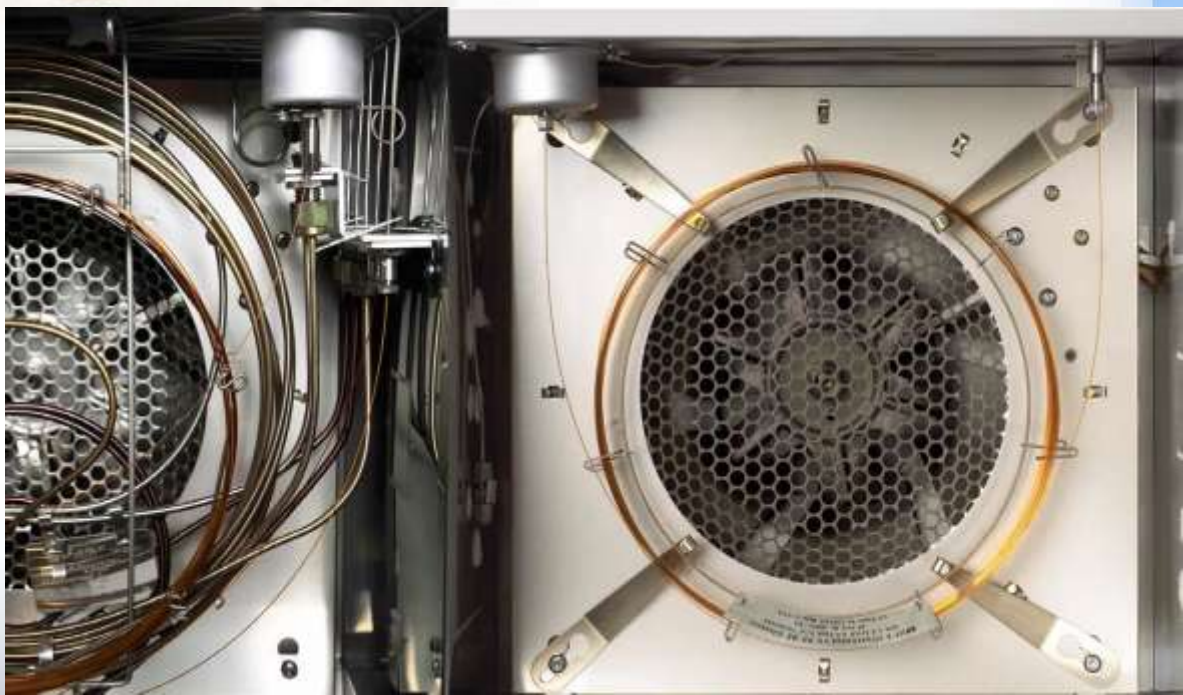
A **packed column** is constructed from glass, stainless steel, copper or aluminum and is typically 2–6 m in length, with an internal diameter of 2–4 mm. The column is filled with a particulate solid support, with particle diameters ranging from 37–44 mm to 250–354 mm.



These tubes are densely packed with a uniform, finely divided packing material, or solid support, that is coated with a thin layer (0.05 to 1 mm) of the stationary liquid phase. The tubes are usually formed as coils with diameters of roughly 15 cm so that they can be conveniently placed in a temperature-controlled oven.

1. Types of GC columns





packed column

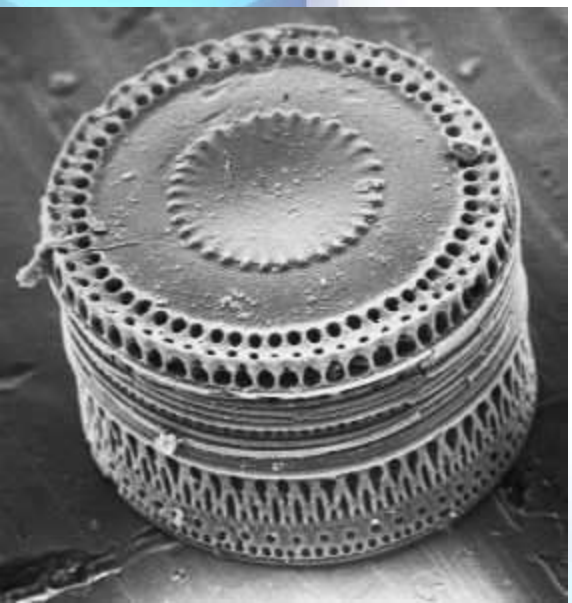
The column is packed with small particles that may themselves serve as the stationary phase (adsorption chromatography) or more commonly are coated with a nonvolatile liquid phase of varying polarity (partition chromatography). ***Gas–solid chromatography*** (GSC) is useful for the separation of small gaseous species such as H_2, N_2, CO_2, CO, O_2, NH_3, and CH_4 and volatile hydrocarbons, using high surface area inorganic packings such as alumina *(Al_2O_3) or porous polymers (e.g., Porapak* Q—a polyaromatic cross-linked resin with a rigid structure and a distinct pore size).

The gases are separated by their size due to retention by adsorption on the particles. Gas–solid chromatography is preferred for aqueous samples.

The efficiency of a gas chromatographic column increases rapidly with decreasing particle diameter of the packing. The pressure difference required to maintain an acceptable flow rate of carrier gas, however, varies inversely as the square of the particle diameter. As a result, the usual support particles are 60 to 80 mesh (250 to 170 mm) or 80 to 100 mesh (170 to 149 mm).

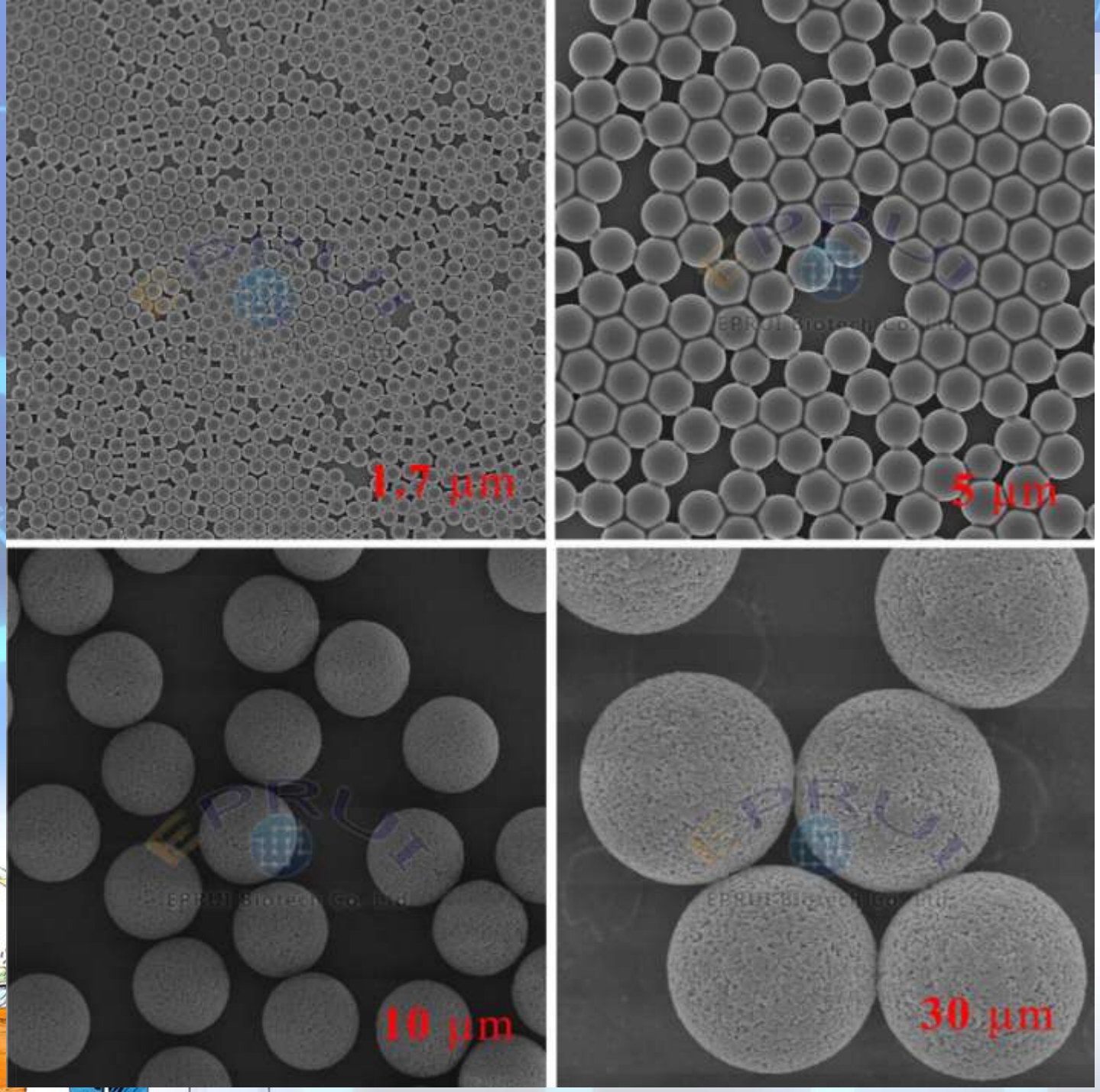
The solid support for a liquid phase should have a high specific surface area that is chemically inert but wettable by the liquid phase. It must be thermally stable and available in uniform sizes. The most commonly used supports are prepared from diatomaceous earth (consists of the skeletons of thousands of species of single-celled plants that inhabited ancient lakes and seas), a spongy siliceous material. They are sold under many different trade names. Chromosorb P, Chromosorb W, Chromosorb G. Generally, all of the above are available in non-acid washed, acid washed, and silanized with dimethylchlorosilane (DMCS, this greatly reduces polarity) which gives a surface layer of methyl groups. This treatment reduces the tendency of the packing to adsorb polar molecules.

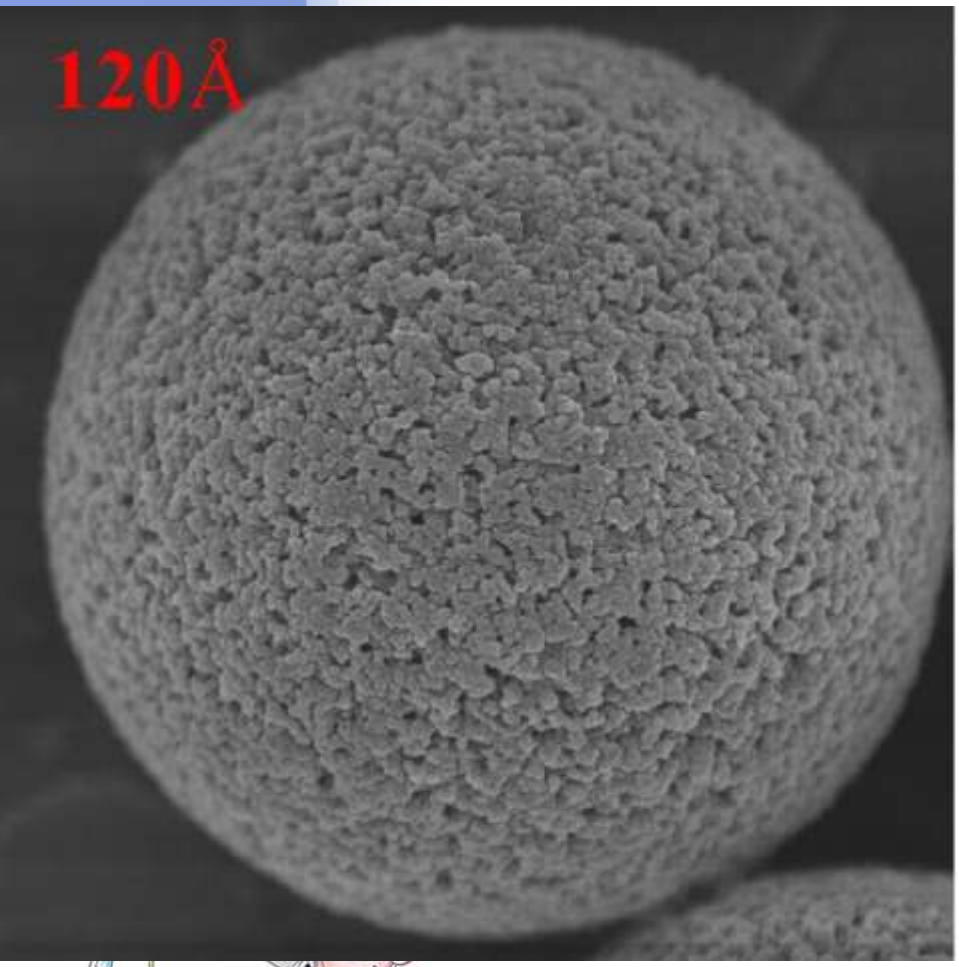
A photomicrograph of a diatom. Magnification 5000

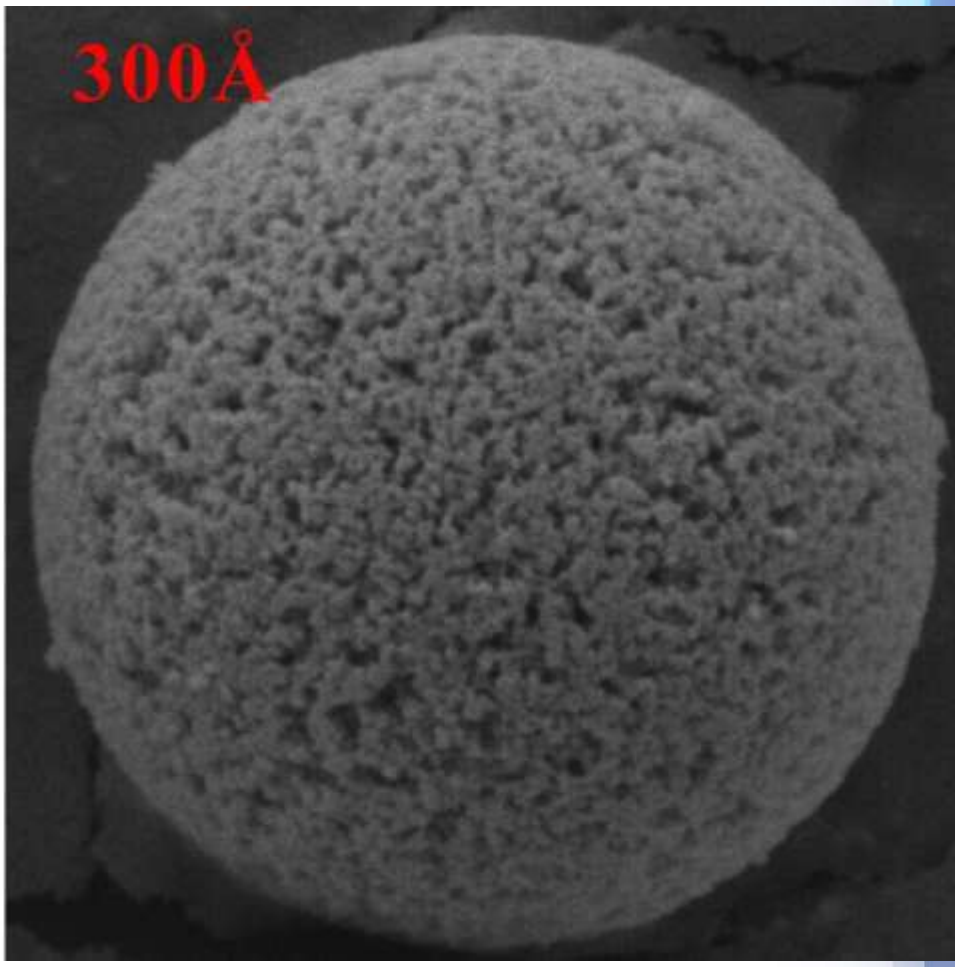


In **gas–liquid chromatography (GLC),** separation is based on the partitioning of solutes between a gaseous mobile phase and a liquid stationary phase coated on the solid packing material.

Column-packing support material is coated by mixing with the correct amount of liquid phase dissolved in a low-boiling solvent such as acetone or pentane. About a 5 to 10% coating (wt/wt) will give a thin layer. After coating, the solvent is evaporated by heating and stirring. A newly prepared column should be conditioned at elevated temperature by passing carrier gas through it for several hours.


1.7 μm
5 μm
10 μm
30 μm


120Å


300Å

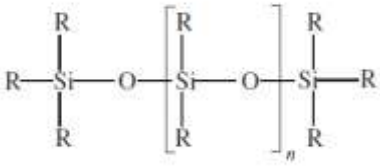
STATIONARY PHASES-THE KEY TO DIFFERENT SEPARATIONS

Selectivity in gas chromatography is influenced by the choice of stationary phase. Elution order in GLC is determined primarily by the solute's boiling point and, to a lesser degree, by the solute's interaction with the stationary phase. Solutes with significantly different boiling points are easily separated. On the other hand, two solutes with similar boiling points can be separated only if the stationary phase selectively interacts with one of the solutes. In general, nonpolar solutes are more easily separated with a nonpolar stationary phase, and polar solutes are easier to separate using a polar stationary phase. The main criteria for selecting a stationary phase are that it should be chemically inert, thermally stable, of low volatility, and of an appropriate polarity for the solutes being separated. Although hundreds of stationary phases have been developed, many of which are commercially available, the majority of GLC separations are accomplished with perhaps five to ten common stationary phases.

General rule: *"like dissolves like"*

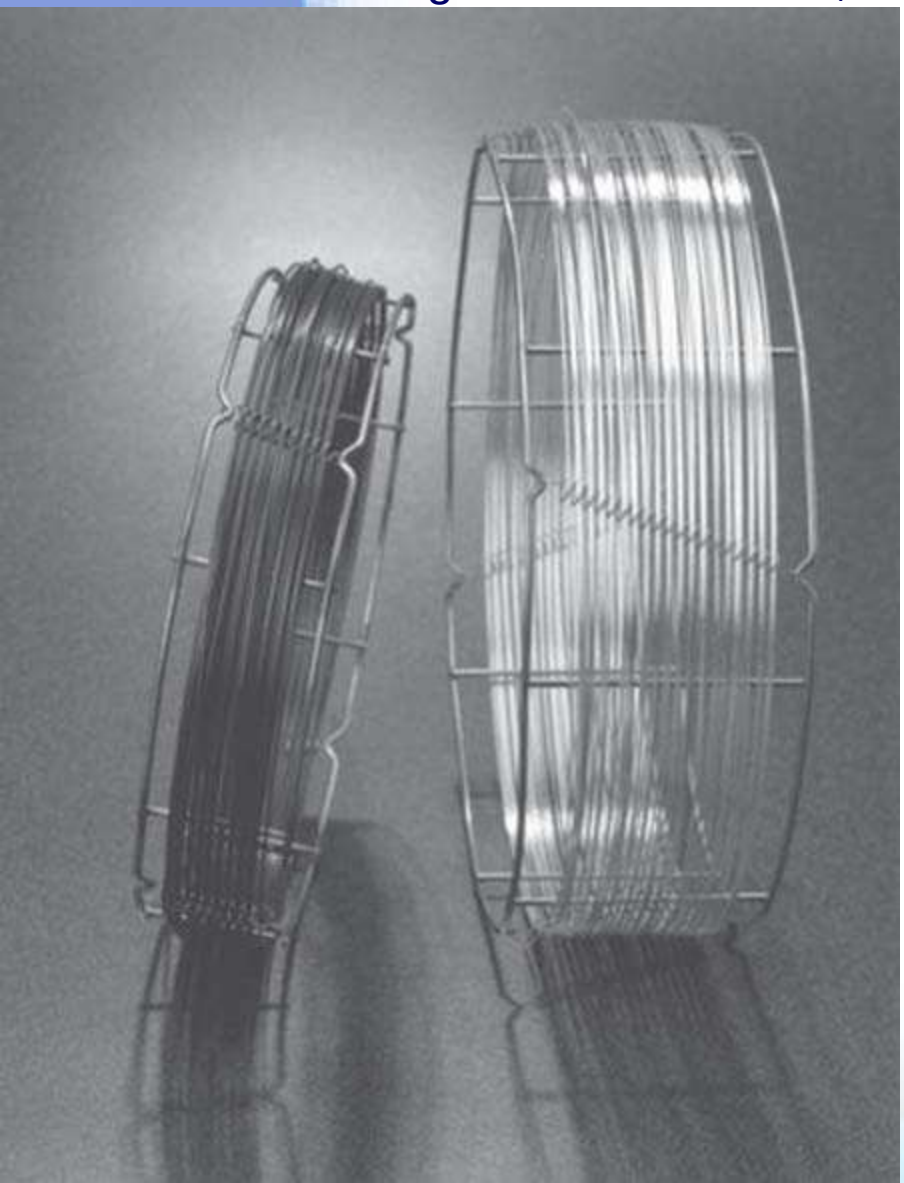
Polar functional groups: -CN, -CO, -OH, -COOH, -NH_2, -CHO, -X, etc.

Nonpolar function groups: saturated alkane –CH, etc.

Stationary Phase	Polarity	Trade Names	Temperature Limit (°C)	Applications
squalane	nonpolar	squalane	150	low-boiling aliphatic hydrocarbons
Apezion L	nonpolar	Apezion L	300	amides fatty acid methyl esters high-boiling aliphatic hydrocarbons terpenoids
polydimethyl siloxane 	slightly polar	SE-30	300–350	alkaloids amino acid derivatives drugs pesticides phenols steroids
50% methyl-50% phenyl polysiloxane	moderately polar	OV-17	375	alkaloids drugs pesticides polyaromatic hydrocarbons polychlorinated biphenyls
50% trifluoropropyl-50% methyl polysiloxane	moderately polar	OV-210	275	alkaloids amino acid derivatives drugs halogenated compounds ketones phenols
50% cyanopropyl-50% phenylmethyl polysiloxane	polar	OV-225	275	nitriles pesticides steroids
polyethylene glycol	polar	Carbowax 20M	225	aldehydes esters ethers phenols

Capillary Columns

In capillary columns, the stationary phase was a film of liquid a few tenths of a micrometer thick that uniformly coated the interior of a capillary tubing. In the late 1950s, such open tubular columns were constructed.



The most significant development in capillary GC occurred in 1979 when fused-silica capillaries were introduced.

Capillary, or **open tubular columns** are constructed from fused silica coated with a protective polymer. Columns may be up to 100 m in length with an internal diameter of approximately 150–300 mm.

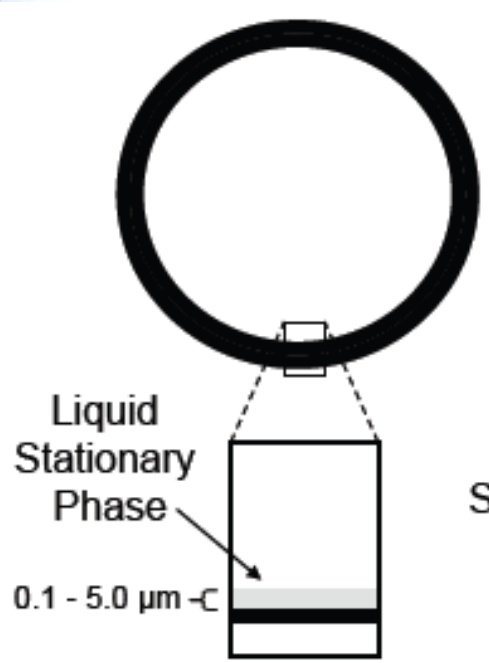
There are three types of open-tubular columns. **Wall-coated open-tubular** (WCOT) columns have a thin liquid film coated on and supported by the walls of the capillary. The walls are coated by slowly passing a dilute solution of the liquid phase through the columns. The solvent is evaporated by passing carrier gas through the columns. Following coating, the liquid phase is cross-linked to the wall. The resultant stationary liquid phase is 0.1 to 0.5 *μm thick. Wall-coated open-tubular columns* typically have 5000 plates/m. So a 50-m column will have 250,000 plates.

In **support coated open-tubular (SCOT) columns,** solid microparticles coated with the stationary phase (much like in packed columns) are attached to the walls of the capillary. These have higher surface area and have greater capacity than WCOT columns. The tubing diameter of these columns is 0.5 to 1.5 mm, larger than WCOT columns. The advantages of low pressure drop and long columns is maintained, but capacity of the columns approaches that of packed columns. Flow rates are faster and dead volume connections at the inlet and detector are less critical.

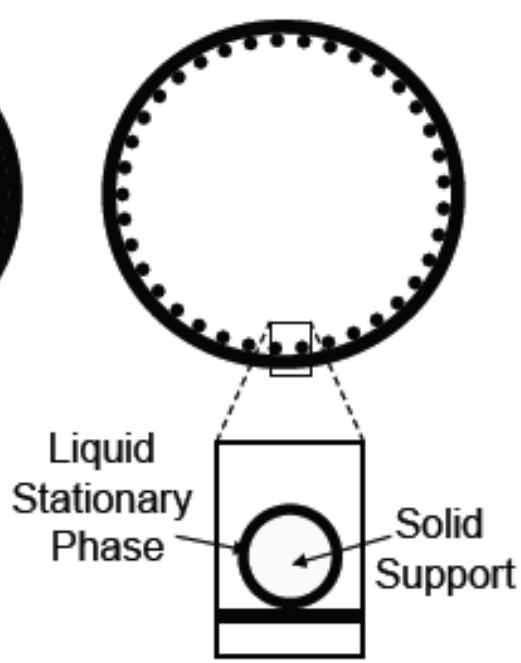
The third type, **porous layer open-tubular (PLOT) columns,** have solid-phase particles attached to the column wall, for adsorption chromatography. Particles of alumina or porous polymers (molecular sieves) are typically used. The resolution efficiency of open-tubular columns is generally in the order: WCOT > *SCOT > PLOT.*

Open-Tubular Columns

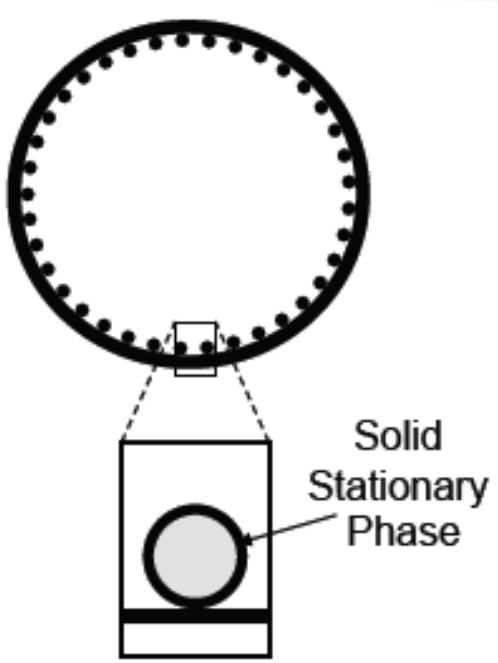
Wall-Coated Open Tubular (WCOT)

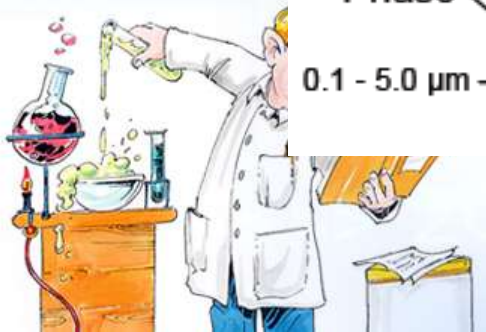


Support-Coated Open Tubular (SCOT)



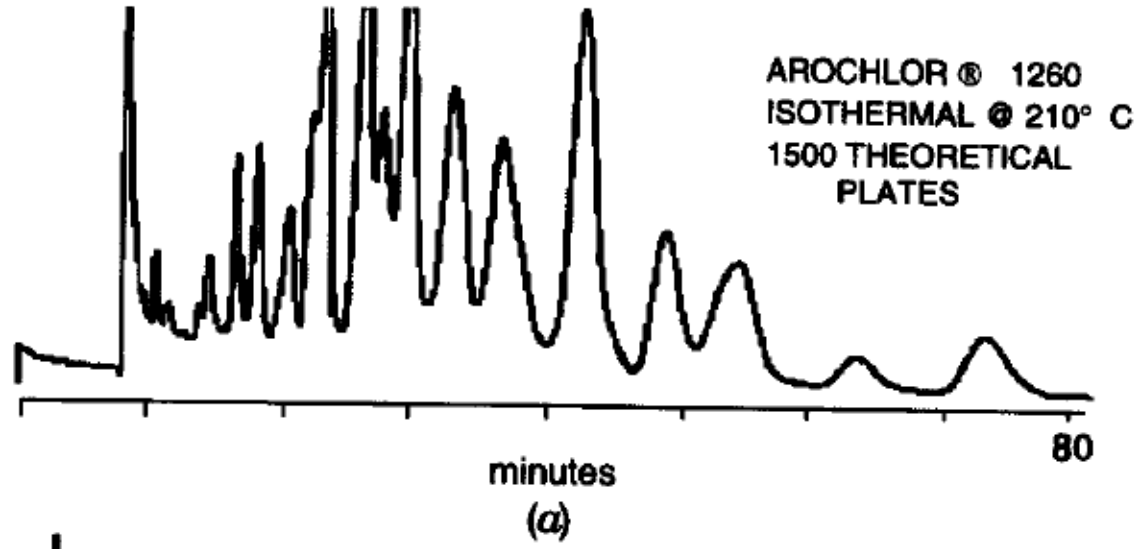
Porous-Layer Open Tubular (PLOT)



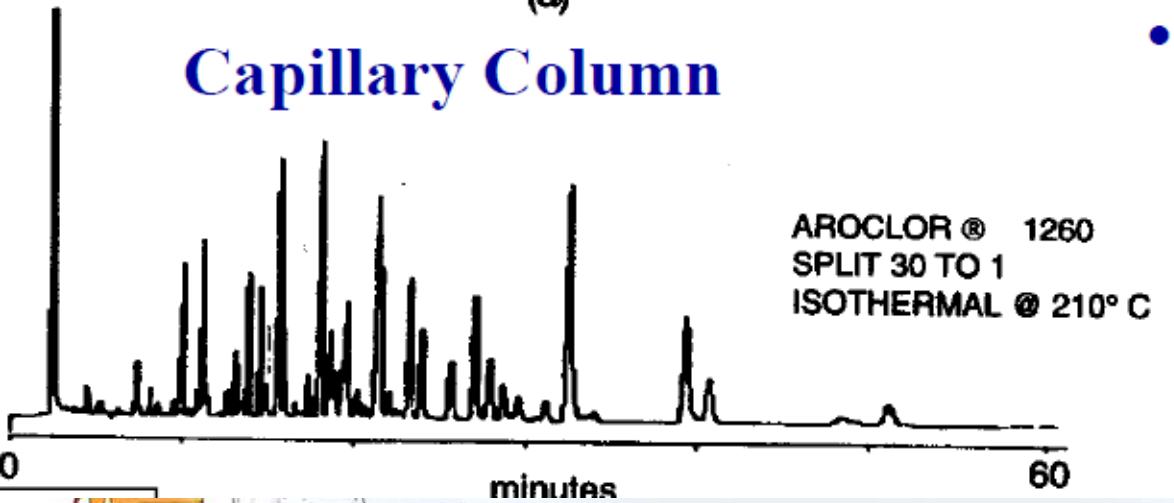


Column Type vs. Separation

Packed Column

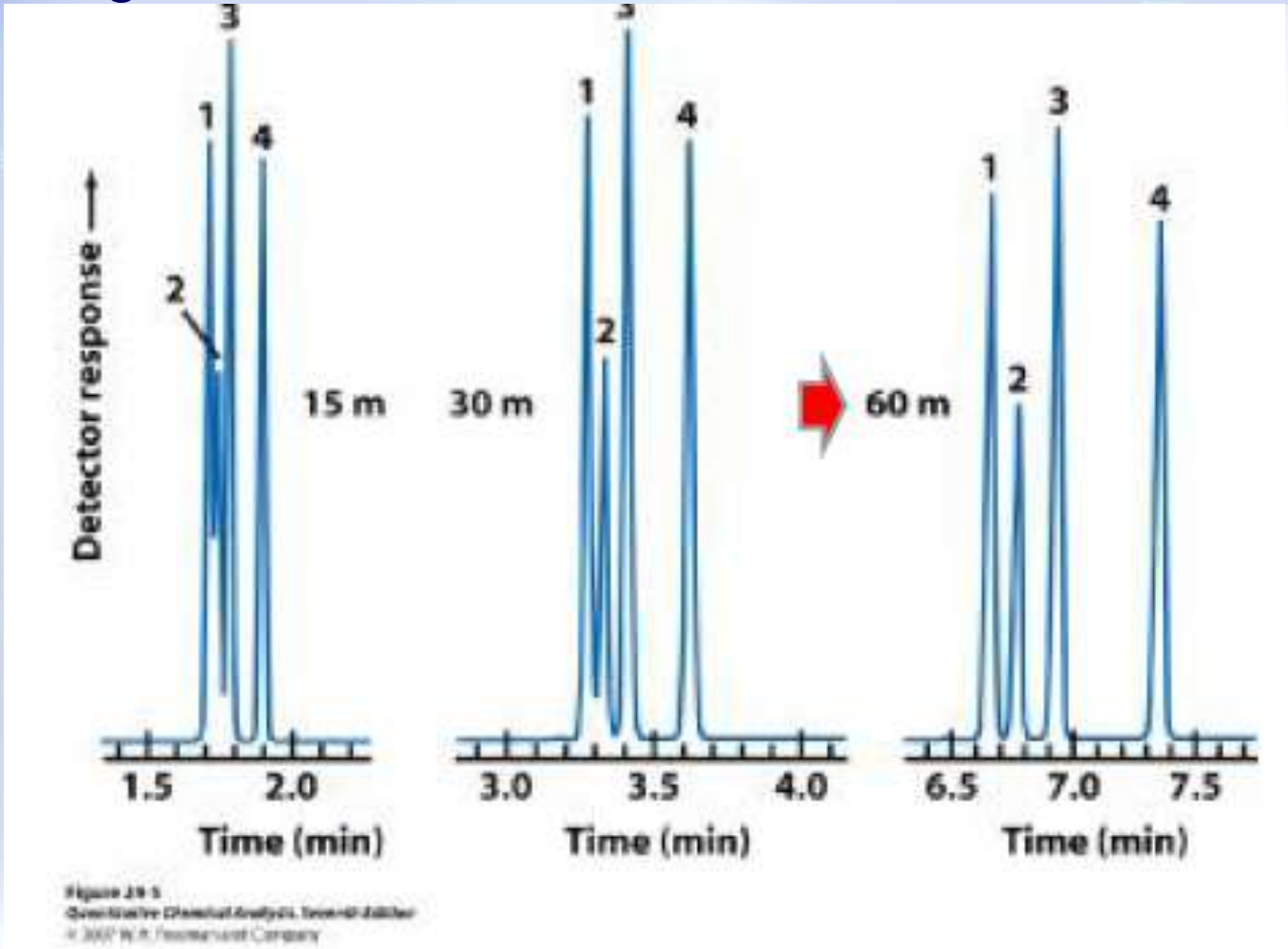


Capillary Column

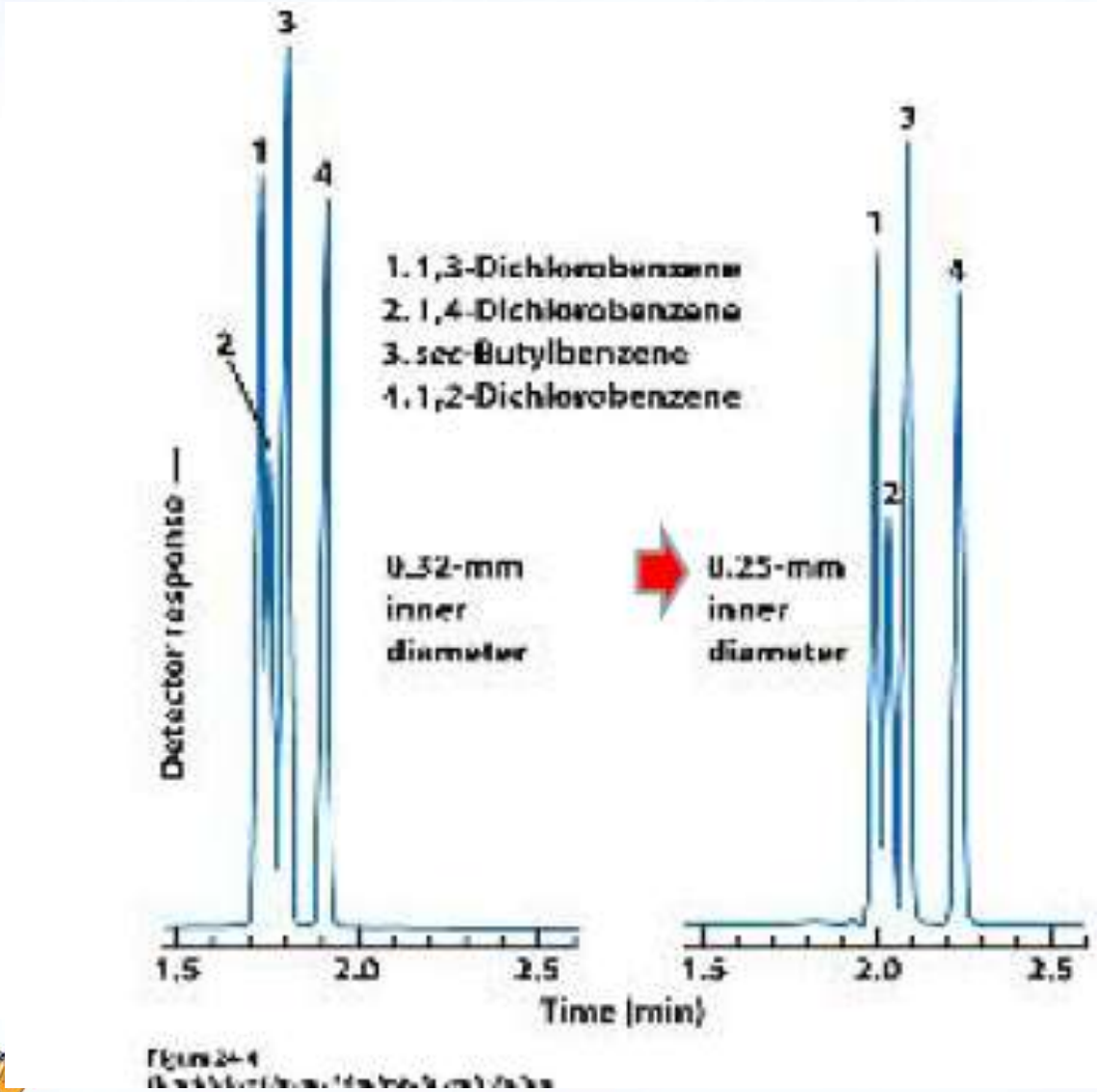


- Packed Column
  - Lower resolution
  - Fewer peaks (16)
  - Fewer plates
- Capillary Column
  - Small sample needed
  - Better resolution
  - More peaks
  - Faster Analysis

Length vs. resolution



Diameter vs. resolution



Column Selection Parameters

- The critical parameters for GC columns:
  - **Dimensions**: Internal diameter, Column length, Film thickness
  - **Conditions**: Temperature, Flow rate
  - **Composition**: Stationary phase composition*, Carrier gas
- Given a sample, you will need to first choose the what stationary phase will work best
  - First pick the type of column, then think about dimensions
  - Conditions can be optimized for given column dimensions
- Choice of stationary phase is very important
  - It determines what kind of sample you can run
  - Critical for packed columns, but less so for OT columns because of high efficiency

Temperature Control

As noted earlier, control of the column's temperature is critical to attaining a good separation in gas chromatography. For this reason the column is located inside a thermostated oven. In an isothermal separation the column is maintained at a constant temperature, the choice of which is dictated by the solutes.

Normally, the temperature is set slightly below that for the lowest boiling solute so as to increase the solute's interaction with the stationary phase.

One difficulty with an isothermal separation is that a temperature favoring the separation of low-boiling solutes may cause unacceptably long retention times for higher boiling solutes. Ovens capable of temperature programming provide a solution to this problem. The initial temperature is set below that for the lowest boiling solute. As the separation progresses, the temperature is slowly increased at either a uniform rate or in a series of steps.

RETENTION INDICES FOR LIQUID STATIONARY PHASES

Selecting the proper packed-column stationary phase from the myriad of possible phases is challenging. Methods have been developed that group phases according to their retention properties, for example, according to polarity. **The Kovats retention index (KRI)** system is designed primarily to provide a means to identify unknowns by comparing their retention with the retention of a set of known standards on the same column. *n-Alkanes (paraffins) are used as* the standards on the Kovats scale.

$$I_{\text{unk}} = 100\,n + \frac{\log t'_{R,\text{unk}} - \log t'_{R,\,C_n}}{\log t'_{R,C_{n+1}} - \log t'_{R,C_n}} \times 100$$

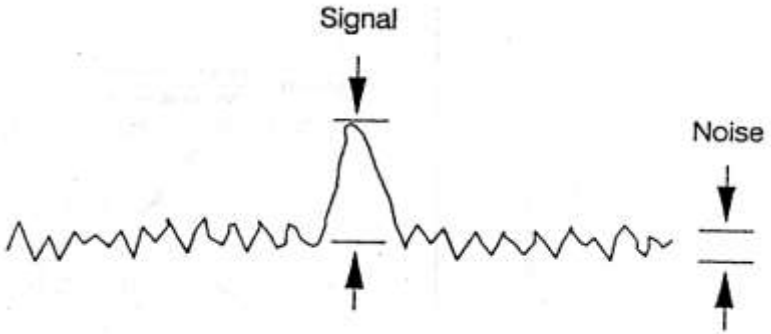
An analyte can be identified by its KRI on different columns, as these indices are tabulated for many analytes on many columns.

IV. GC Detection Systems/Detectors

Characteristics of the Ideal Detector:

• Adequate sensitivity (application specific, i.e. adequate for certain tasks)

S/N >3



• Good stability and reproducibility

• A linear response to solutes that extends over several orders of magnitude (calibration purposes)

• A wide temperature range

• A short response time independent of flow rate

• High reliability and ease of use (unfortunately, usually not the case)

• Similarity in response toward all solutes/most classes of solutes

• The detector should be nondestructive

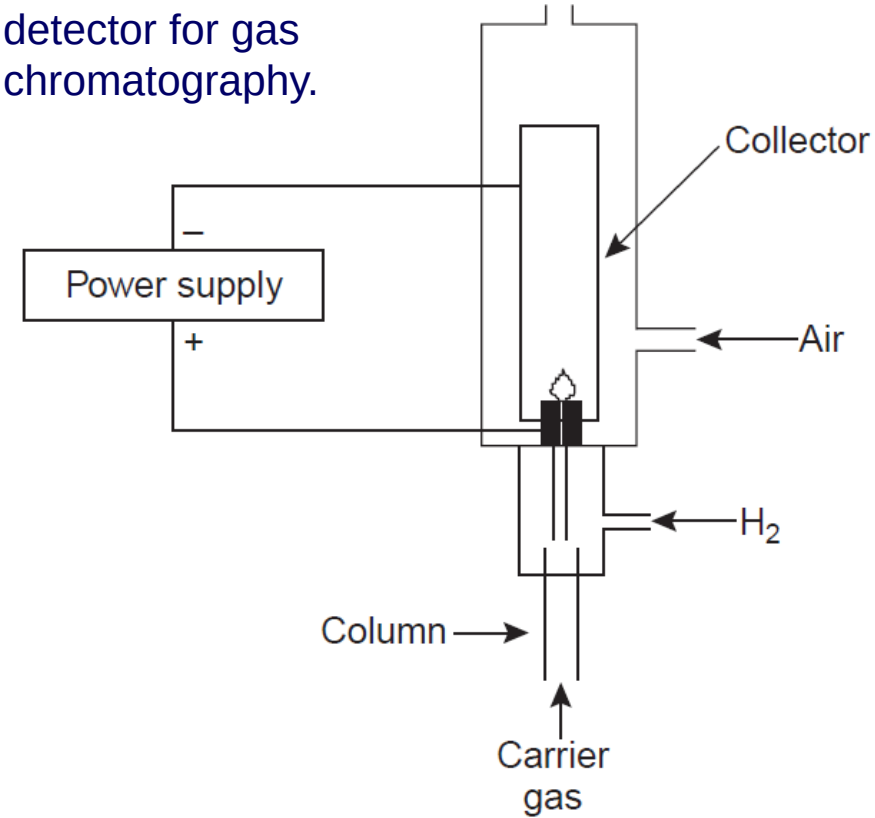
Typical GC Detectors

Type	Approximate limit of detection ($g\ s^{-1}$)	Approximate linear range	Comments
Thermal conductivity (TCD)	10^{-5}–10^{-6}	10^{3}–10^{4}	Universal detector Measures changes in heat conduction
Flame ionization (FID)	10^{-12}	10^{6}–10^{7}	Universal detector Measures ion currents from pyrolysis
Electron capture (EC or ECD)	10^{-14}	10^{2}–10^{3}	Selective detector for compounds containing atoms with high electron affinities
Flame photometric (FPD)	10^{-13}	10^{2}	Selective detector for compounds containing S, P
Nitrogen-phosphorus	10^{-8}–10^{-14}	10^{5}–10^{7}	Selective for N, P containing compounds
Photoionization (PID)	10^{-8}–10^{-12}	10^{5}	Universal (some selectivity due to to identity of gas in lamp)
Hall Detector	10^{-11}	10^{5}	Specific detector for compounds which contain halogen, S, or N
Mass spectrometer (MS)	10^{-12}	*a*	Universal detector
Fourier-transform infrared (FTIR)	10^{-10}	10^{2}	Polar molecules

1. Flame Ionization Detector (FID)

Combustion of an organic compound in an H_2/air flame results in a flame rich in electrons and ions. If a potential of approximately 300 V is applied across the flame, a small current of roughly 10^{-9}–10^{-12} A develops. When amplified, this current provides a useful analytical signal. Most carbon atoms, except those in carbonyl and carboxylic groups, generate a signal, making the FID an almost universal detector for organic compounds.

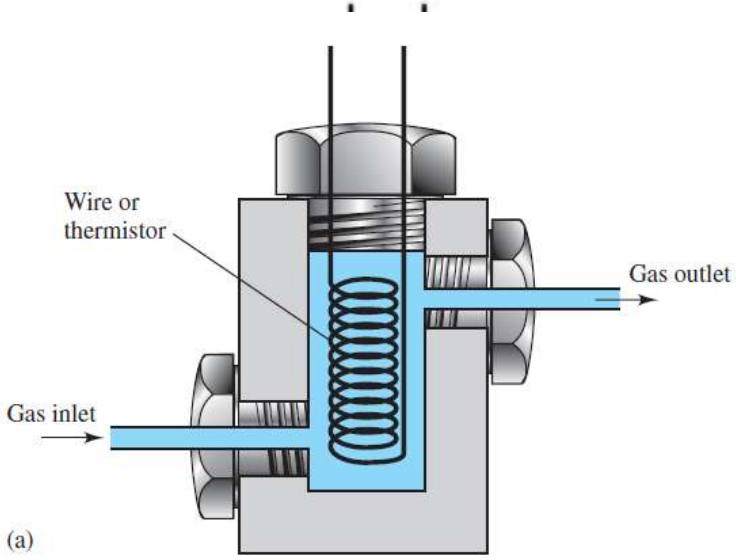
Schematic diagram of a flame ionization detector for gas chromatography.



Most inorganic compounds and many gases, such as H_2O and CO_2, cannot be detected, making the FID detector ideal for the analysis of atmospheric and aqueous environmental samples. Advantages of the FID include a detection limit that is approximately two to three orders of magnitude smaller than that for a thermal conductivity detector and a linear response over 10^6–10^7 orders of magnitude in the amount of analyte injected. The sample, of course, is destroyed when using a flame ionization detector.

Thermal Conductivity Detector (TCD)

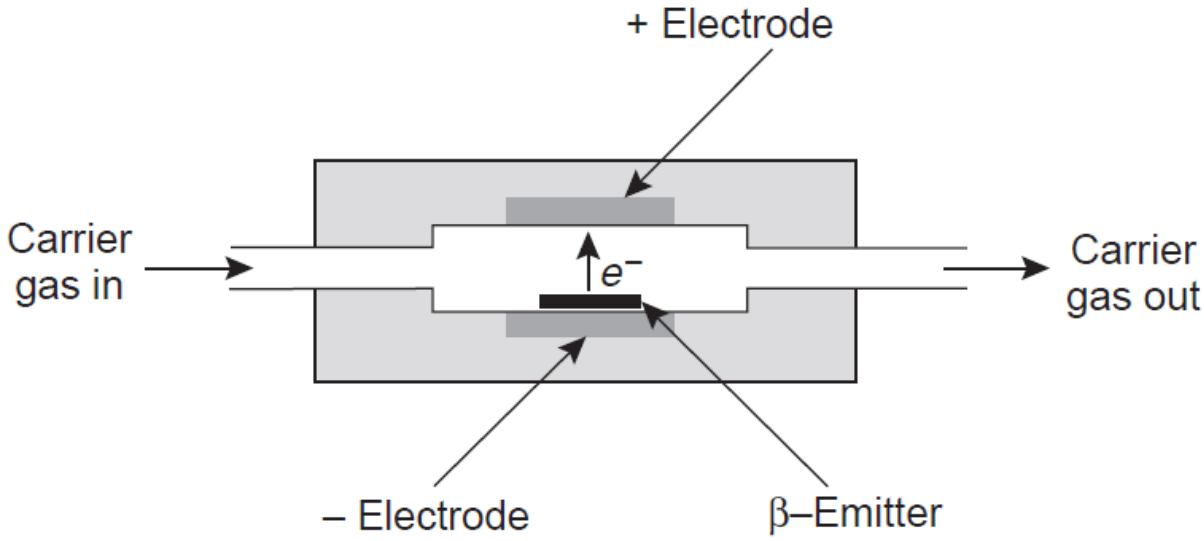
- TCD was one of the earliest detectors for GC and still finds wide application.
- TCD consists of an electrically heated source whose temperature at constant electric power depends on the thermal conductivity of the surrounding gas.



As the mobile phase exits the column, it passes over a tungsten–rhenium wire filament. The filament's electrical resistance depends on its temperature, which, in turn, depends on the thermal conductivity of the mobile phase. Because of its high thermal conductivity, helium is the mobile phase of choice when using a **thermal conductivity detector (TCD).** When a solute elutes from the column, the thermal conductivity of the mobile phase decreases and the temperature of the wire filament, and thus its resistance, increases. A reference cell, through which only the mobile phase passes, corrects for any time-dependent variations in flow rate, pressure, or electrical power, all of which may lead to a change in the filament's resistance.

A TCD detector has the advantage of universality, since it gives a signal for any solute whose thermal conductivity differs from that of helium. Another advantage is that it gives a linear response for solute concentrations over a range of 10^4–10^5 orders of magnitude. The detector also is nondestructive, making it possible to isolate solutes with a postdetector cold trap. Unfortunately, the thermal conductivity detector's detection limit is poor in comparison with other popular detectors.

Electron Capture Detector The electron capture detector is an example of a selective detector. The detector consists of a beta emitter (a beta particle is an electron) such as ^{63}Ni. The emitted electrons ionize the mobile phase, which is usually N_2, resulting in the production of additional electrons that give rise to an electric current between a pair of electrodes. When a solute with a high cross section for the capture of electrons elutes from the column, the electric current decreases. This decrease in electric current serves as the signal.



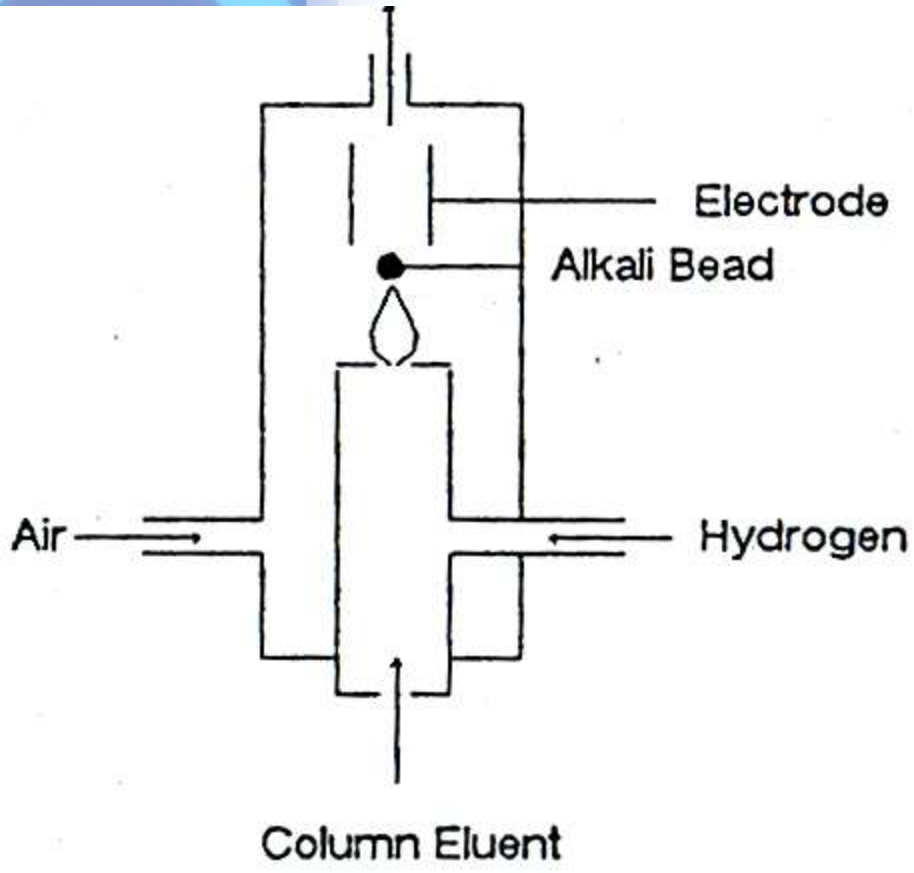
The ECD is highly selective toward solutes with electronegative functional groups, such as halogens, and nitro groups and is relatively insensitive to amines, alcohols, and hydrocarbons. Although its detection limit is excellent, its linear range extends over only about two orders of magnitude.

Thermionic Detector/Nitrogen-Phosphorous Detector (NPD)

A NPD is based on the same basic principles as an FID.

However, a small amount of alkali metal vapor in the flame, which greatly enhances the formation of ions from nitrogen and phosphorus-containing compounds.

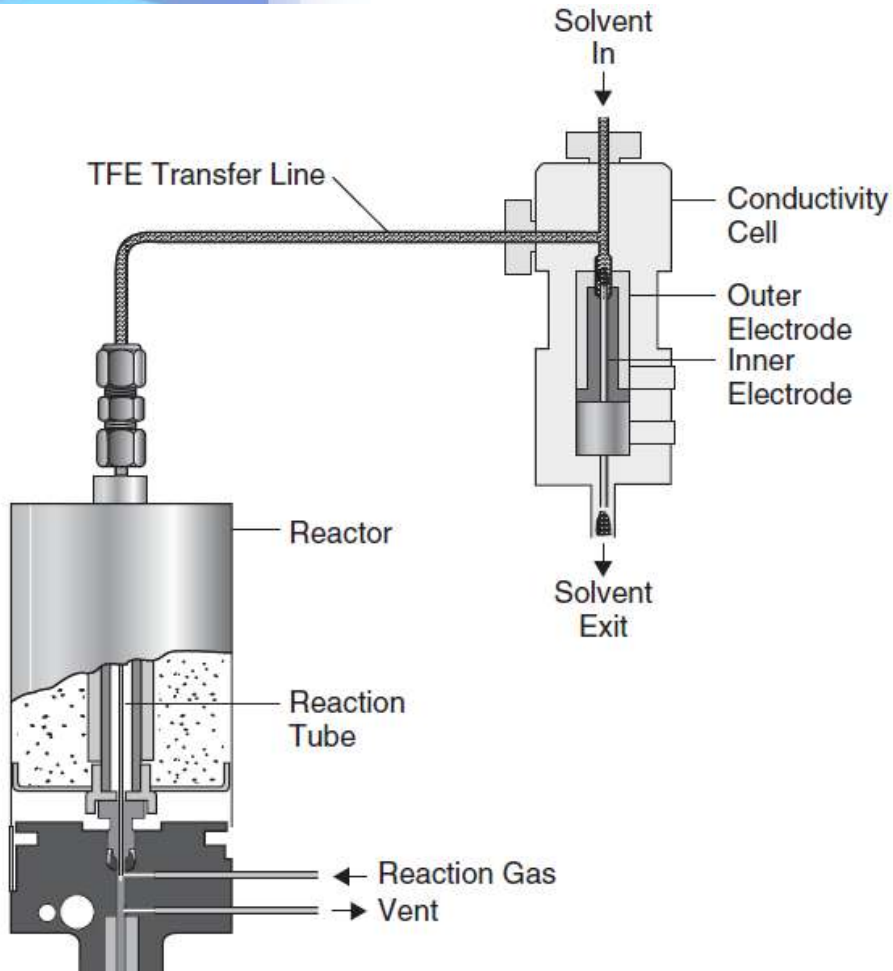
The NPD is about 500-fold more sensitive that an FID in detecting phosphorous-containing compounds, and 50-fold more sensitive to nitrogen-containing compounds



Applications: Organophosphate in pesticides and in drug analysis for determination of amine-containing or basic drugs

Electrolytic Conductivity Detector

• Element-selective detector for halogen-, sulfur- and nitrogen-containing compounds.



•Compounds containing halogens, sulfur, or nitrogen are mixed with a reaction gas in a small reactor tube, usually made of Ni. The products from the reaction tube are then dissolved in a liquid, which produces a conductive solution. The change in conductivity as a result of the ionic species is then measured.

FPD: Flame Photometric Detector

sample exits the analytical column into a hydrogen diffusion flame. Ions and atoms (excited) produced by organic compounds during combustion. When compounds are burned in the FPD flame, they emit photons of distinct wavelengths photons that are within the frequency range of the filter specifications can pass through the filter to the PMT. The PMT generates a proportional analog signal (***current)*** uses band pass filters over the PMT to selectively detect compounds containing S and P.

Two additional detectors are similar in design to a flame ionization detector. In the ***flame photometric detector*** optical emission from phosphorus and sulfur provides a detector selective for compounds containing these elements. The ***thermionic detector*** responds to compounds containing nitrogen or phosphorus.

Two common detectors, which also are independent instruments, are ***Fourier transform infrared spectrophotometers (FT–IR) and mass spectrometers (MS). Photoionization Detector-aromatic hydrocarbons organosulfur/organophosphorous. Atomic Emission Detector -*** element-selective detector.

Mass Spectrometry Detector (MS)

One of the most powerful detectors for gas chromatography

•**SAVE FOR LATER**

What Compounds Can Be Determined by GC?

Many, many compounds may be determined by gas chromatography, but there are limitations. They must be volatile and stable at operational temperatures, typically from 50 to 300°C. GC is useful for:

- All gases
- Most nonionized organic molecules, solid or liquid, containing up to about 25 carbons
- Many organometallic compounds (volatile derivatives of metal ions may be prepared)

If compounds are not volatile or stable, often they can be derivatized to make them amenable to analysis by GC. GC cannot be used for macromolecules nor salts, but these can be determined by HPLC and ion chromatography.

The principal ***advantages*** of GC are enumerated below, namely:

• It has high frequency of separation and even complex mixtures may be adequately resolved into constituents,

• It has a very high degree of sensitivity in detection of components *i.e., only a few mg of sample is* enough for complete analysis,

• Speed of analysis is quite rapid,

• Gives reasonably good accuracy and precision,

• The technique is fairly suitable for routine analysis because its operation and related calculations do not require highly skilled personnel, and The overall cost of equipment is comparatively low and its life is generally long.

Disadvantages of GC

• Limited to volatile samples

– T of column limited to ~ 380 °C

– Need Pvap of analyte ~ 60 torr at that T

– Analytes should have b.p. below 500 °C

• Not suitable for thermally labile samples

• Some samples may require intensive preparation

– Samples must be soluble and not react with the column

• Requires spectroscopy (usually MS) to confirm the peak identity