GAS CHROMATOGRAPHY

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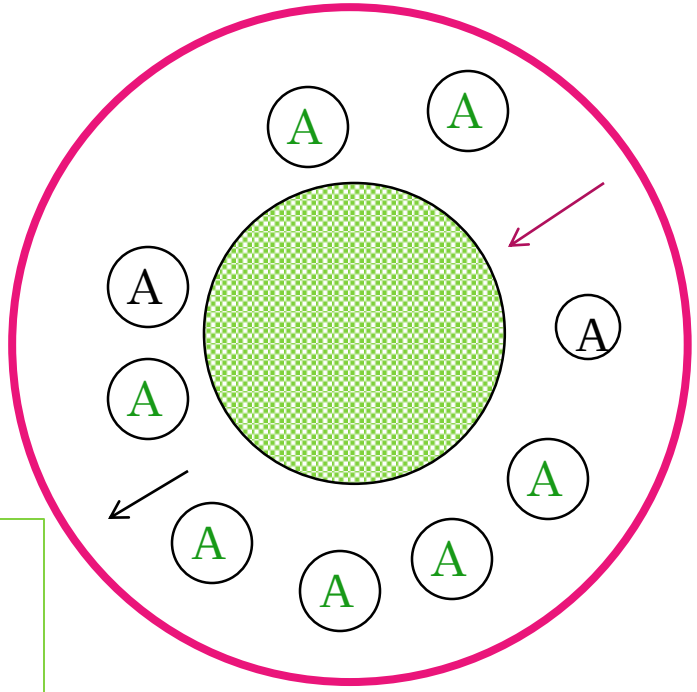
Chromatography is based on a physical equilibrium that results when a solute is transferred between the mobile phase and a stationary phase.

K = distribution coefficient or partition ratio

$$K = \frac{C_S}{C_M}$$

Where C_S is the molar concentration of the solute in the stationary phase and

C_M is the molar concentration of solute in the mobile phase.

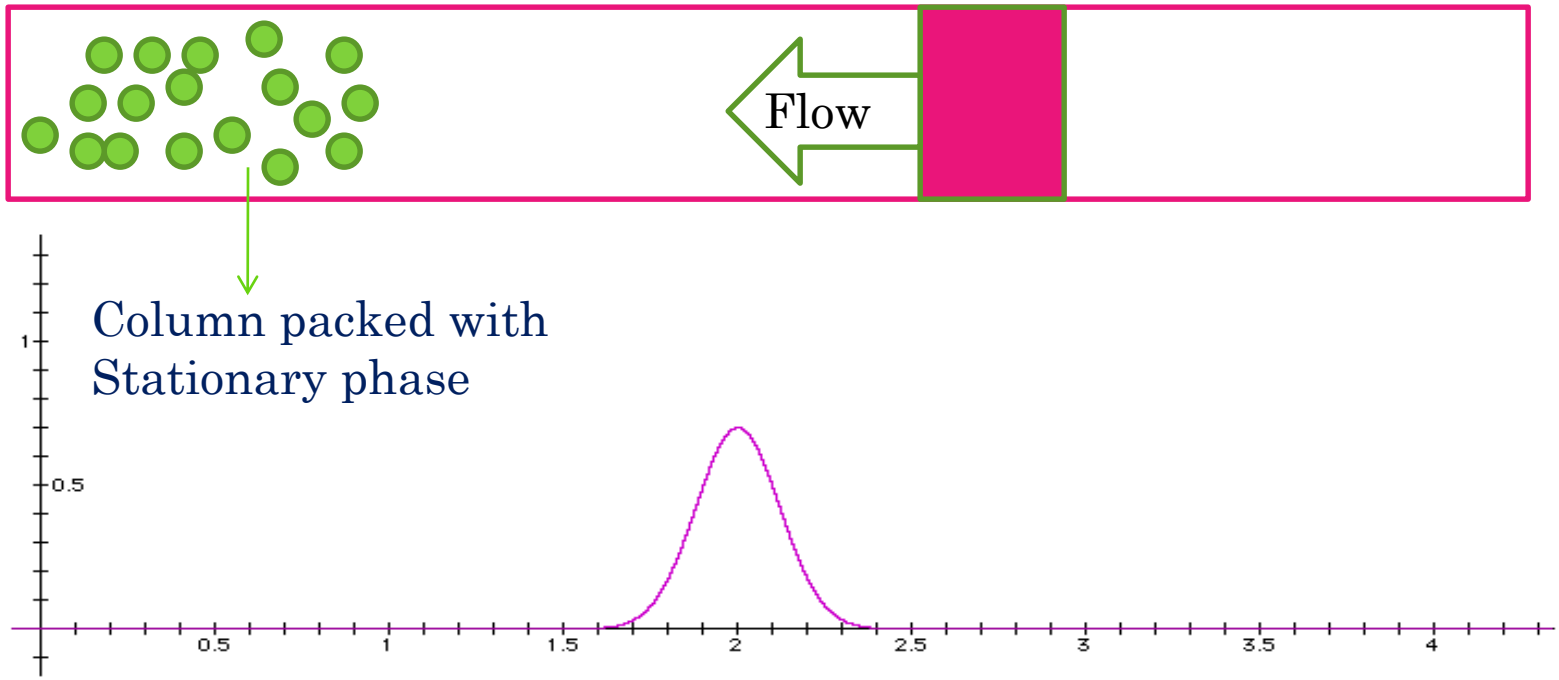


Cross Section of Equilibrium in a column.

"A" are adsorbed to the stationary phase.

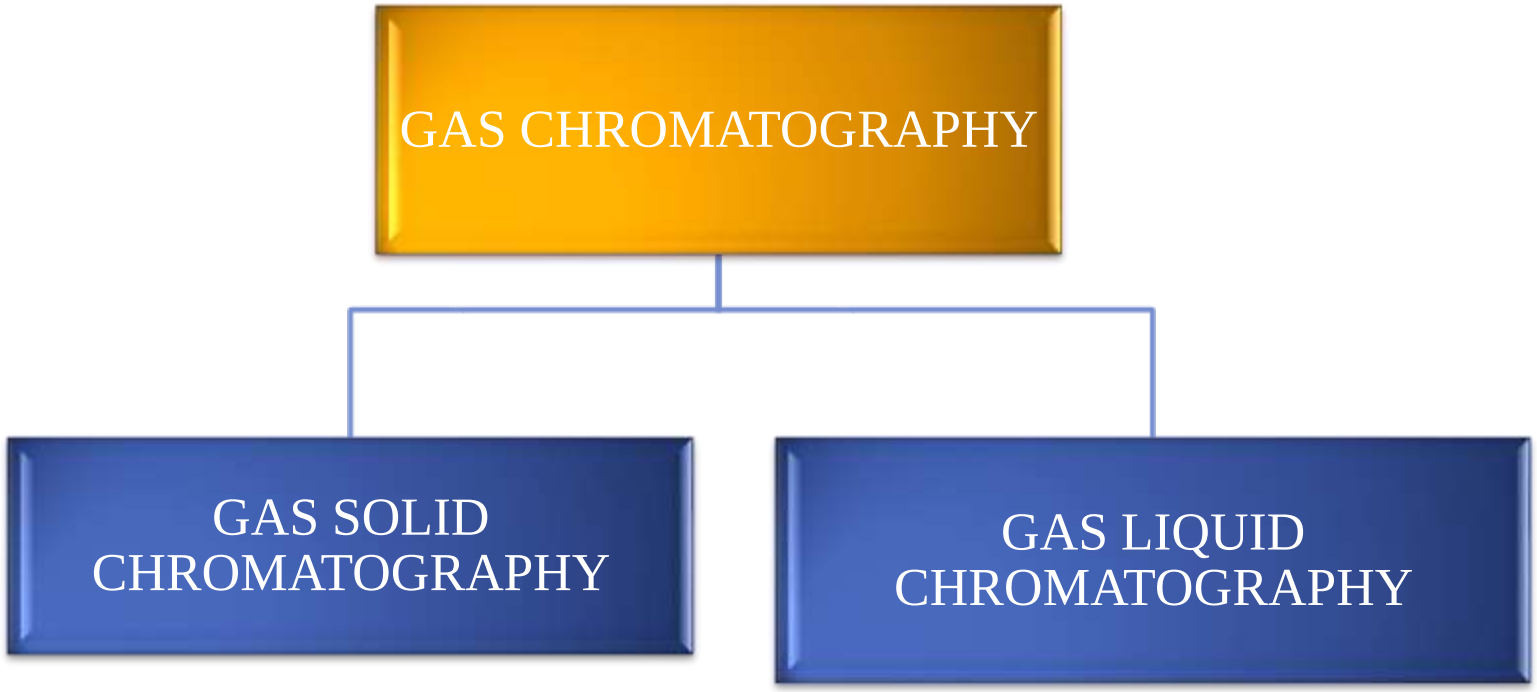
"A" are traveling in the mobile phase.

In a chromatography column, flowing gas or liquid continuously replaces saturated stationary phase and results in movement of sample through the column.



As a material travels through the column, it assumes a Gaussian concentration profile as it distributes between the stationary packing phase and the flowing mobile gas or liquid carrier phase.

Gas chromatography is probably the most utilized of the chromatographic techniques.



In both types, gas is used as mobile phase and either solid or liquid is used as stationary phase.

GAS SOLID CHROMATOGRAPHY

- Based upon the solid stationary Phase in which retention of analytes is a consequence of Physical adsorption.
- Limited application owing to semi permanent retention of active or polar molecules and severe tailing of elution peaks.
- Thus this technique has not found wide application except for the separation of certain low-molecular-weight gaseous species.

First enunciated in 1941 by Martin and Synge.

GAS LIQUID CHROMATOGRAPHY

- Based on the partition of the analyte between a gaseous mobile phase and a liquid phase immobilized on the surface of an inert solid.
- It is applicable to substances or their derivatives which are volatilized under the temperatures employed.
- GC is based on mechanisms of adsorption, mass distribution or size exclusion

PRINCIPLE OF SEPARATION

STATIONARY PHASE

- Liquid coated on to the solid support(e.g., diatomaceous earth)

MOBILE PHASE

- GAS

PARTITION-COEFFICIENT

- Ratio of solubility of the substance distributed between two immiscible liquids at a constant temperature.

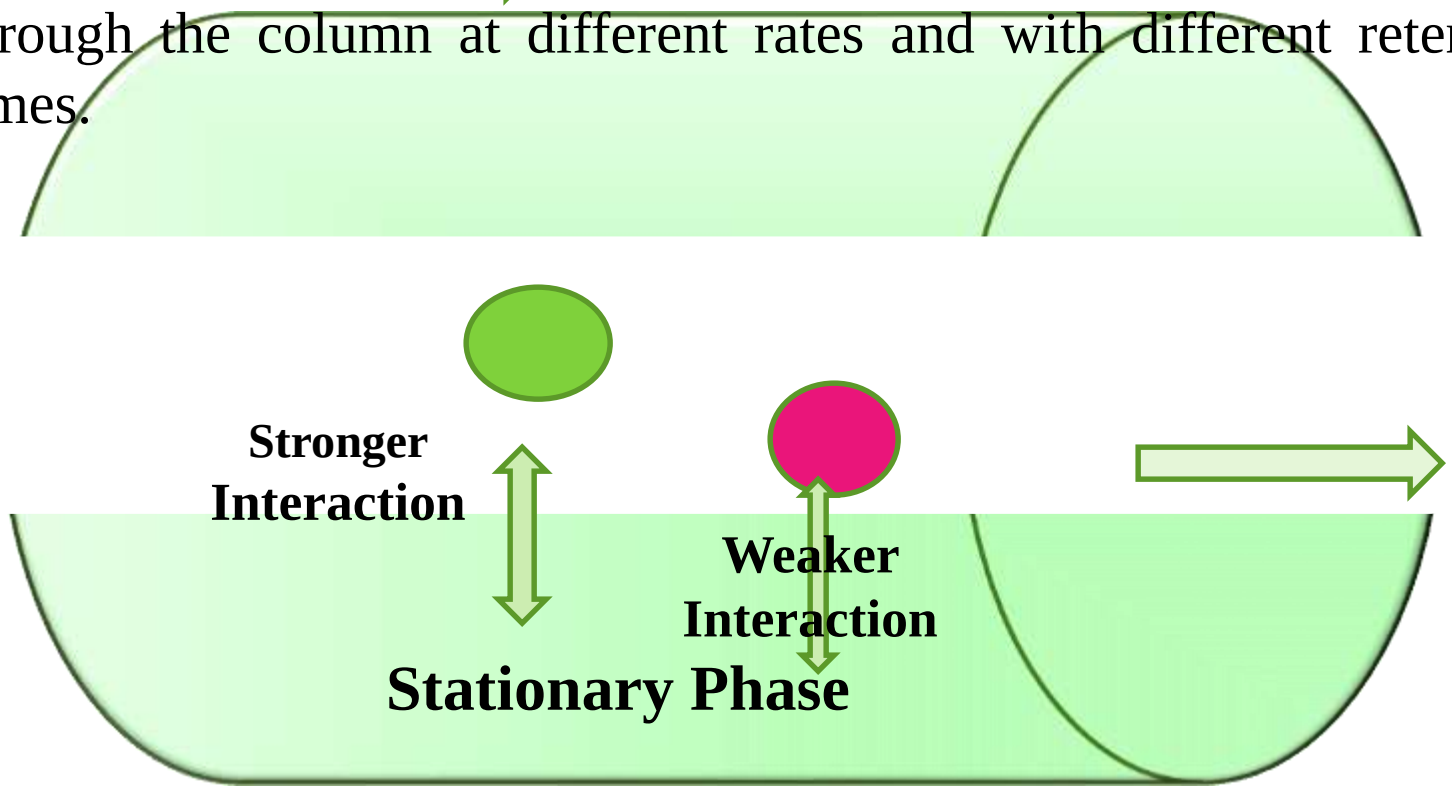
Typically the stationary phase has a sufficient low pressure (mm) at the column temperature so that it can be considered as a non-volatile.

$$\text{Partition - coefficient} = \frac{\text{Amount of solute in the stationary phase}}{\text{Amount of solute in the mobile phase}}$$

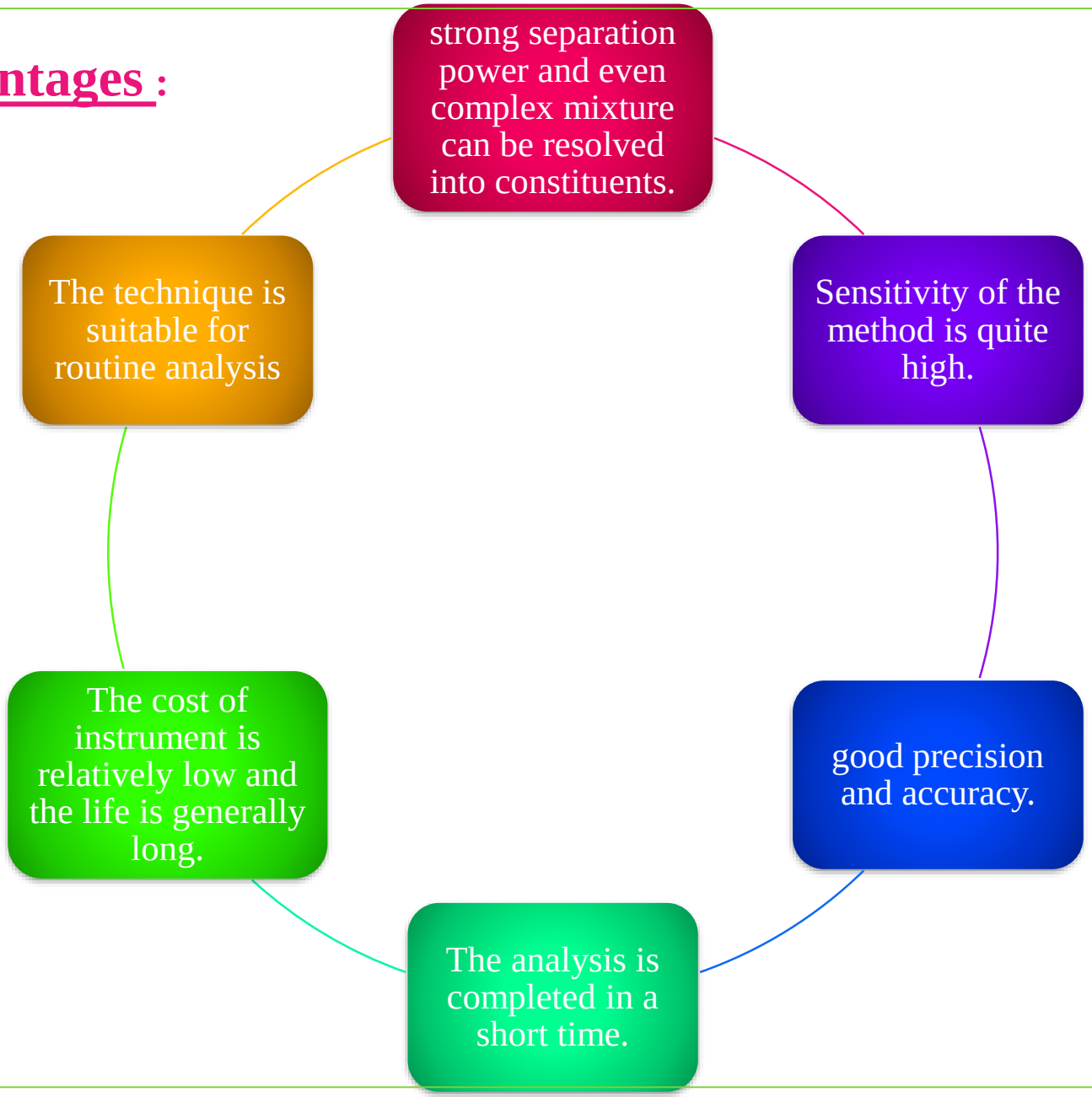
Schematic representation of Separation

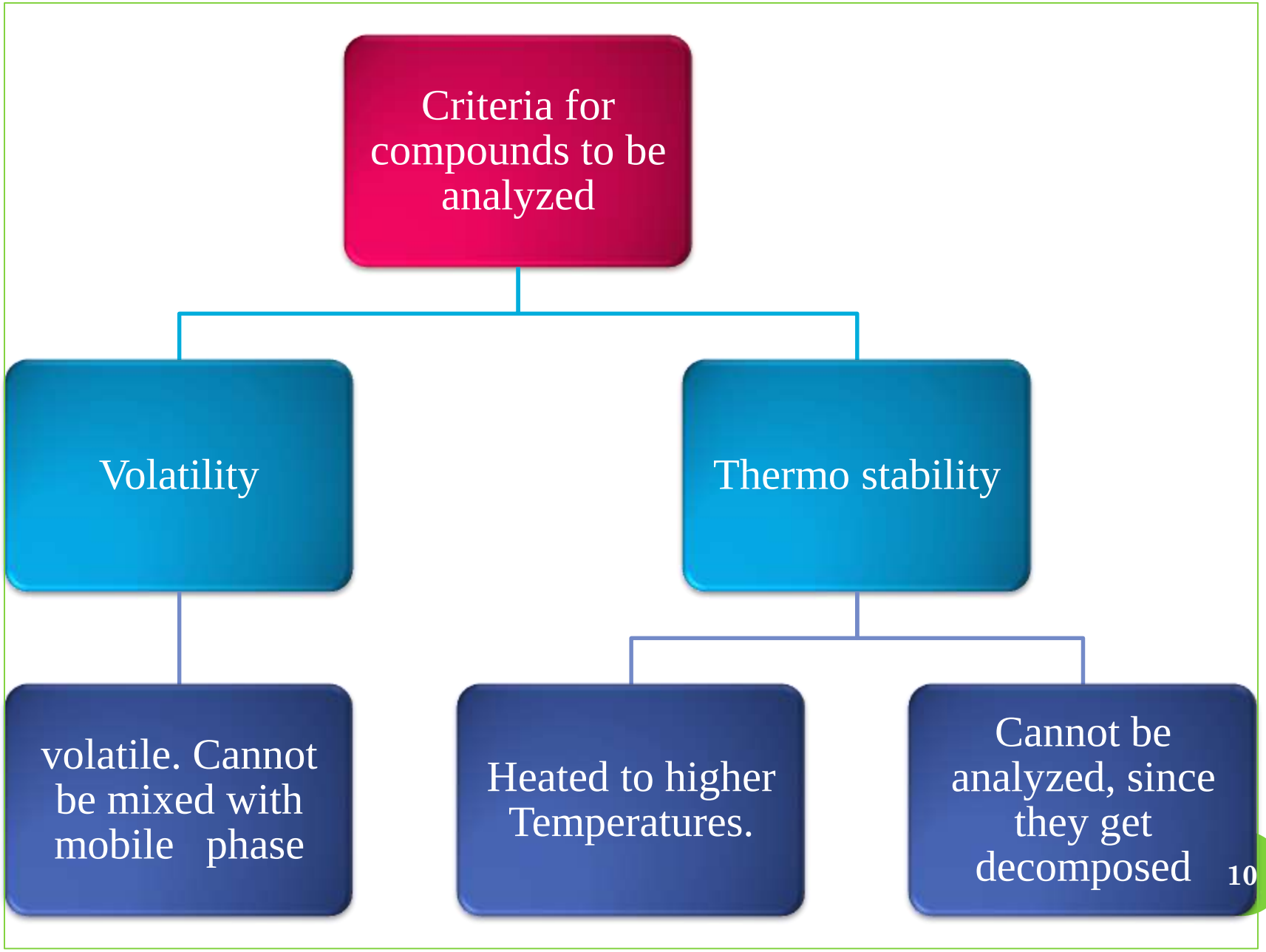
The sample mixture in gaseous form is run through the column with a carrier gas.

Separation can be achieved by the differences in the distribution ratios of the components of the sample between the mobile (gaseous) and stationary (liquid) phases causing them to move through the column at different rates and with different retention times.



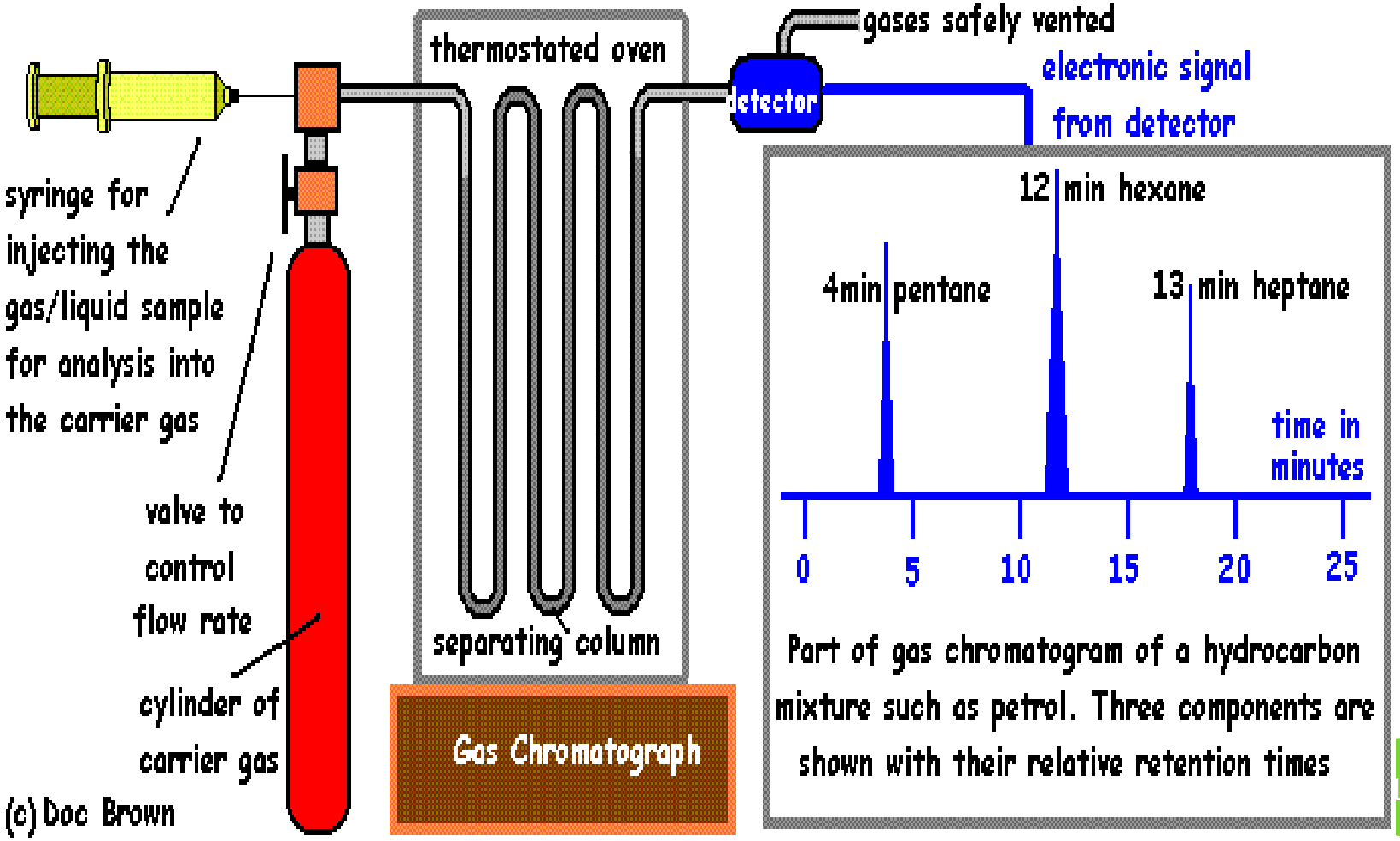
Advantages :

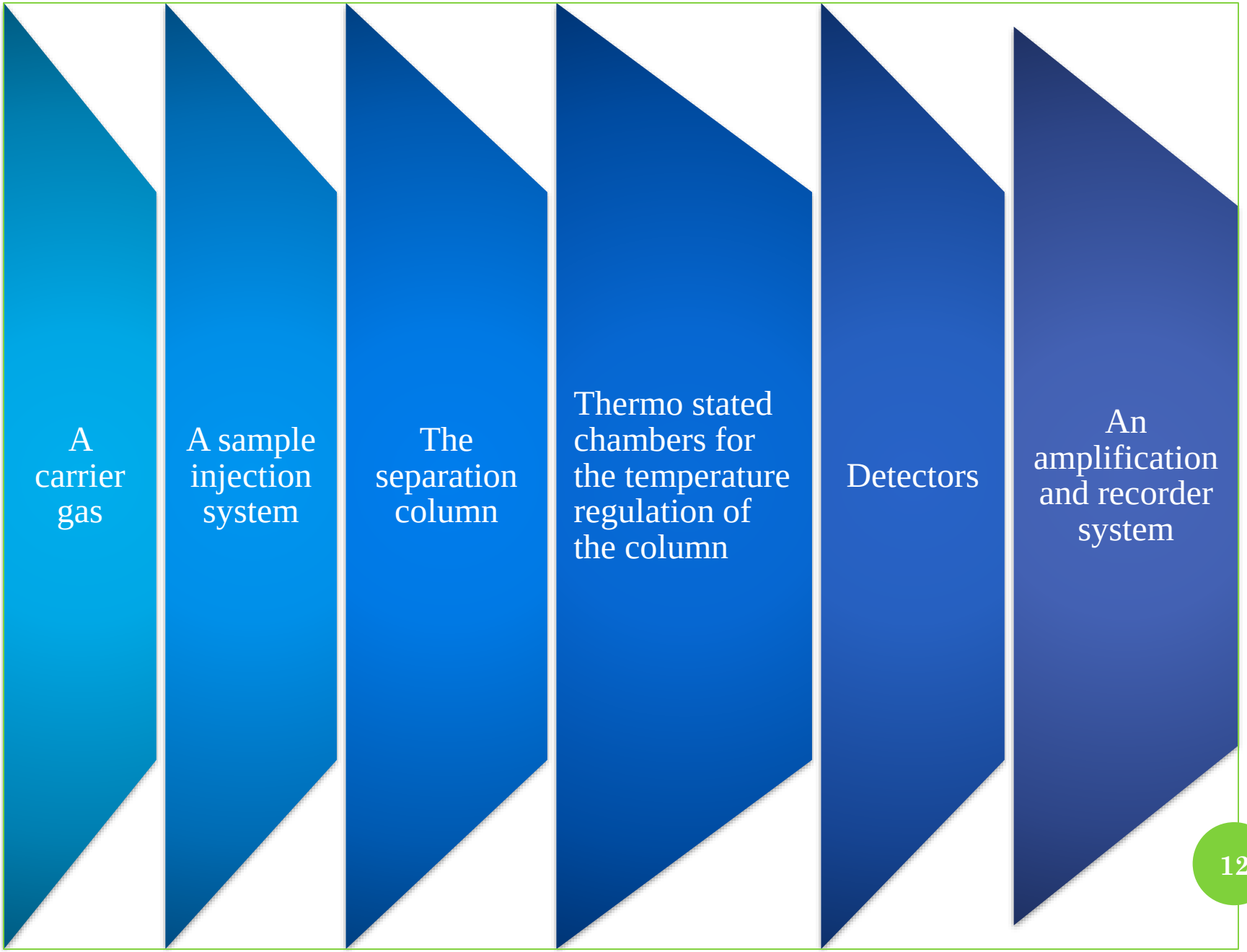

strong separation power and even complex mixture can be resolved into constituents.
Sensitivity of the method is quite high.
good precision and accuracy.
The analysis is completed in a short time.
The cost of instrument is relatively low and the life is generally long.
The technique is suitable for routine analysis


Criteria for compounds to be analyzed
Volatility
Thermo stability
volatile. Cannot be mixed with mobile phase
Heated to higher Temperatures.
Cannot be analyzed, since they get decomposed

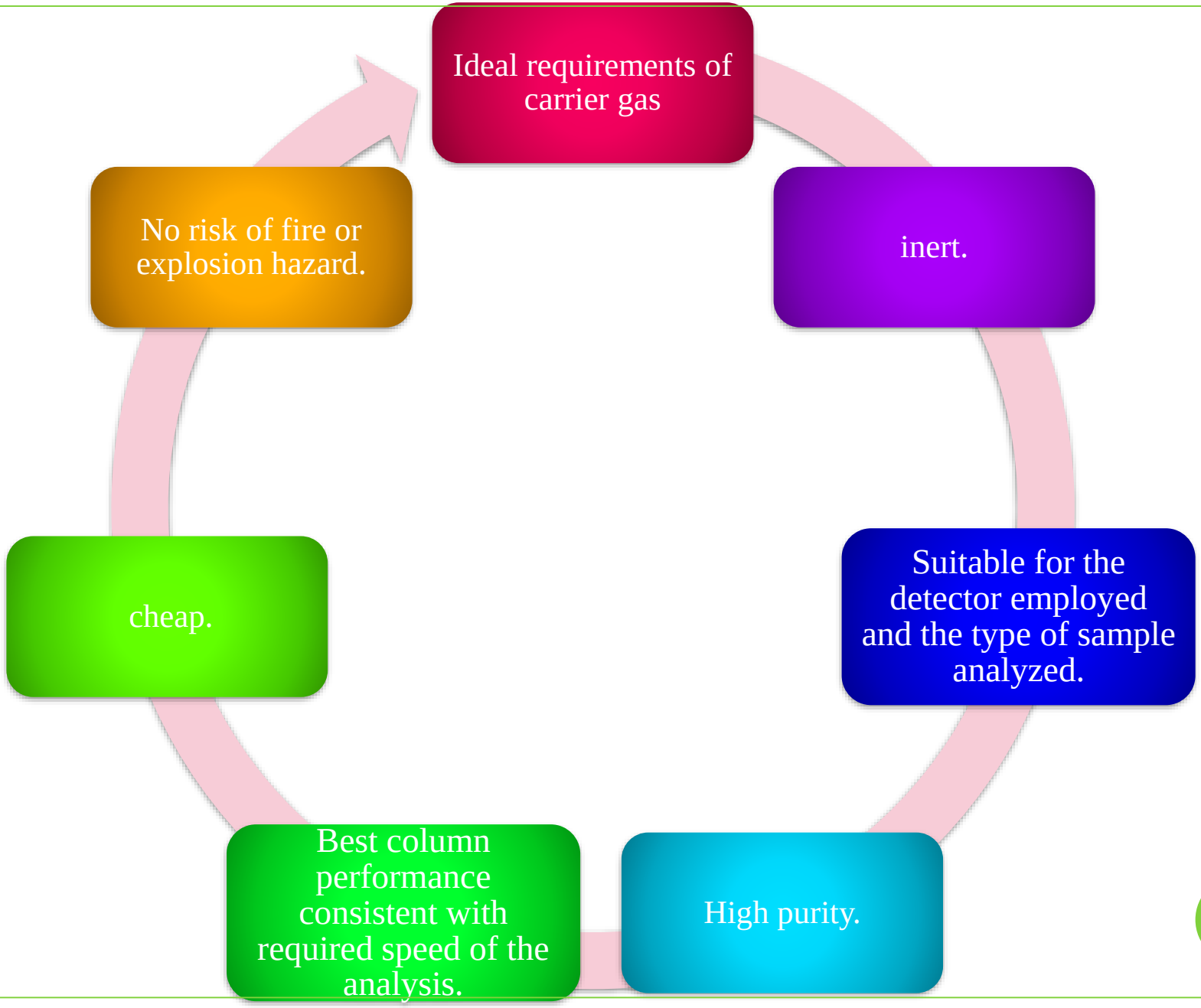
Instrumentation

Essential components of the apparatus used for the Gas Chromatography :




A carrier gas
A sample injection system
The separation column
Thermo stated chambers for the temperature regulation of the column
Detectors
An amplification and recorder system

Hydrogen	• Better thermal conductivity , lower density and greater flow rates. But is it highly Inflammable.
Helium	• Excellent thermal conductivity, inertness, low density and it allows greater flow rates. But it is very expensive.
Nitrogen	• Inexpensive but gives reduced sensitivity.
Air	• when the oxygen in the air is useful to the detector or separation.
Oxygen	• Avoided since it oxidizes the stationary phase.


Ideal requirements of carrier gas
inert.
Suitable for the detector employed and the type of sample analyzed.
High purity.
Best column performance consistent with required speed of the analysis.
cheap.
No risk of fire or explosion hazard.

Pyrolysis Gas Chromatography is employed for substances that are not volatile and cannot be easily derivatized to volatile forms.

The solid is heated in a controlled way to break it into smaller, more volatile pieces separated by the column to form a pyrogram, which can be matched to known standards.

The sample is placed directly into a small coil of platinum wire so that it can be heated to several hundred degrees in few seconds while the carrier gas is flowing over it.

Better temperature control can be achieved by the use of a heater of nickel or other magnetic material operated at its Curie point.

Sample Introduction System

SOLIDS	GASES	LIQUIDS
Must be dissolved in volatile liquids for introduction directly if they can be liquefied.	Require special gas sampling valves for introduction into the carrier gas stream.	Hypodermic syringe through a self-sealing rubber septum into small inlet chamber, which may be heated to cause flash evaporation.
		The temperature must not be so high that it decomposes the sample.

FLOW METERS & FLOW REGULATORS

FLOW REGULATORS

Deliver the gas with uniform pressure of flow rate

FLOW METERS

Measure the flow rate of carrier gas.

Eg : ROTAMETER ,Soap Bubble meter

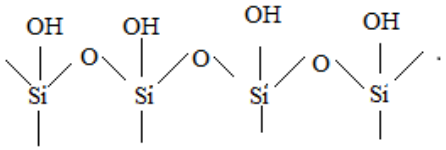
The Stationary Phase:

- Low volatility (ideally, the boiling point of the liquid should be at 100^0 C higher than the maximum operating temperature for the column)
- Thermally stable
- Chemical inert towards the sample

Stationary Phase	Maximum Temperature
Polydimethyl siloxane	350
Poly (biphenyl dimethyl) siloxane	350
Polyalkalene glycol	250
Polyethylene glycol	250
Poly (dicyanoallyldimethyl) Siloxane	240

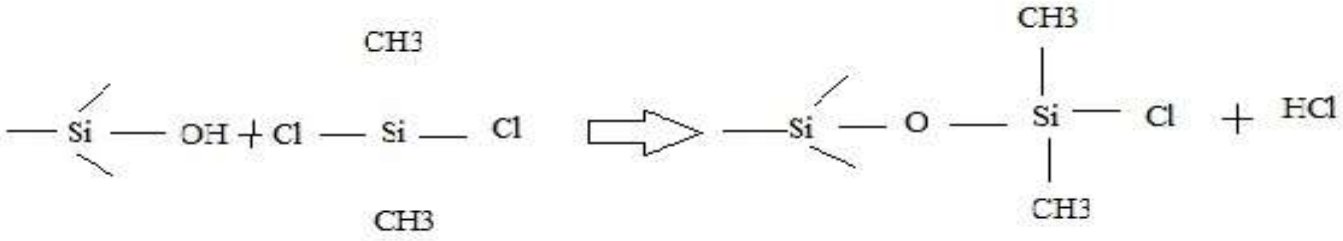
Adsorption on column Packing or Capillary walls

- A problem that has overwhelmed gas chromatography from its beginning has been the physical adsorption of polar or Polarizable analyte species, such as alcohols or hydrocarbons, on the silicate surfaces of the column packings or capillary walls.
- Adsorption results in distorted peaks, which are broadened and often exhibit a tail.
- It has been established that adsorption is the consequence of silanol groups that form on the surface of silicates by reaction with moisture.
- Thus, a fully hydrolyzed silicate surface has the structure



The SiOH groups on the support surface have a strong affinity for polar organic molecules and tend to retain them by adsorption

Support materials can be deactivated by silanization with dimethylchlorosilane. The reaction is



Silanized surfaces of column packing may still show a residual adsorption, which apparently arise from metal oxide impurities in the diatomaceous earth. Acid washing prior to silanization removes these impurities.

DERIVATIZATION :

Derivatization prior to gas chromatography is often desirable to improve the process of separation by column or detection by detector.

There are two types of Derivatization based upon its need:

Pre Column Derivatization

- Improve the thermal stability of compounds, particularly compounds that contain polar functional groups
- Change the separation properties of compounds by the purposeful adjustment of their volatility.

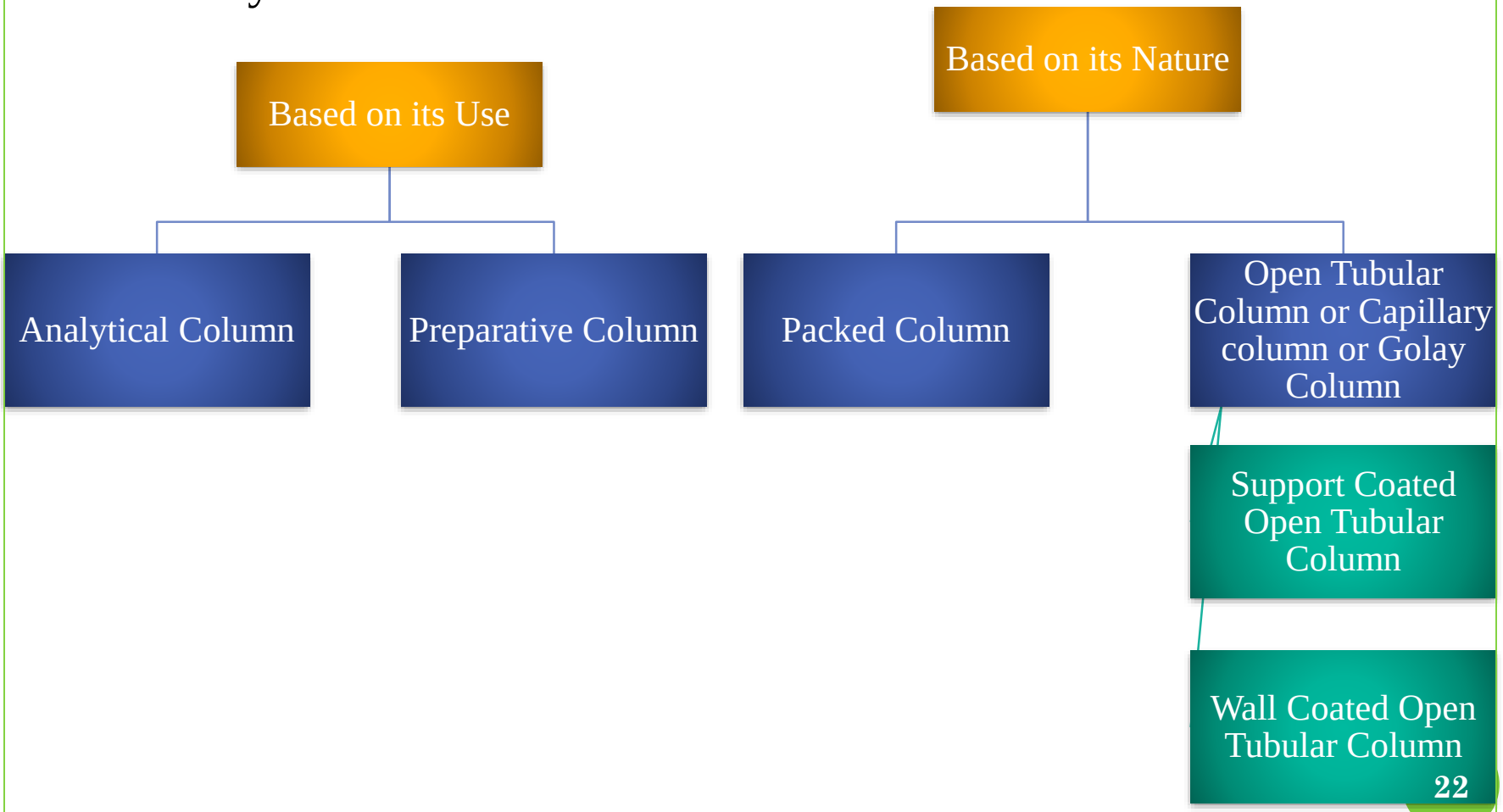
  silylating agents, such as N,O-bis(trimethylsilyl)acetamide convert one active hydrogen in polar groups such as -OH, -COOH, $-NH_2$, =NH , and -SH to an -O- $Si(CH_3)_3$ group.

Post Column Derivatization

- Improve the response shown by the detector.
- The components are converted in such a way that their ionization or affinity towards electrons is increased.

COLUMNS

Column is one of the important part which decides the separation efficiency.



Columns:

The column can be constructed of glass or metal tubing .

length from a few centimeters to over a hundred meters and can be coiled, bent or straight.

Packed Column:

Packed columns are prepared by packing metal or glass tubing's with granular stationary phase.

For Gas Solid Chromatography the columns are packed with **size graded adsorbent or porous polymer,** whereas for

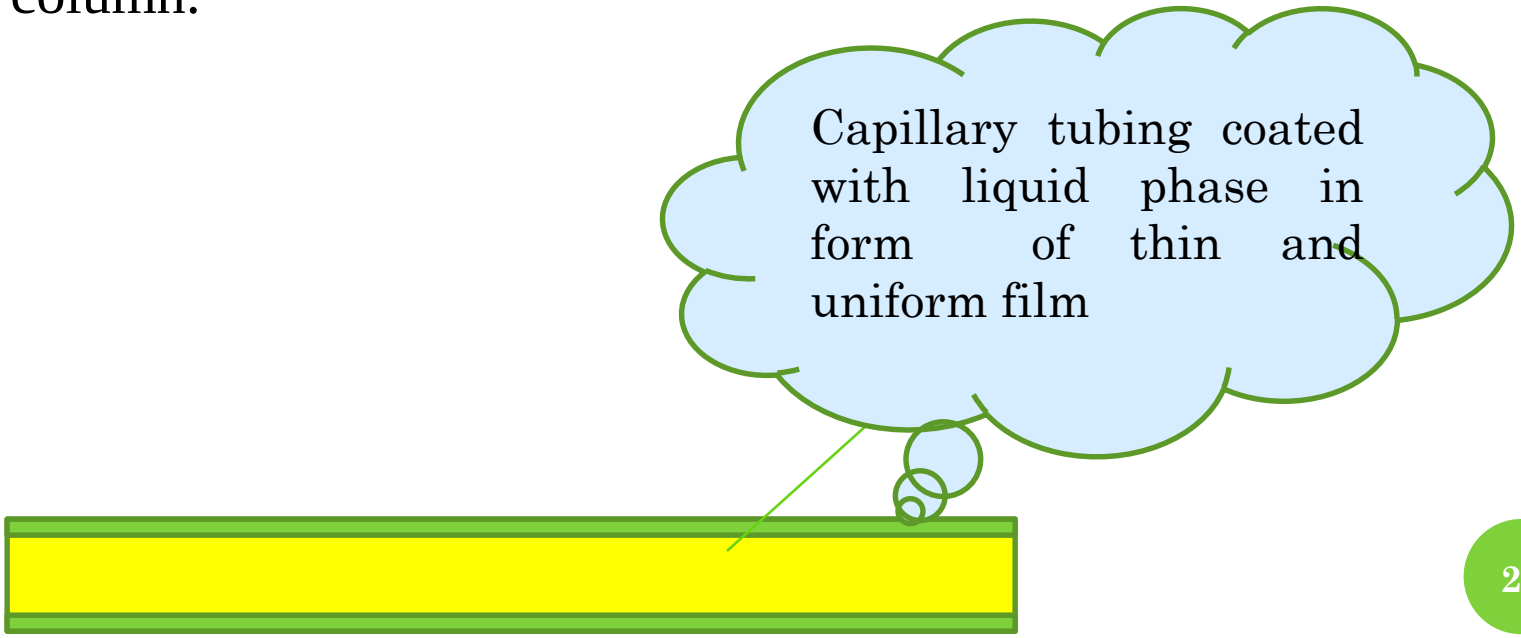
Gas Liquid Chromatography the packing is prepared by **coating the liquid phase over a size graded inert solid support.**

The advantages of porous polymer as packing material :

- Porous polymer beads are mechanically strong and can be easily packed on columns.
- There is no column bleed.
- Most of the porous polymer are stable at 250^0C and cause no base line drift and therefore allows a use of highly sensitive detector.
- There is no adsorption of polar compounds such as water, alcohols or acids and they are eluted rapidly as sharp symmetrical peaks.
- Thus it is quite useful in preparative and trace work analysis because Sample over load recovery is rapid and tailing is not seen.
- Retention data are highly reproducible.

Wall-Coated Open tubular Columns:

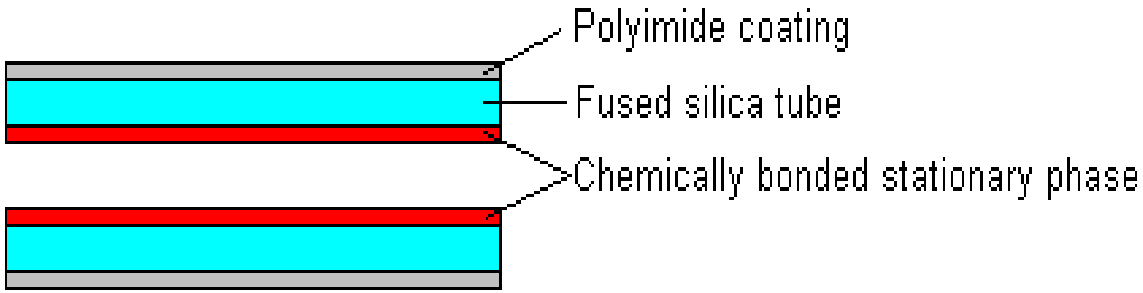
- Long capillary tubing (30-90 meters)
- Uniform and narrow internal diameter (0.025-0.075 cm).
- Made of stainless steel, copper, nylon, or glass etc., the stainless steel being the most popular.
- Carrier gas flow faces least resistance because there is no packing in the column.



Fused-Silica Open Tubular Column:

- Fused-silica capillaries are drawn from specially purified silica that contains minimal amounts of metal oxides.
- These capillaries have much thinner walls than their glass counterparts.
- The tubes are given added strength by an outside protective polyimide coating, which is applied as the capillary tubing.
- The resulting columns are quite flexible and can be bent into coils having diameters of few inches.

Cross section of a Fused Silica Open Tubular Column

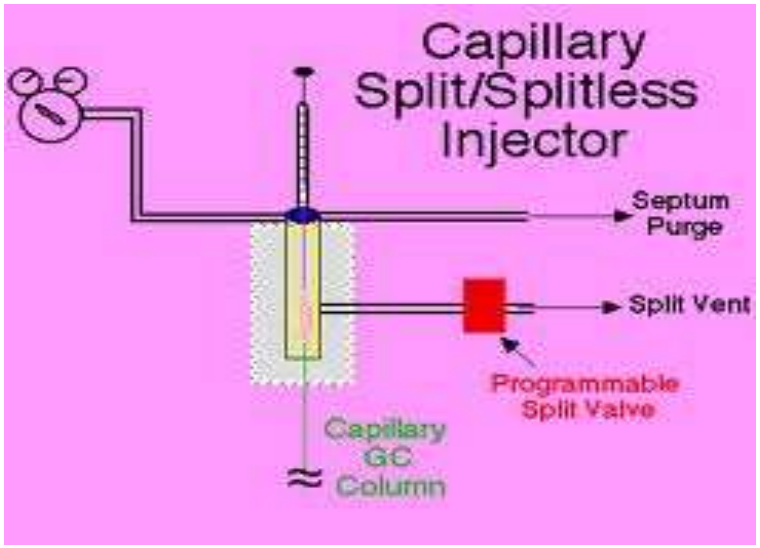


Adantages:

- Offer several important advantages such as physical strength, lower reactivity and flexibility towards sample Components

Disadvantages

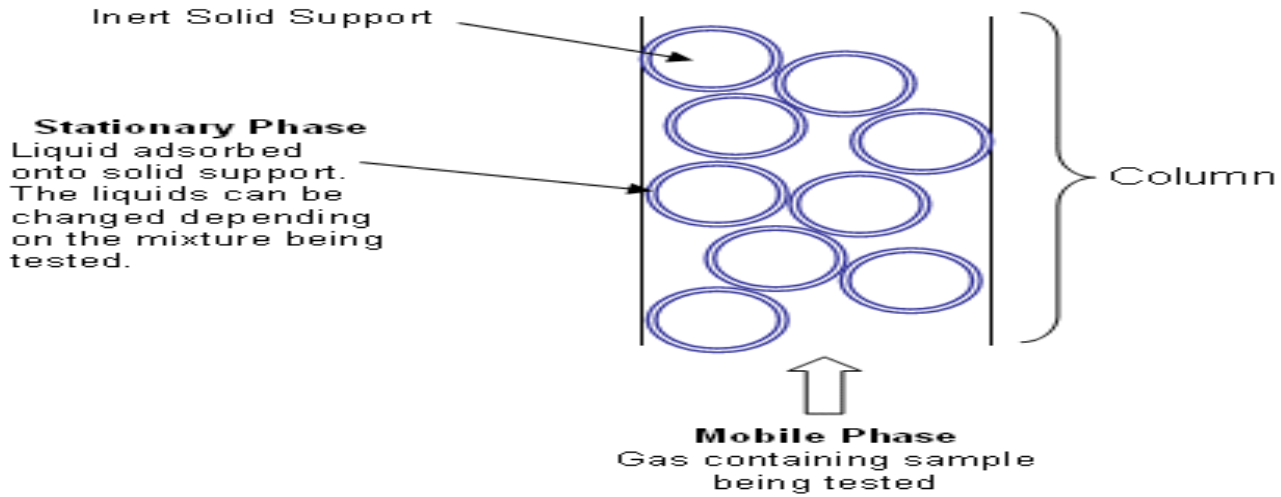
- Troublesome to use and are more demanding of the injection and detection systems.
- Thus, a sample splitter must be used to reduce the size of the sample injected onto the column and a more sensitive detector system with a rapid response time is required.



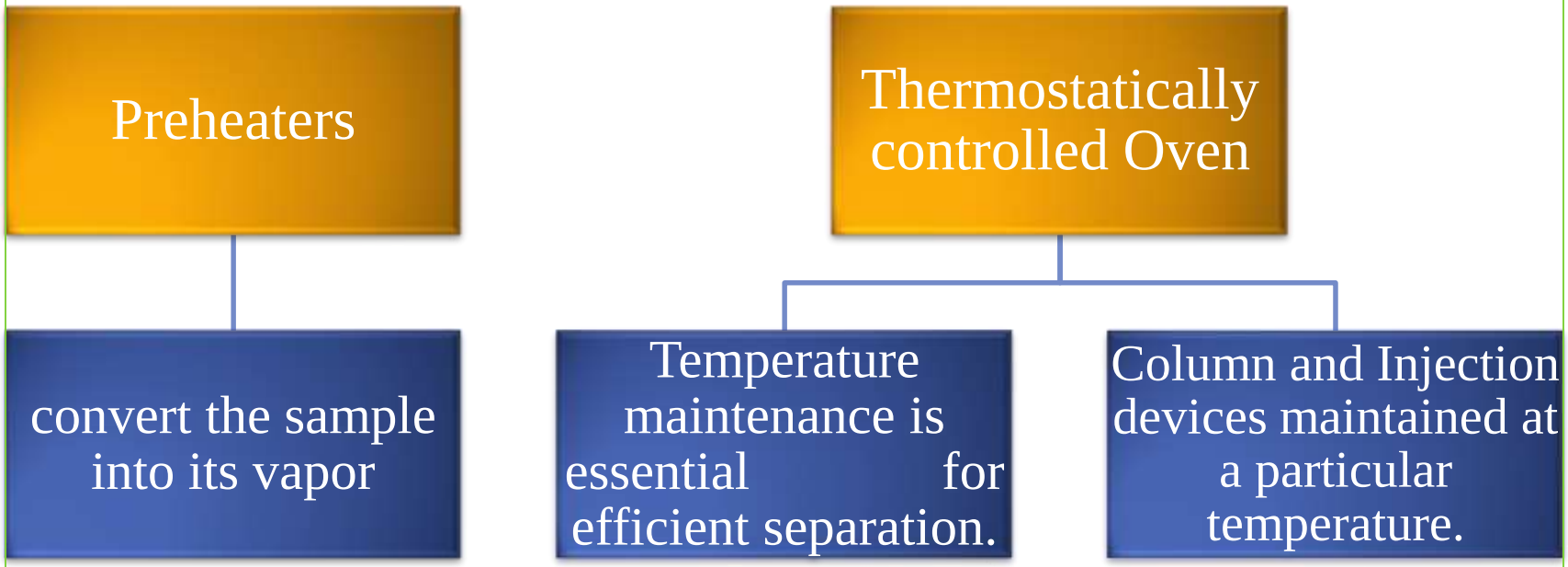
Support Coated open Tubular columns

- prepared by depositing a micron size porous layer of support material on the inside wall of a capillary column and then coated with a thin film of liquid phase.
- These columns have more sample capacity and an inlet splitter may not be required.
- Support Coated Open Tubular is preferred for trace analysis.

Cross section of column with micron size porous polymer



TEMPERATURE CONTROL DEVICES



Types of operation performed

Isothermal Programming

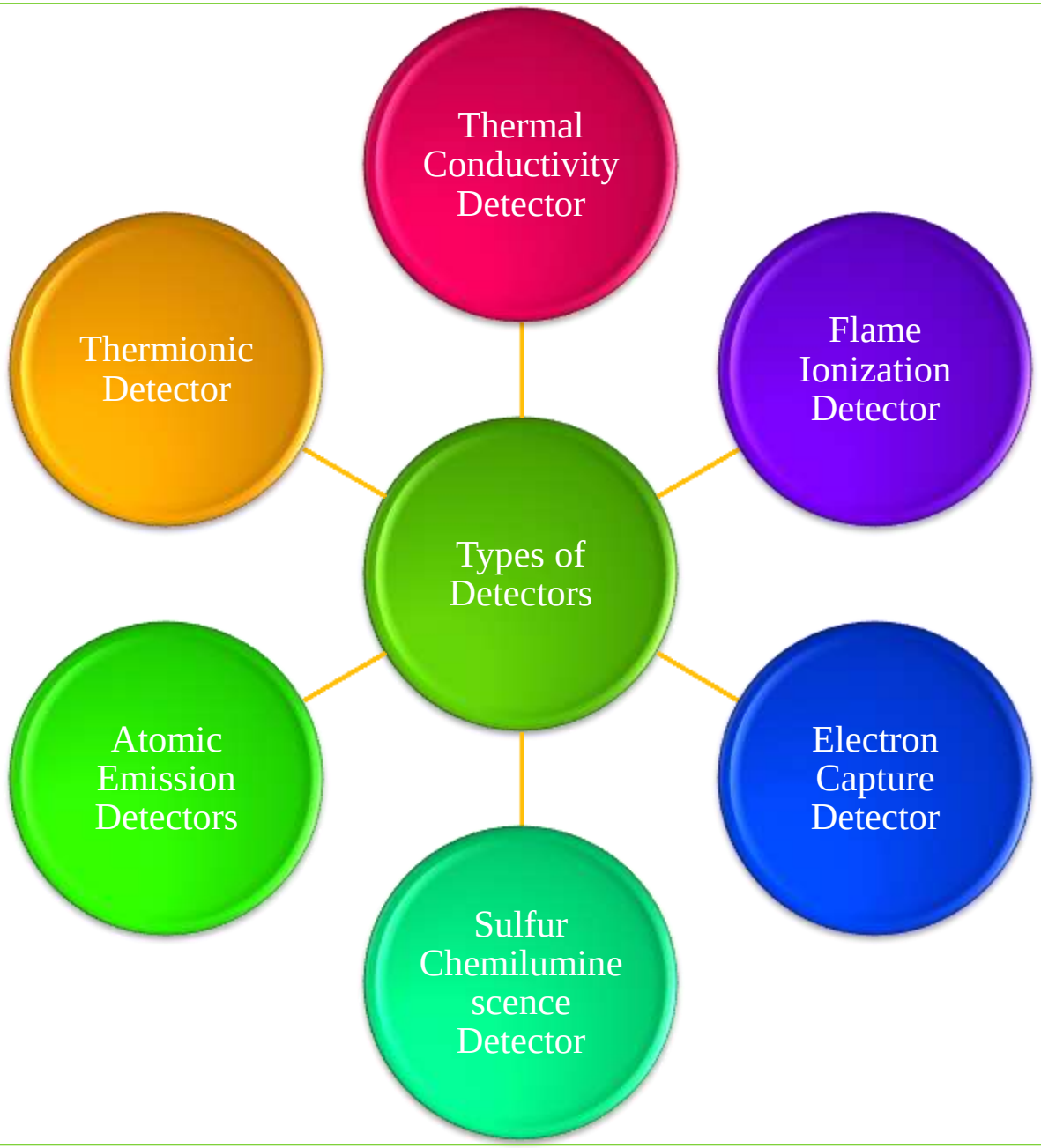
same temperature is maintained throughout the process of separation.

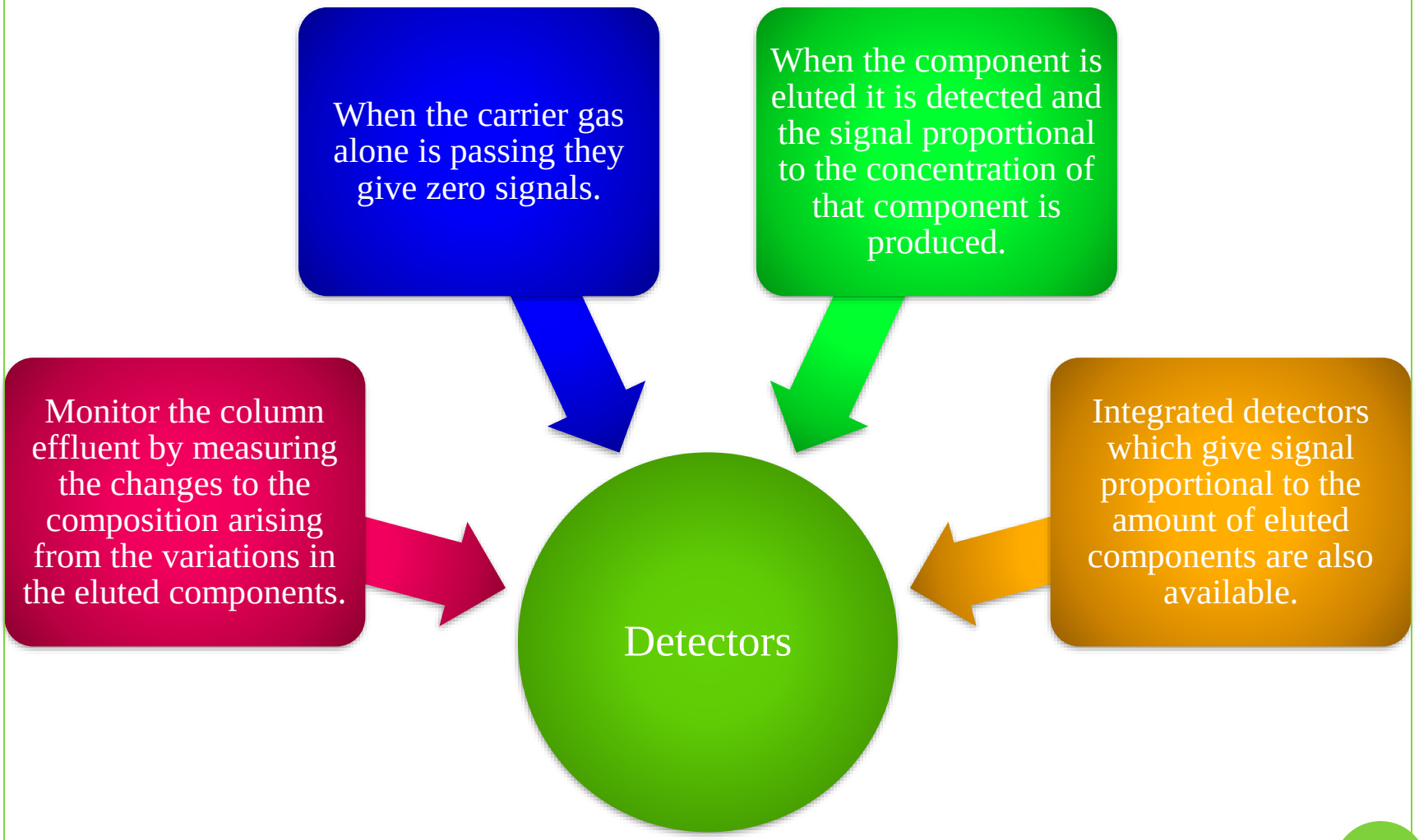
Linear Programming

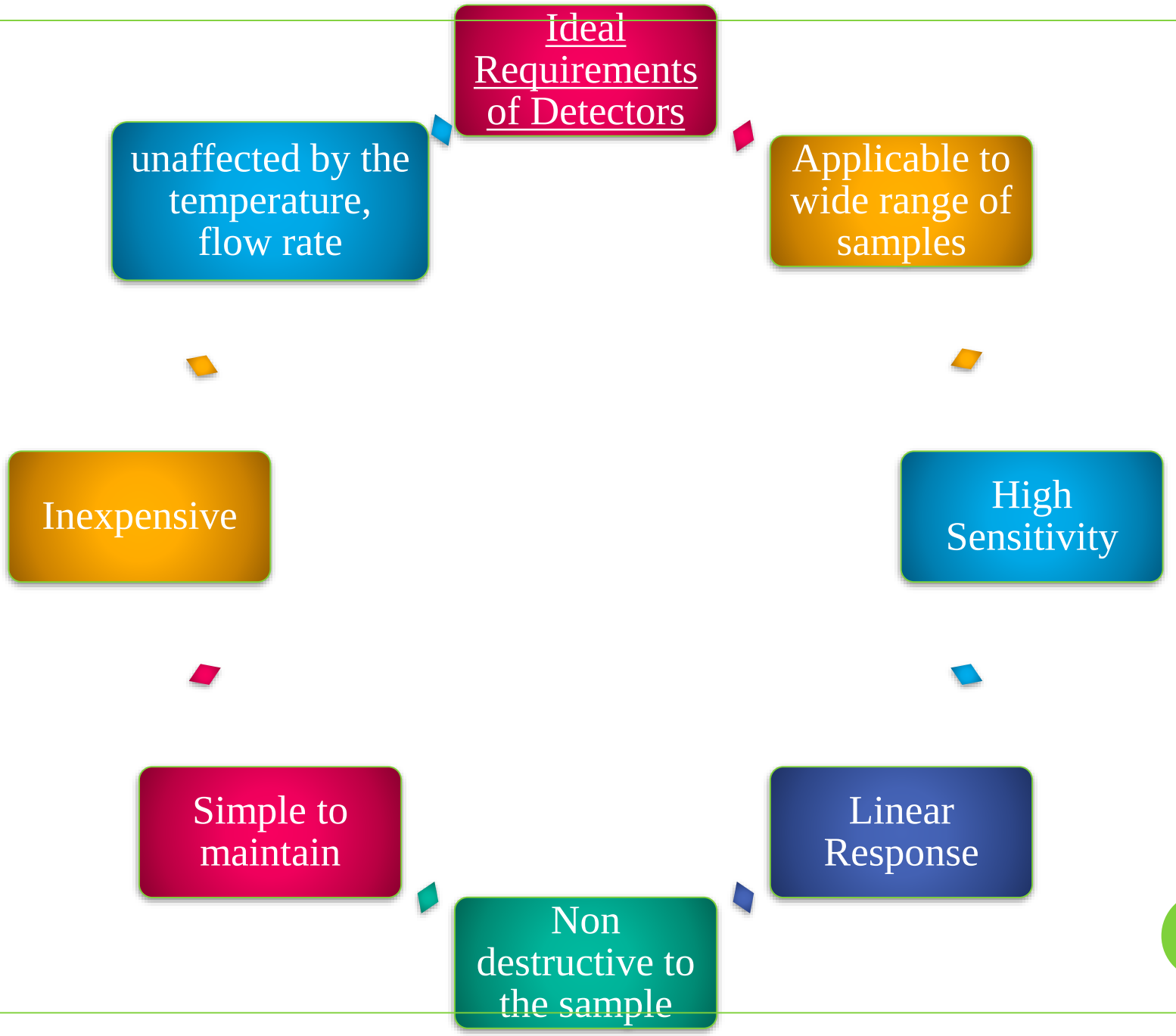
oven is heated over a period of time.

Required when a sample has a mixture of low boiling and high boiling point compounds.

Efficient for separation of complex mixtures


Thermal Conductivity Detector
Thermionic Detector
Flame Ionization Detector
Types of Detectors
Atomic Emission Detectors
Electron Capture Detector
Sulfur Chemilumine scence Detector


When the carrier gas alone is passing they give zero signals.
When the component is eluted it is detected and the signal proportional to the concentration of that component is produced.
Monitor the column effluent by measuring the changes to the composition arising from the variations in the eluted components.
Detectors
Integrated detectors which give signal proportional to the amount of eluted components are also available.


Ideal Requirements of Detectors
unaffected by the temperature, flow rate
Applicable to wide range of samples
Inexpensive
High Sensitivity
Simple to maintain
Linear Response
Non destructive to the sample

Differential Thermal Conductivity Detector or Katharometer

Principle:

- Based upon the thermal conductivity difference between carrier gas and that of the component in the sample.
- Hydrogen and helium have higher thermal conductivity and they are the best carrier gases for katharometer.

Merits:

- Responds to all types of organic and Inorganic compounds
- Linearity is good
- Non-destruction of the sample and hence used in preparative analysis.

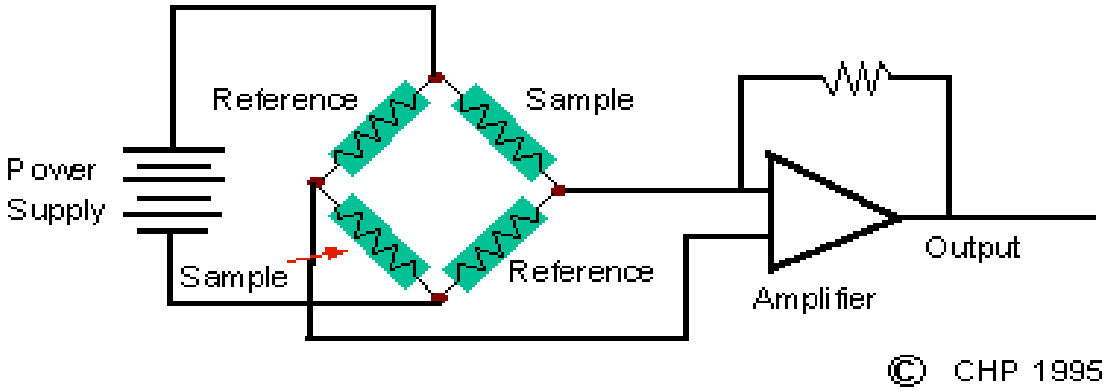
Demerits:

- Low sensitivity
- Biological samples are not analyzed
- Affected by fluctuations in temperature and flow rate.

Procedure:

- Has two platinum wires of uniform dimensions that form part of Wheatstone bridge.
- Carrier gas always passes through one of them and effluents of the column passes through the other column.
- The two wires are heated electrically and hence assume equilibrium conditions of temperature and electrical resistance.
- When the component emerges, it alters the thermal conductivity and the resistance of the wire.
- Hence this produces a difference in resistance and so the conductivity between two wires, is amplified and recorded as a signal.

Schematic Representation of Thermal conductivity Detector



Flame Ionization Detector

Principle :

- The ionization detectors are based on the electrical conductivity of gases.

Advantages:

- Extremely sensitive
- Linearity is excellent
- Insensitive to small changes in water vapor and flow rate of carrier gas.

Drawbacks :

- Different response to different substances and to the fact that the sample is destroyed.
- Responds to all organic compounds except formic acid, the response being greatest for hydrocarbons.

Procedure:

At normal temperature and pressure gases act as insulators but will become conductive of ions if electrons are present.

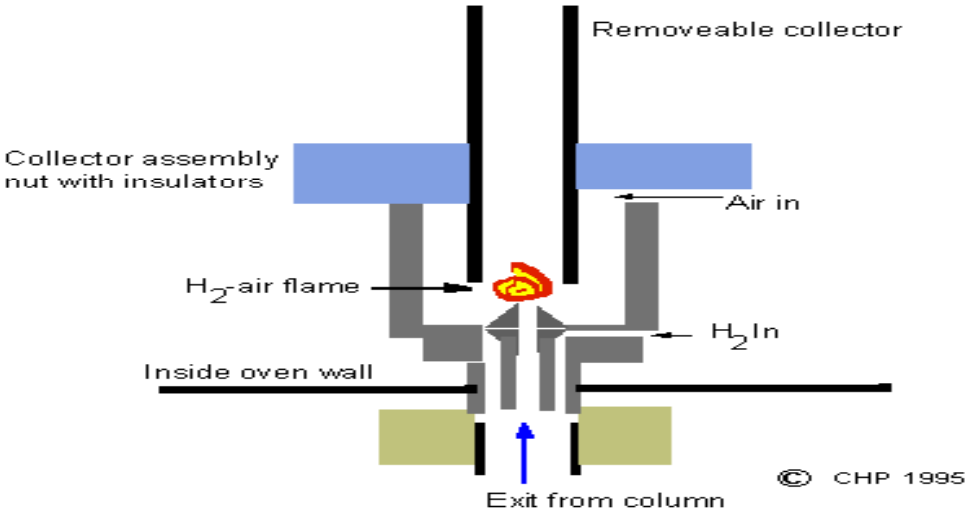
The carrier gas used with this detector is hydrogen.

The mixture is burnt in air (or oxygen) in the detector. The platinum jet serves as one electrode of the cell and the anode is a silver gauge placed above the burner tip.

When the component emerges from the column, the number of ions are produced because of the ionization by the thermal energy of the flame,.

This produces potential difference and causes a flow of current which is amplified and recorded as signal.

Schematic Representation of Flame Ionization Detector



Electron Capture Detector

Principle:

The electron affinity of different substances can be used as the basis for an ionization detector.

Advantages:

- Electron Capture Detector is extremely sensitive to certain compounds, e.g., chlorinated pesticides can be determined down to sub picogram levels.

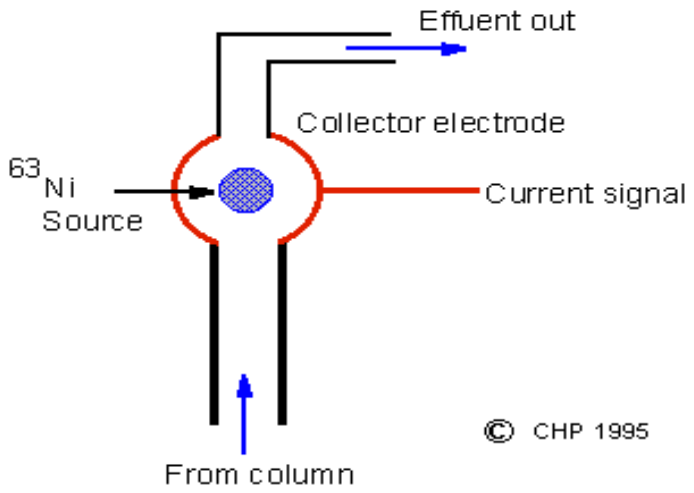
Disadvantages:

- It responds to only those compounds, whose molecules have affinity for electrons. E.g., chlorinated compounds, alkyl leads etc.

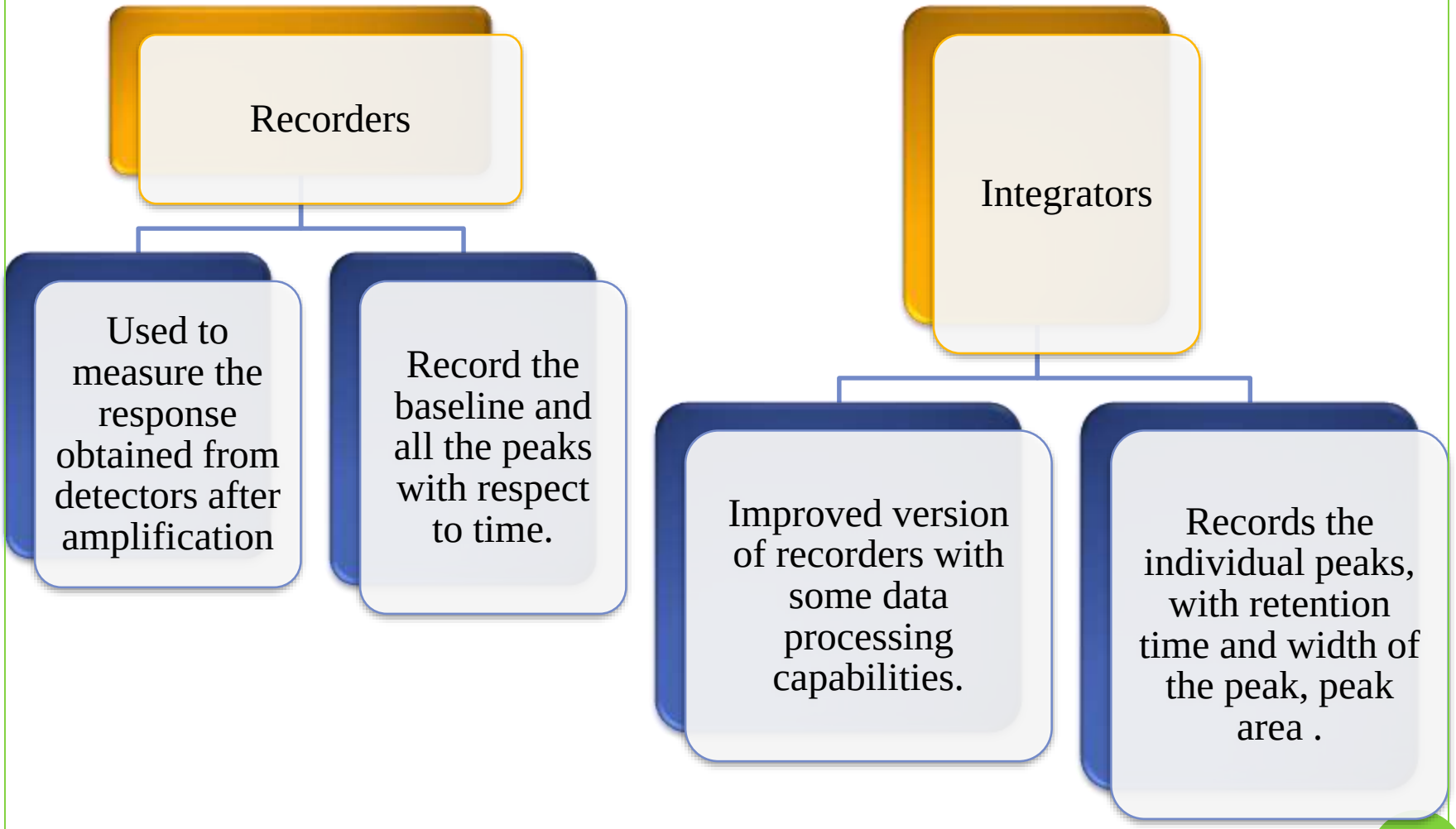
- It has high sensitivity for halogenated compounds and is therefore used for the detection of herbicides, pesticides, SF6 traces in fuel gases, organometallics (e.g. lead tetraethyl), poly nuclear aromatic carcinogenic NO_2 and SO_2 in chimney stack gases.

Procedure:

- It has two electrodes, with the column effluent passing between them.
- One is treated with radioactive isotope, which emits electrons as it decays.
- These emitted electrons produce secondary electrons which are collected by the anode, when a potential of 20 V are applied between them.
- Effluent molecules which have affinity for electrons capture these electrons when they pass through the electrodes. Hence the amount of steady state current is reduced. The difference is amplified and recorded as output signal.
- The carrier gas used depends upon the electron affinity of the compounds to be analyzed.



Recorders and Integrators



Evaluation Parameters:

A theoretical plate is hypothetical or imaginary unit of a column where distribution of a solute between stationary phase and mobile phase has attained equilibrium.

The efficiency of the column is measured (or) expressed by the Number of Theoretical Plates (N) in the column or by the Height Equivalent of the Theoretical Plate (HETP).

A theoretical plate is the distance on the column in which equilibrium is attained between the solute in the gas phase and the solute in the liquid phase.

The larger the number of theoretical plates or the smaller the HETP, more efficient is the column for separations.

It is calculated using the following formula:

$$\text{HETP} = \frac{\text{length of the column}}{\text{Number of Theoretical Plates}}$$

Factors effecting the Efficiency of the column

Particle size and surface area

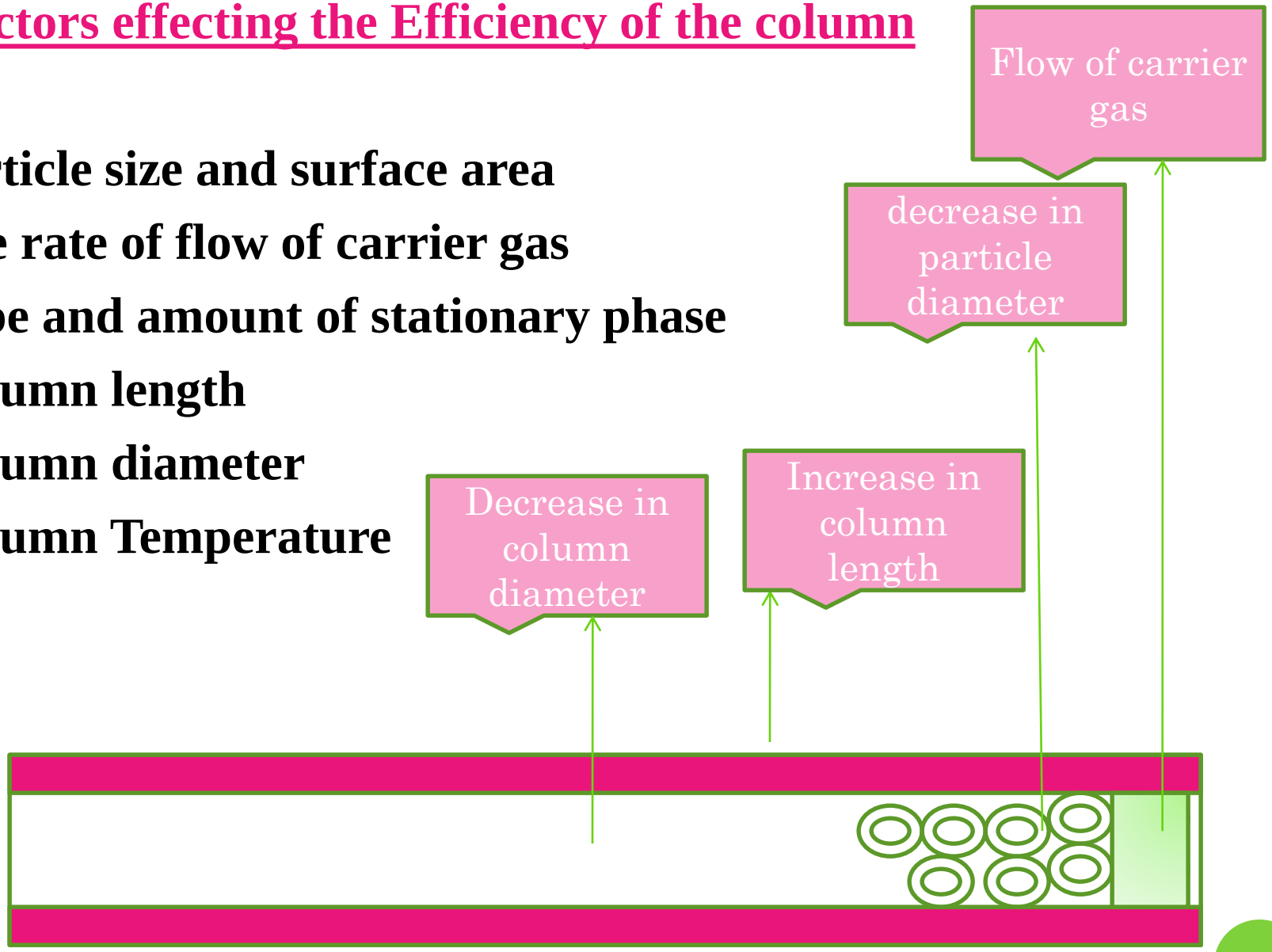
The rate of flow of carrier gas

Type and amount of stationary phase

Column length

Column diameter

Column Temperature

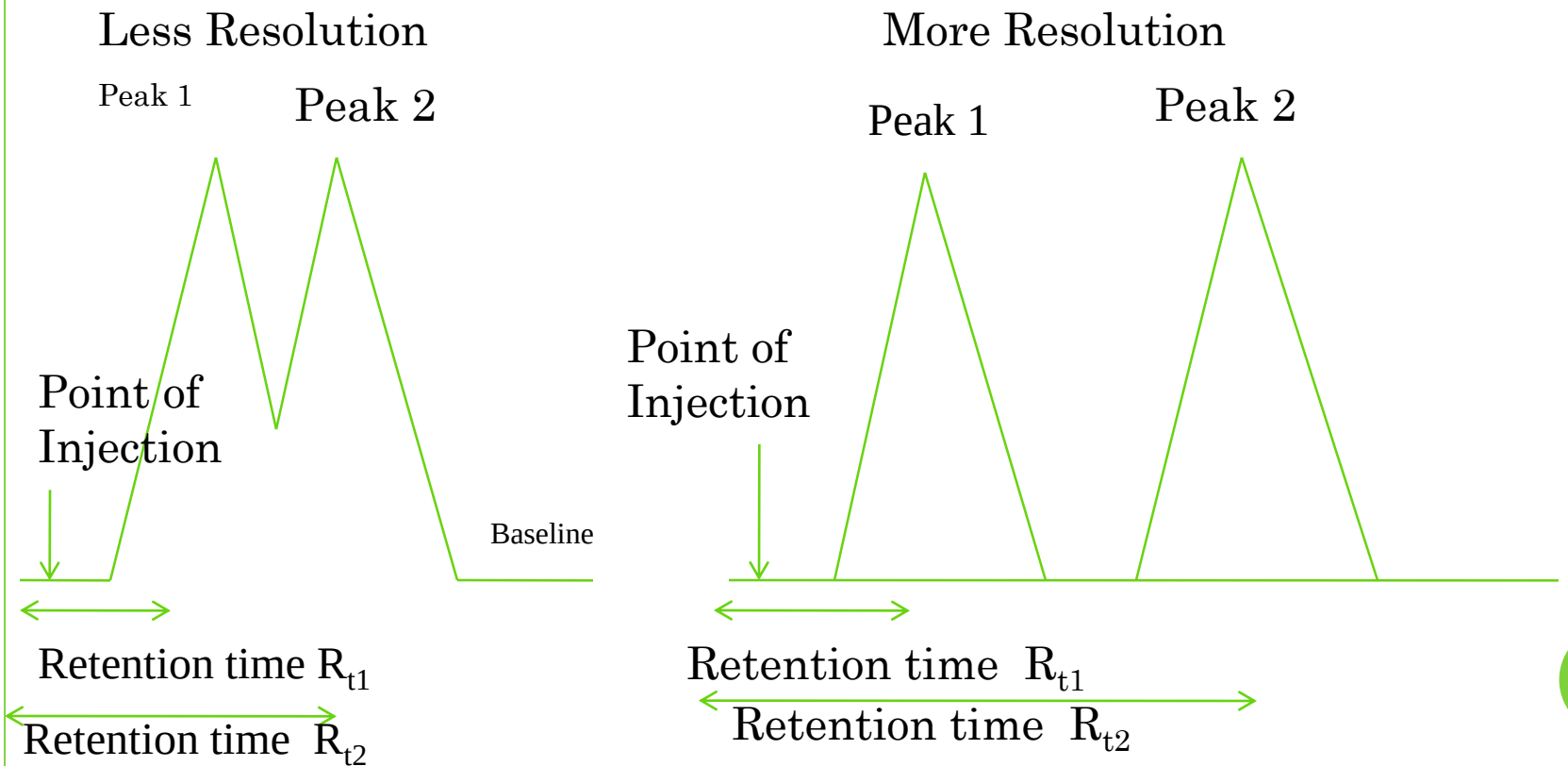


Resolution

The Resolution is a measure of extent of separation of two components and the baseline separation achieved.

It is determined by using the following formula:

$$R = \frac{2(R_{t1} - R_{t2})}{W_2 + W_1}$$



Retention time(R_t):

Retention is the difference in time between the point of Injection and the appearance of peak maxima.

Retention is the time required for 50% of a component to be eluted from a column.

Retention time is measured in minutes or seconds and is also proportional to the distance which can me measured in terms of cm or mm.

Retention volume(V_r):

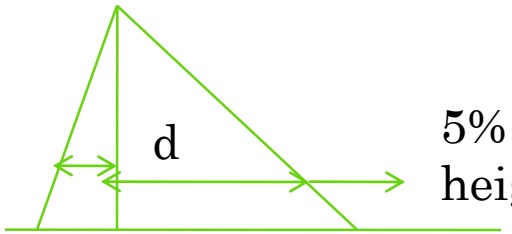
Retention volume is the volume of the carrier gas required to elute 50% of the component from the column.

It is the product of retention time and flow rate

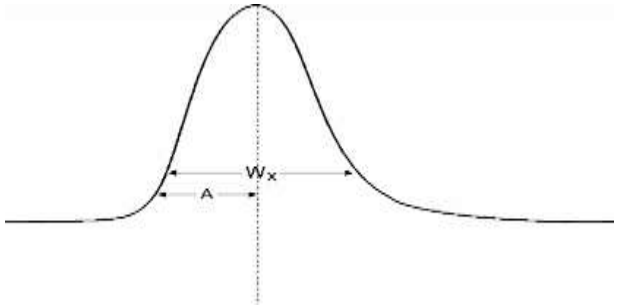
Retention volume = Retention time × flow rate.

Asymmetry factor :

A chromatographic peak should be symmetrical about its centre and said to follow Gaussian distribution



5% of the peak height



The symmetry factor or tailing factor of a peak is calculated from the expression:

$$A_s = \frac{W_{0.05}}{2d}$$

$W_{0.05}$ = width of the peak calculated at the one-twentieth of peak height

d = Distance between the perpendicular dropped from the peak maximum and the leading edge of the peak at one-twentieth of the peak height.

A value of 1.0 signifies complete or ideal symmetry.

Column efficiency

The efficiency of the column is expressed by the number of Theoretical plates.

$$N = 5.54(t_r / W_h)$$

N = Number of Theoretical Plates.

t_r = retention time or

W_h = width of the peak at half height.

If the number of theoretical plates is high , the column is said to be highly efficient.

If the number of theoretical plates is low, the column is said to less efficient.

Separation factor:

It is the ratio of partition-coefficient of the two components to be separated.

It is usually expressed and determined by using the following equation:

$$S = \frac{K_b}{K_a} = \frac{K'_a}{K'_b} = \frac{t_b - t_0}{(t_a - t_0)}$$

S = Depends on the liquid phase and column temperature.

If there is more difference in the partition- coefficient between the two compounds, the peaks are far apart and the separation factor is more.

If the partition coefficient is similar, then the peaks are closer and the separation factor is less.

Applications

- The Gas liquid chromatography is mainly used as tool for performing separations and for providing means of completion of an analysis.
- When the technique is used for providing means for completion of an analysis,
- qualitative identification is determined by retention times or retention volumes, and whereas
- Quantitative identification is determined by peak heights and peak areas.

Qualitative analysis:

- Some qualitative identification can be obtained from the observation of the relative sensitivity of various column liquids and various detectors.
- Further identification can only be gained from an observation of retention time, which is constant, for a given column, flow rate and temperature.
- Retention data should be useful for identification of component mixtures
- It is widely used for studying the purity of the organic compounds by analyzing the gas chromatograms. Contaminants if present, will give additional peaks.
- The areas under these peaks give rough estimates of contamination.
- This technique is also useful for evaluating the efficiency of purification techniques.

QUANTITATIVE ANALYSIS

- Based either on peak height or on peak Ares and hence for known substances, quantitative determinations are generally performed by comparing their peak areas.
- Peak height is often a better analytical parameter than peak area for solutes with low retention time, although both parameters are dependent upon various experiment variables.
- The peaks for solutes with low retention time are narrow and tall and hence very difficult to determine their areas accurately.

Calibration curve method

- Standards of various concentrations are used to determine their peak areas

Other Miscellaneous applications:

- Chromatography is most widely used chemical techniques to separate particles and contaminates in chemical plants.
- For example, in the chemical industries, pesticides and insecticides like DDT in the groundwater and PCBs (Polychlorinated biphenyls) are removed by the process of chromatography.
- As a major testing tool, chromatography is used by government agencies to separate toxic materials from the drinking water and also to monitor air quality.
- Chromatography is used by pharmaceutical companies to prepare large amounts of pure materials that are further required in making medicines.

- In the field of organic chemistry and pharmacy, chiral compounds are very close to each other in terms of atomic or molecular weight, element composition, and the physical properties.
- However, they exist in two different forms, called the enantiomers and optical isomers.
- Both these compounds though may appear to be same, have very different chemical properties.
- So, in pharmacy, chromatography becomes crucial to analyze the exact chiral compound so that correct medicines can be manufactured.
- For instance, a compound called thalidomide has two optical isomers and one of the isomers can cause birth defect if a pregnant women consumes it in early stages of pregnancy. So, it is important to carefully separate the isomers.
- Chromatography is used as a technique to separate the additives, vitamins, preservatives, proteins and amino acids.
- Some other uses are in the detection of drugs or medications in the urine and the separation of traces of chemicals in the case of fire in houses or buildings.

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- I Appreciate your Patience and valuable Time
- Thank you