Chromatographic Methods of Analysis

Section: 5 Gas Chromatography (GC)

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Gas Chromatography (GC)

- In gas chromatography, the sample is vaporized and injected onto the head of a chromatographic column. Elution is made by the flow of an inert gas as a mobile phase (He, N_2 and sometimes H_2).
- The mobile phase, does not interact with molecules of the analyte (components to be separated); its function is only to transport the analyte through the column, so it is called a carrier gas.

There are two type of such technique:

1- Gas-liquid chromatography (GLC) which is based upon the partition of the components between the gaseous mobile phase and a nonvolatile liquid phase immobilized on the surface of an inert solid support .

Most common stationary phase in GLC:

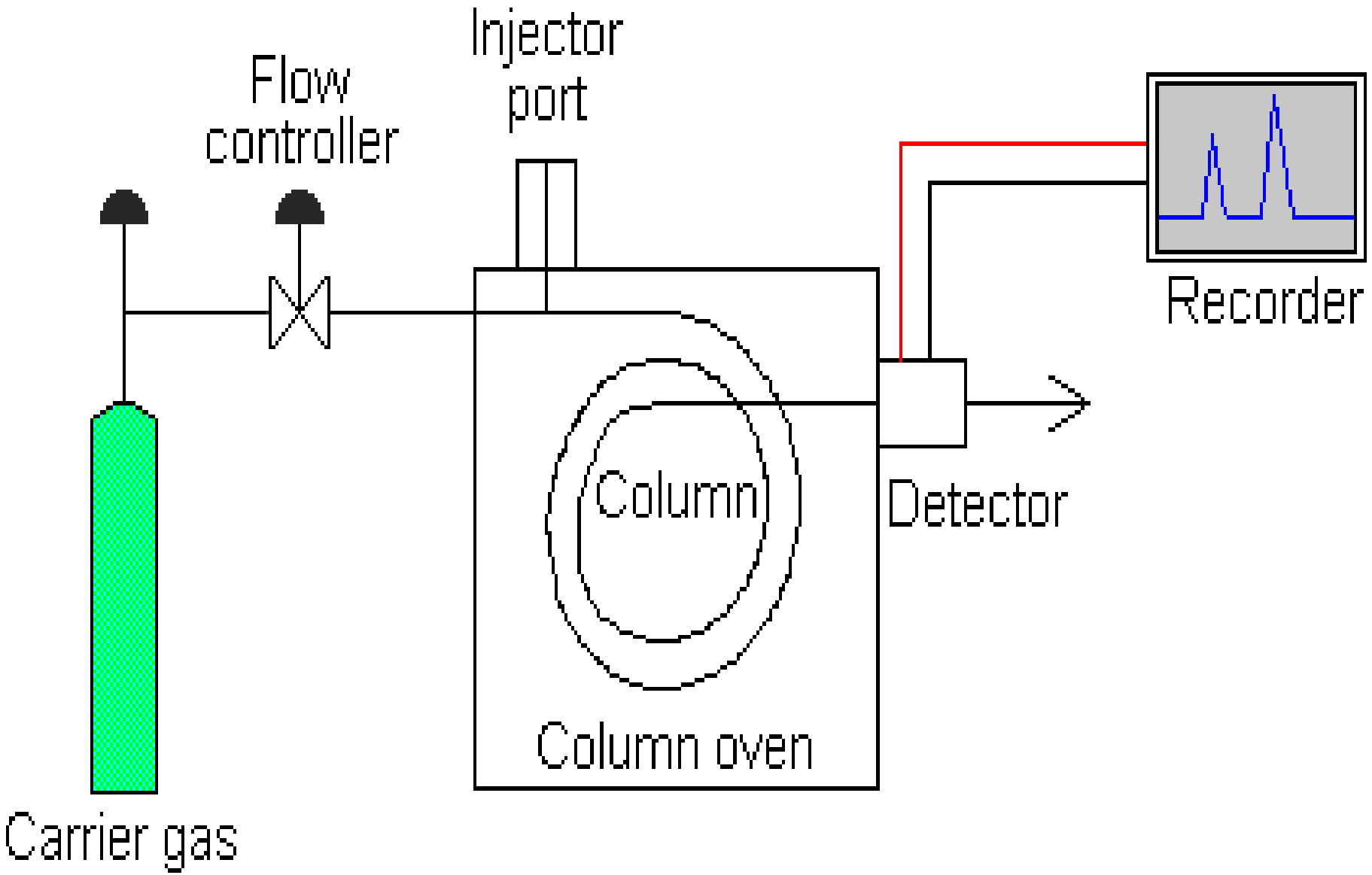
1- For separation of mixture of polar compounds poly-ethylene glycol is used.

2. For separation of mixtures of non-polar compounds polymer of methylsilicone is used.

2- Gas solid chromatography (GSC) which is based on the adsorption of components on the solid stationary phase (as silica or alumina).

- The principle of GC is the separation of volatile components based on the difference in adsorption characters of components or partition of them between the carrier gas and the solid or liquid stationary phase, respectively.

Instrument for GC (gas chromatograph)



Components of a Gas Chromatograph

Gas Supply: Cylinder containing a chemically inert gas like N_2, He or CO_2 , the flow rate is controlled by a regulator.

Sample Injector: Contains heated chamber through which the sample is injected by a micro-syringe (from steel or Teflon). Sample sizes vary from about 1/10 µl to 20 µl

Column: Typically stainless or glass, 1.5 - 10 m long and 2 - 4 mm in diameter. packed with small uniform size, inert support coated with thin film of nonvolatile liquid. Column is housed in a thermo-stated oven. Temperature must be strictly controlled, slightly higher than its boiling point of components.

Detector: There are several types of detectors to monitor the outlet of the column, each respond to different compounds or properties.

Recorder: Converts the detector signal into a sequence of peaks (known as a chromatogram).

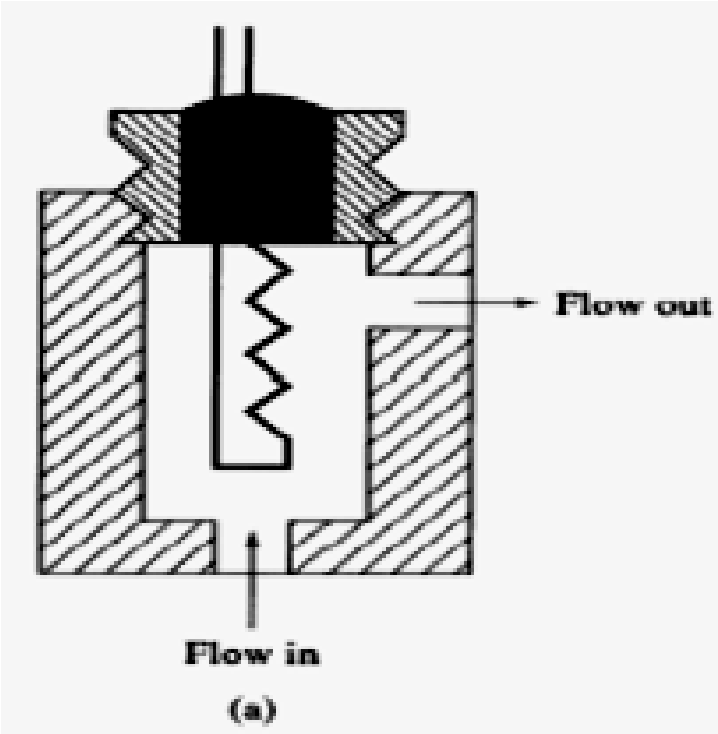
Detectors:

They are used to indicate the presence and measure the amount of the components in the column effluent.

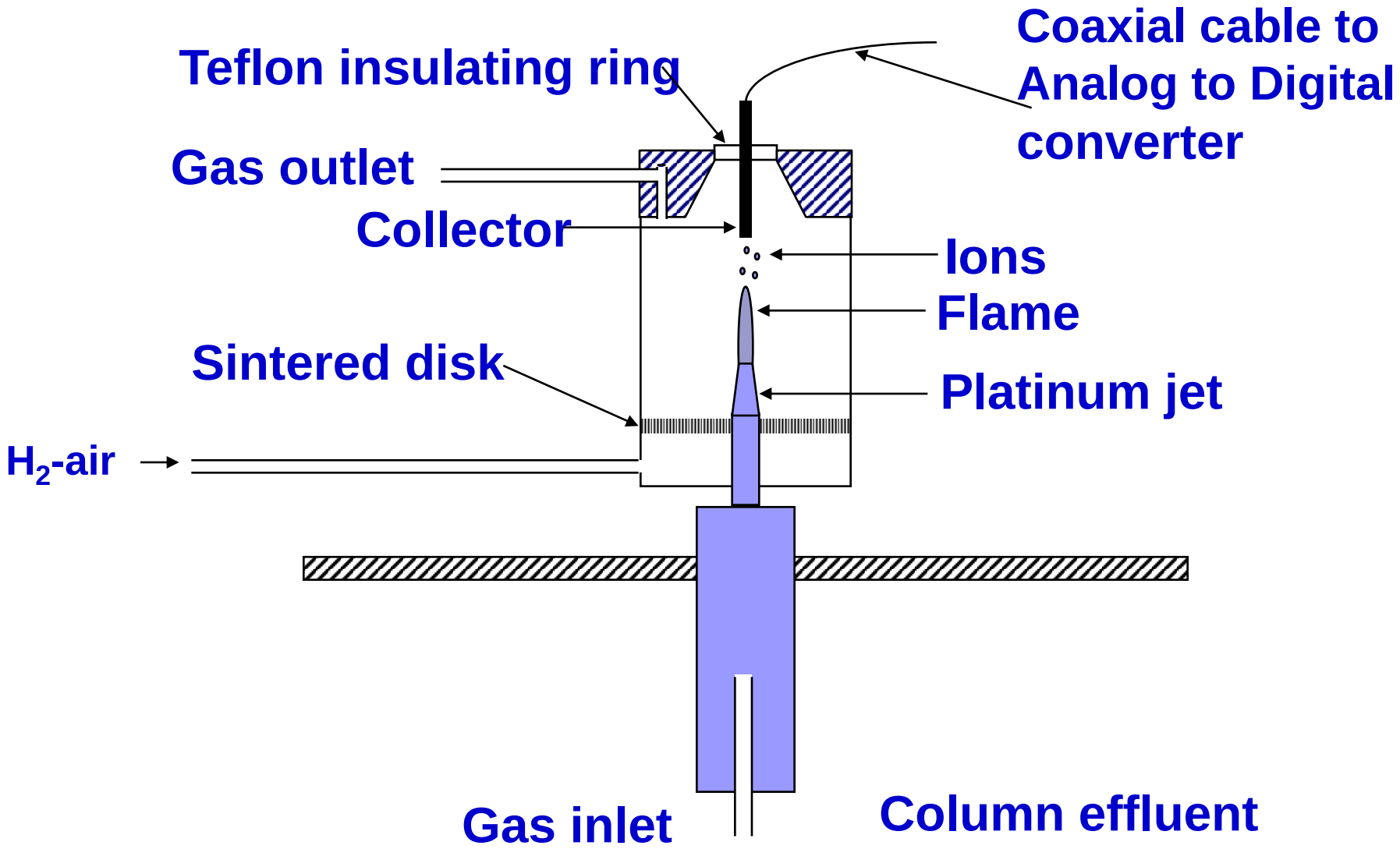
Examples of the commonly used detectors are:

1- Thermal Conductivity Detector (TCD):

- Measures the changes of thermal conductivity of the carrier gas.
- Non specific (responds to all compounds) with suitable sensitivity.
- Good linear range of signal and stable signal.
- Nondestructive detector
- High detection limit.



2- Flame Ionization Detector (FID)

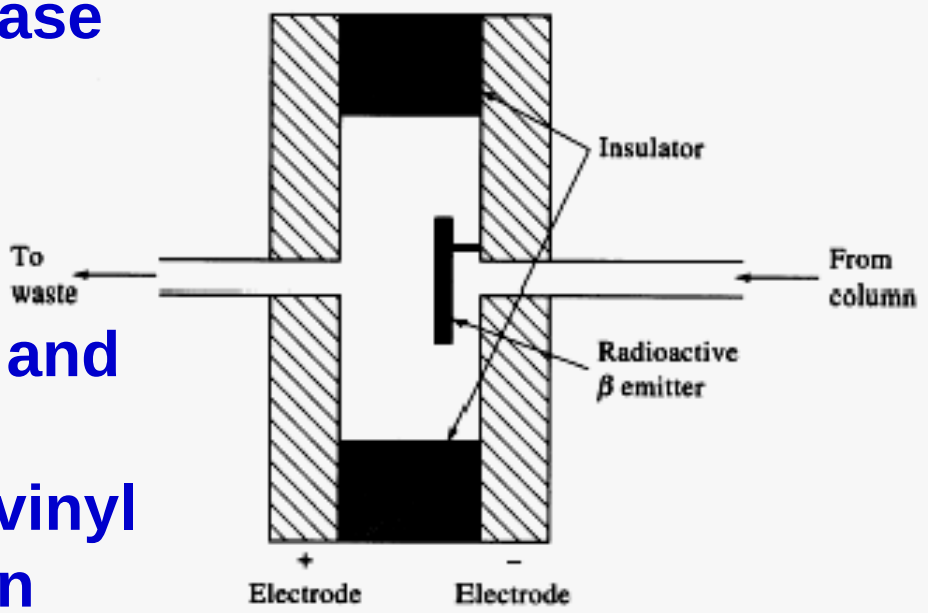


- In FID, high temperature of hydrogen flame (H_2 + O_2 + N_2) ionizes compounds eluted from column into flame.

  The ions collected on collector or electrode and were recorded on recorder due to electric current.
- Responds to compounds that produce ions when burned in an H_2-air flame (all hydrocarbons).
- Small or no response to inert gasses.
- High sensitivity, linear from the minimum detectable limit through concentrations to 10^{-7}.
- Destructive for detected components.

3- Electron capture detector ECD

- In ECD, electrons from radioactive source is captured by organic molecules and decrease current.
- Simple and reliable detector.
- Sensitive to electronegative groups (halogens, peroxides and cyano or nitro-derivatives) as in pesticide, Insecticides, vinyl chloride, and fluorocarbons in foods.
  Insensitive to amines or alcohols
- Non-destructive and low detection limit (10^{-12}).



Factors affecting separation by GC

1- Nature of stationary phase:

- Solid with adsorption characters or non-volatile liquid.
- Particles size which controls the surface area (300-600 or 150-300 μm). The efficiency of a gas-chromatographic column increases rapidly with decreasing particle size.

2- Carrier gas:

- Flow rate; high flow rate leads to higher speed and low resolution due to longitudinal diffusion.
- Pressure; the pressure difference between column in and out, required to maintain a given flow rate of carrier gas is 1 atm.

3- Nature of sample:

- More volatile compounds are easily to be carried by carrier gas.
- Injection time (longer time leads to low resolution).
- Polarity of compounds and column (like dissolves like).

4- temperature:

- As column temperature increases, faster movement and lesser separation takes place.
- Temperature of the column should be controlled near the boiling point of the components.

5- Column dimensions:

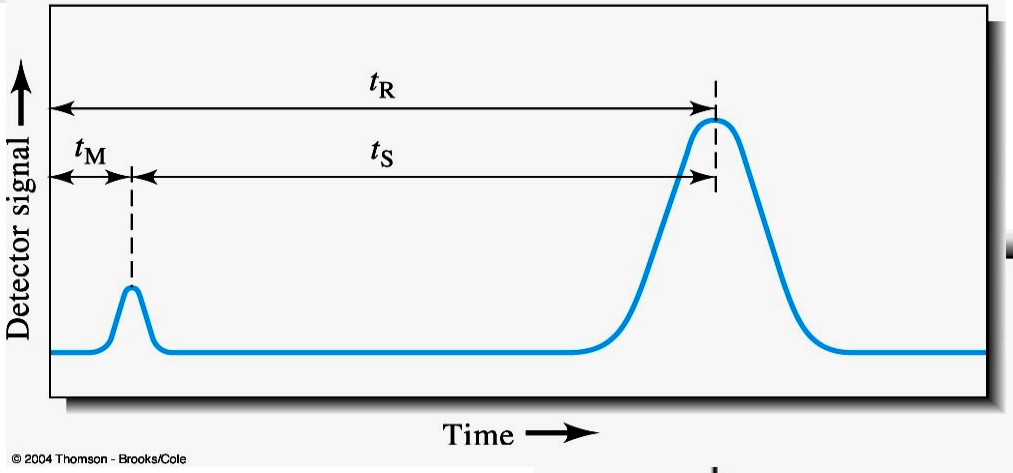
- Length of the column- greater separation using longer column.
- Diameter of column; small diameter leads to slow low rate and high resolution.

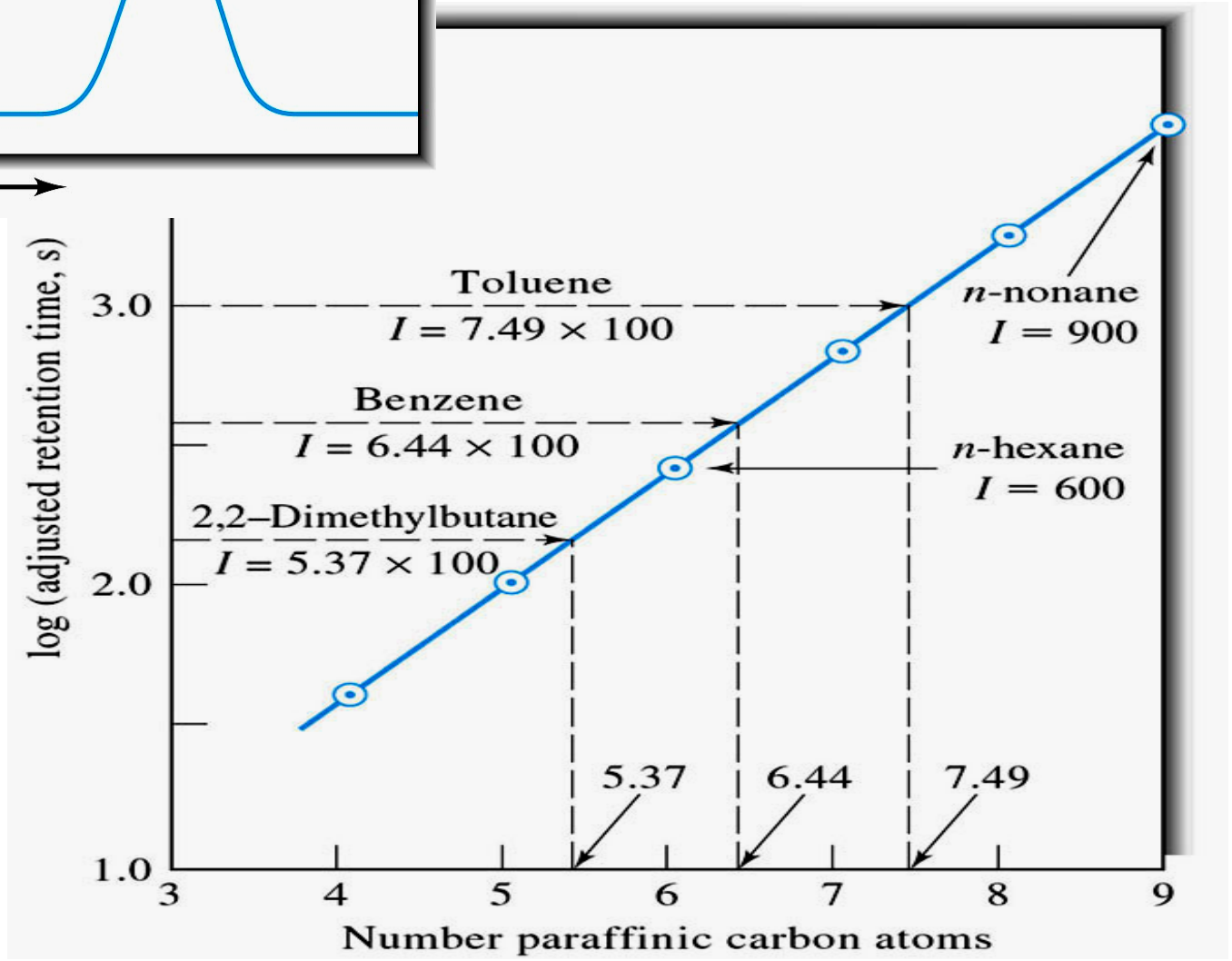
Applications of GC

1- Qualitative Analysis

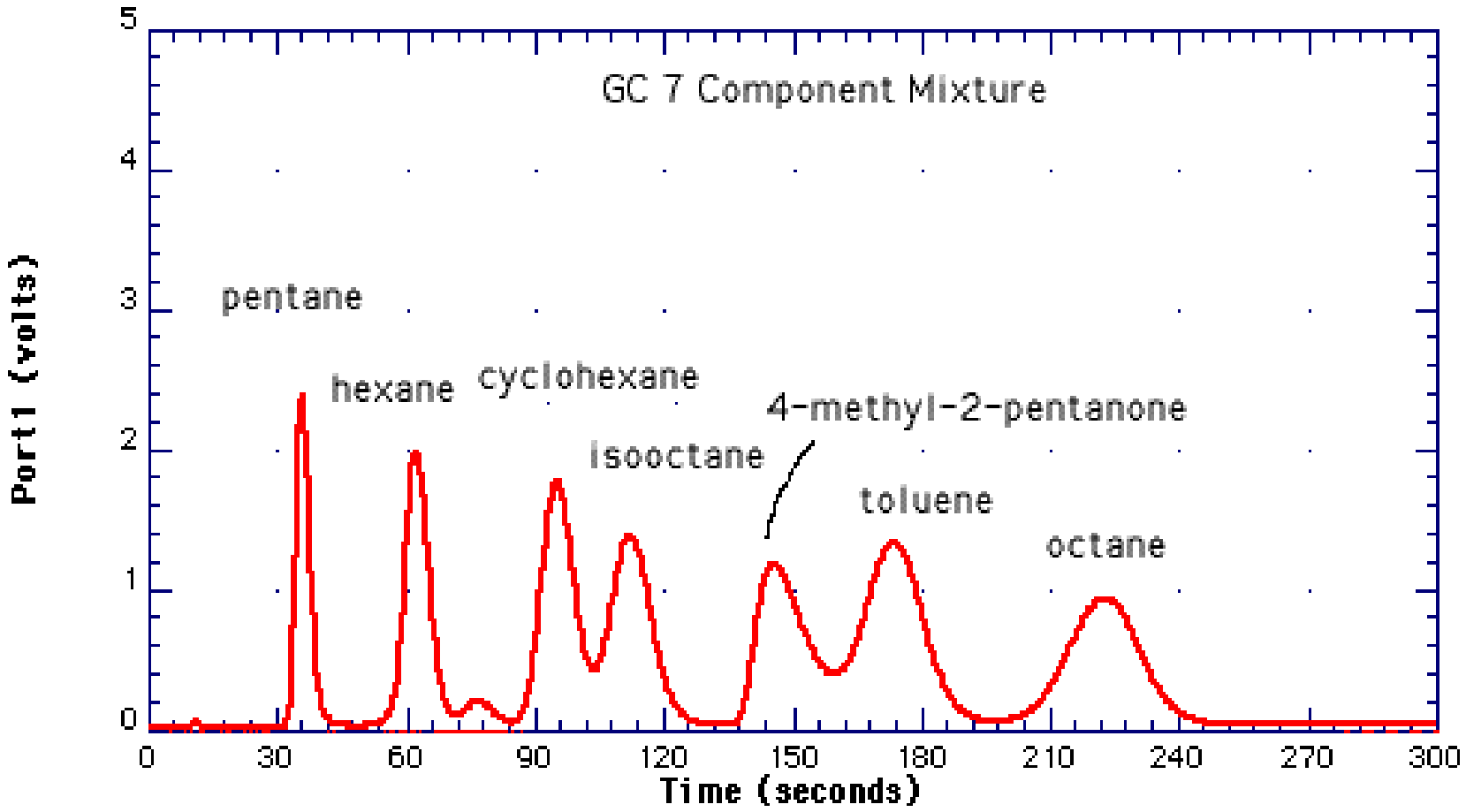
- Gas chromatograms are widely used as criteria of purity for organic compounds. impurities, if present, are revealed by the appearance of additional peaks. The components elapsed from the GC are subjected to analysis by IR or mass spectrometer to determine their structure, molecular weight and fragmentation pattern for the identification purpose.
- It can also be made by determination of retention time.

The identification based on the comparison of the retention time of the substance not only with one standard but with series of hydrocarbons (n-paraffins C_nH_{2n+2}).


t_R
t_M
t_S
Detector signal
Time
© 2004 Thomson - Brooks/Cole

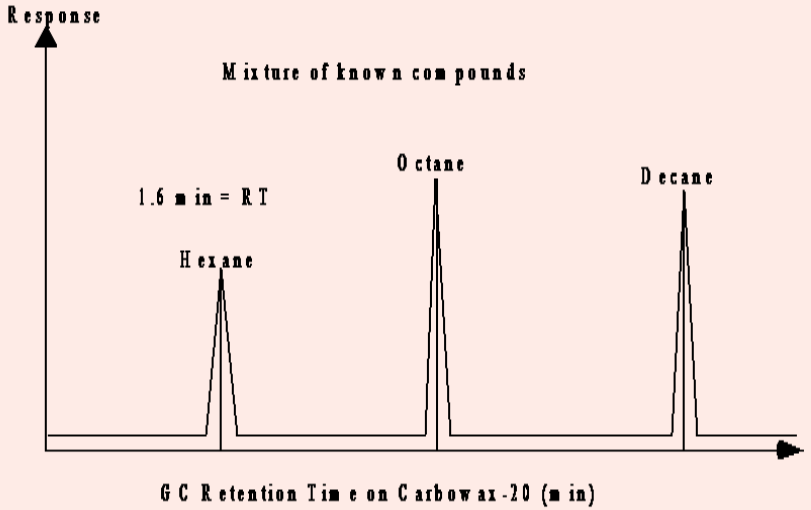

log (adjusted retention time, s)
3.0
2.0
1.0
Toluene
$I = 7.49 \times 100$
Benzene
$I = 6.44 \times 100$
2,2–Dimethylbutane
$I = 5.37 \times 100$
n-nonane
$I = 900$
n-hexane
$I = 600$
5.37
6.44
7.49
3
4
5
6
7
8
9
Number paraffinic carbon atoms

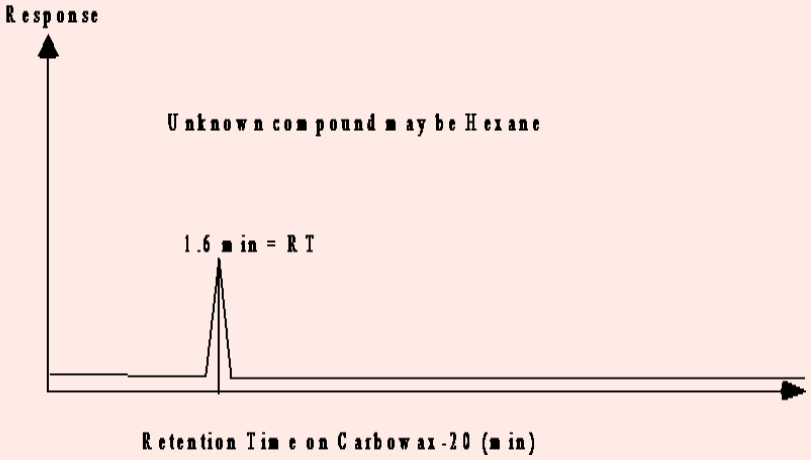
GC Chromatogram of hydrocarbon mixture



Compound	Boiling Point (^{0}C)	Compound	Boiling Point (^{0}C)
pentane	36	toluene	110
hexane	69	4-methyl-2-pentanone	117
cyclohexane	80	octane	126
isooctane	99		

Identification of unknown compounds by comparison of retention time

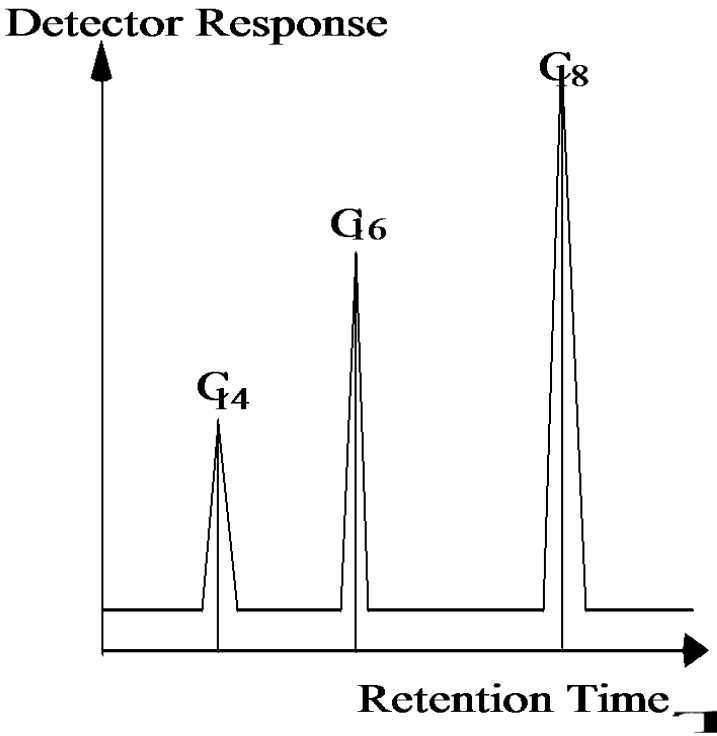


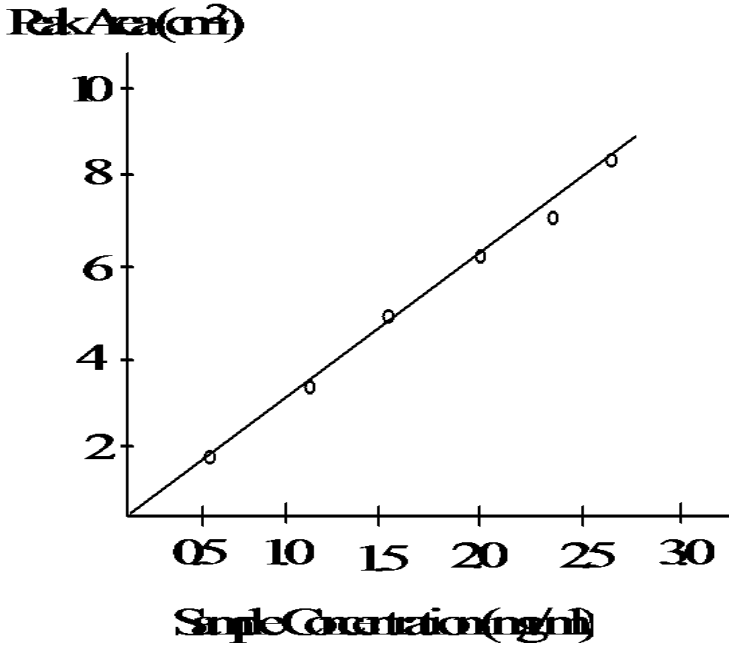


2- Quantitative Analysis

A) From the area under peak: The area under peak is proportional to the amount (moles) of the compounds eluted.

- The molar percentage composition of a mixture can be approximated by comparing the relative areas of the peaks in the chromatogram.





Determination of the area under peak

- Multiply the height of peak (in mm) above the baseline by the width of the peak at half the height.
- Baseline is a straight line connecting side arms of the peak.
- Add the individual areas to get the total area
- Divide each area by total area to get mole fraction
- Multiply mole fraction by 100 to get adjusted mole %

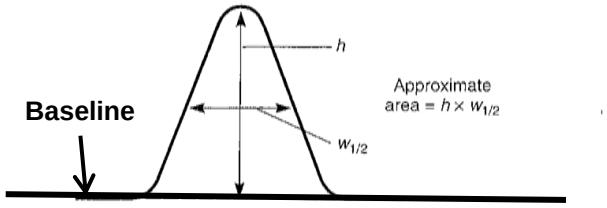
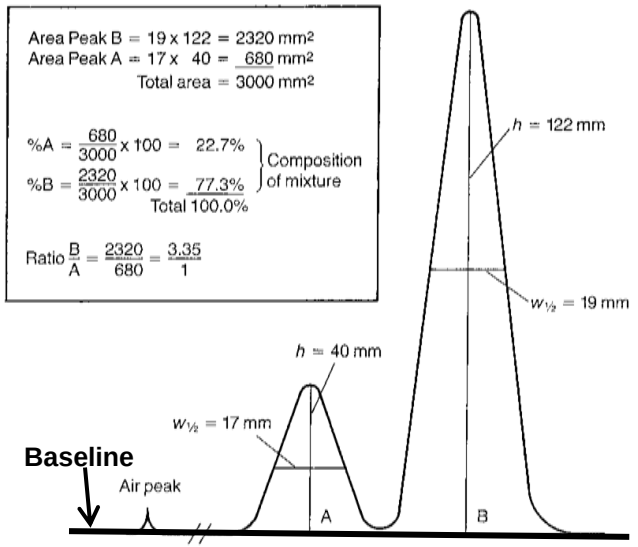


Figure 22.12 Triangulation of a peak.



B) Internal Standard Method

- In this approach, an internal standard is added to the sample, and the response from the analyte peak is compared to the internal standard.
- Response Factor (R_F)

The response factor accounts for differences in the detector response between the analyte and standard. It is measured by injecting a mixture containing known amounts of analyte and standard. For some analyte X, the response factor is

$$R_{x/is} = \frac{\left(A_x / m_x\right)}{\left(A_{is} / m_{is}\right)} = \frac{\left(A_x / A_{ix}\right)}{\left(m_x / m_{is}\right)}$$

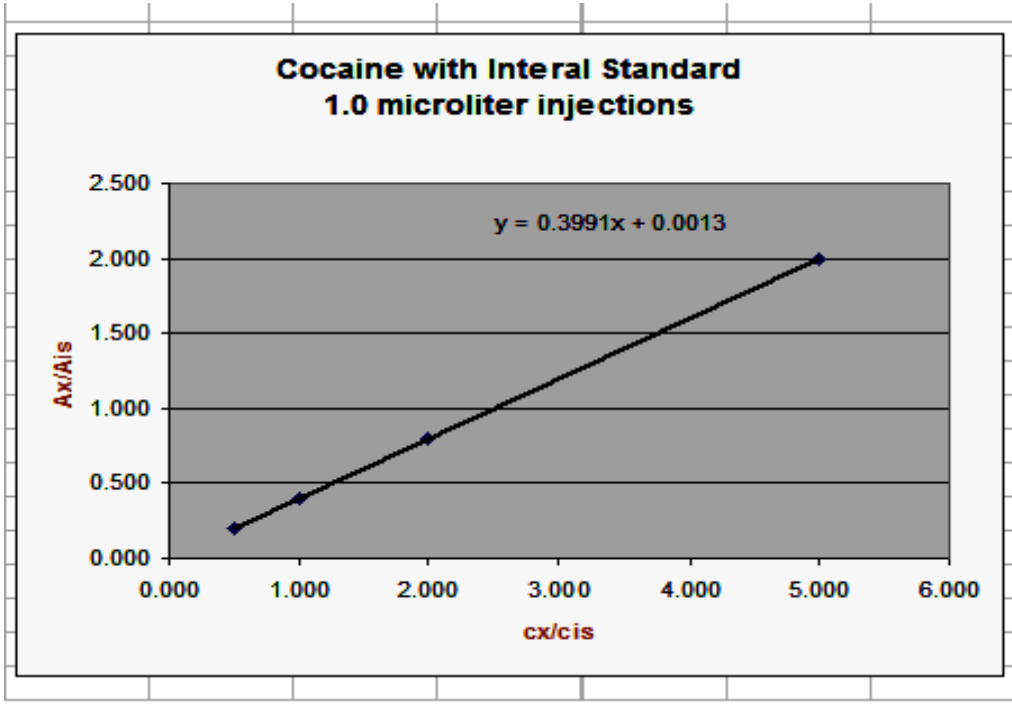
A_x, A_{is} are the peak areas for the analyte and internal standard.

m_x, m_{is} are the masses of analyte and internal standard.

C) Calibration Curve with Internal Standard

Standards

- **Each contains fixed mass of internal standard, various masses of std analyte**
- **Calibration curve shows linear response.**



Unknown

- **Add known amount of internal standard**
- **Inject and measure A_x/A_{is}**
- **Determine c_x/c_{is} for your unknown from calibration curve. Since c_{is} is known, c_x for your unknown is simply**

$$c_x = (c_x/c_{is})c_{is}$$

$$R_{x/is} = \frac{A_x/A_{is}}{c_x/c_{is}} \qquad R_{x/is} = \frac{A_x/A_{is} - (y\ \text{intercept})}{c_x/c_{is}}$$

Examples for GC applications:

1- Forensic Applications

- Analysis of bodily fluids (for example: semen or urine) to test for illegal substances such as alcohols.
- Test fibers and blood recovered at a crime scene.
- Detect residue from explosives and ignitable fluids/ presence of accelerants

2- Separation of racemic mixtures in drugs industry using optically active stationary phase

3- Separation and determination of hydrocarbons in oil industry.

4- Separation and determination of pesticides in foods, water and soil.

5- Extraction and analysis of carbonyl compounds (like aldehydes) from natural products (as sunflower oils), which are formed due to lipid peroxidation.

6- Quality and production control of diesel fuel, beer constituents and aditives in foods and soft-drinks.