Solution

B.Tech VIIth sem. Leather Technology (2015 Batch)

Mid Semester Examination-2018 Code:LT-071719

Subject: Instrumentation and process control

Max. Mark:20 **Time** :2Hours

Note: Attempt any **Four** questions. All questions have equal marks. Assume any missing data

Q1. (a)State the full form of HPLC.

Ans. High performance liquid chromatography

(b) State the full form of AAS.

Ans. **Atomic absorption spectroscopy**

(c) State the full form of GC-MS.

Ans. **Gas chromatography–mass spectrometry**

(d) Write down the full form of GC.

Ans. Gas chromatography

Q2. Explain Beer- Lambert law and its limitation.

Ans. The **Beer-Lambert law (or Beer's law)** is the linear relationship between absorbance and concentration of an absorbing species. The general Beer-Lambert law is usually written as:

$A = a(\lambda) * b * c$

where A is the measured absorbance, $a(\lambda)$ is a wavelength-dependent absorptivity coefficient, **b** is the path length, and **c** is the analyte concentration. When working in concentration units of molarity, the *Beer-Lambert law* is written as:

$A = \varepsilon * b * c$

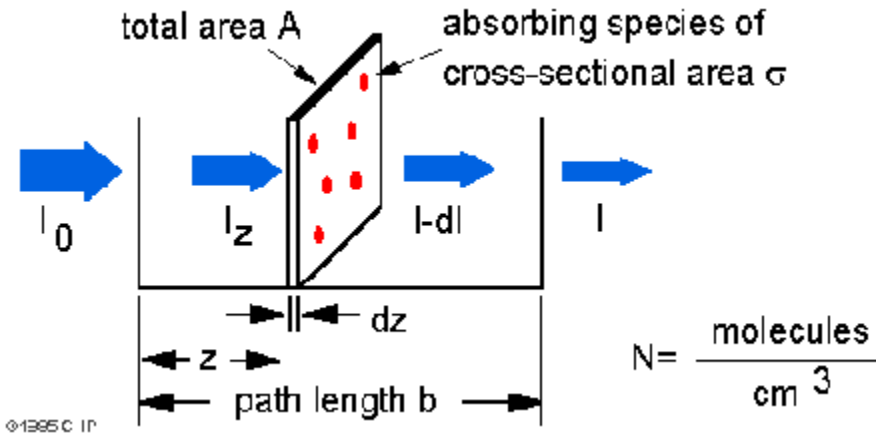
where ε is the wavelength-dependent molar absorptivity coefficient with units of M^{-1} cm^{-1}. Data are frequently reported in percent transmission ($I/I_0 * 100$) or in absorbannce [$A = \log (I/I_0)$]. The latter is particularly convenient. Sometimes the extinction coefficient is given in other units; for example,

$A = E^{1\%} * b * c$

where the concentration C is in gram per 100 ml of solution. This useful when the molecular weight of the solute is unknown or uncertain.

Derivation of the Beer-Lambert law

The Beer-Lambert law can be derived from an approximation for the absorption coefficient for a molecule by approximating the molecule by an opaque disk whose cross-sectional area, σ, represents the effective area seen by a photon of frequency *w*. If the frequency of the light is far from resonance, the area is approximately 0, and if *w* is close to resonance the area is a maximum. Taking an infinitesimal slab, dz, of sample:



I_o is the intensity entering the sample at z=0, I_z is the intensity entering the infinitesimal slab at z, dI is the intensity absorbed in the slab, and I is the intensity of light leaving the sample. Then, the total opaque area on the slab due to the absorbers is $\sigma * N * A * dz$. Then, the fraction of photons absorbed will be $\sigma * N * A * dz / A$ so,

$dI / I_z = - \sigma * N * dz$

Integrating this equation from z = 0 to z = b gives:

$\ln(I) - \ln(I_o) = -\ \sigma * N * b$

or $-\ln(I / I_o) = \sigma * N * b$.

Since N (molecules/cm^3) * (1 mole / 6.023×10^{23} molecules) * 1000 cm^3 / liter = c (moles/liter) and 2.303 * log(x) = ln(x) then

$-\log(I / I_o) = \sigma * (6.023 \times 10^{20} / 2.303) * c * b$

$-\log(I / I_o) = A = \varepsilon * b * c$

where $\varepsilon = \sigma * (6.023 \times 10^{20} / 2.303) = \sigma * 2.61 \times 10^{20}$

Typical cross-sections and molar absorptivities are:

	σ (cm^2)	ε (M^{-1} cm^{-1})
absorption - atoms	10^{-12}	3×10^{8}
molecules	10^{-16}	3×10^{4}
infrared	10^{-19}	3x10
Raman scattering	10^{-29}	3×10^{-9}

Limitations of the Beer-Lambert law

The linearity of the Beer-Lambert law is limited by chemical and instrumental factors. Causes of nonlinearity include:

- deviations in absorptivity coefficients at ***high concentrations (>0.01M)*** due to electrostatic interactions between molecules in close proximity
- **scattering of light due to particulates** in the sample
- **fluoresecence or phosphorescence** of the sample
- changes in refractive index at high analyte concentration
- shifts in chemical equilibria as a function of concentration

- non-monochromatic radiation, deviations can be minimized by using a relatively flat part of the absorption spectrum such as the maximum of an absorption band
- stray light

Q3. Discuss the process to determine the concentration of Cr (VI) in given sample of water by UV- Spectrophotometer.

Ans. Methods for analysis:

A. Instrumental method (ICPMS/AAS)

B. Colorimetric method

Colorimetric method

Apparatus and equipment:

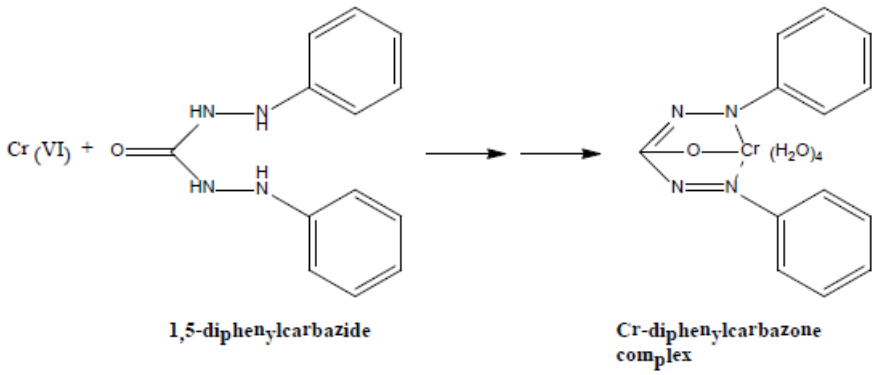
- UV –visible spectrophotometer with an operation range of 400-700 nm.
- Volumetric flask

Reagents:

- Potassium Dichromate Solution – $K_2Cr_2O_7$ (mol. wt. 294, Cr 52.0) 11.3 mg $K_2Cr_2O_7$/250 ml D.W. – Stock solution
- Diphenyl Carbazide Solution - 100 mg 1,5-Diphenyl Carbazide in 100 ml Acetone)
- Sulphuric Acid-6N -10 ml Conc. H_2SO_4 '36N' in 60 ml D.W.

Principle:

The hexavalent chromium is determined colorimetrically by reaction with diphenylcarbazide in acid solution. A red-violet colour of unknown composition is produced which is measured at 540 nm by UV- spectrophotometer. The scanned wavelength for determination of absorbance is 540 nm.



1,5-diphenylcarbazide Cr-diphenylcarbazone complex

Procedure:

- Prepare standard solution by taking 25 ml of stock $K_2Cr_2O_7$ solution by pipette and dilute to 250 ml in volumetric flask with distilled water.
- Take 5, 10, 15, 20, 25 and 30 ml of the standard solution in different 100 ml volumetric flask by pipette.
- In each flask add 2.0 ml of Diphenyl carbazide reagent followed by 2.0 ml of 6N H_2SO_4.
- Make up the volume to 100 ml by D.W.
- Prepare a blank solution in 100 ml volumetric flask.
- Take the absorbance of the blank and standard solutions as 540 mm on spectrophotometer.
- Plot the calibration curve between absorbance on ordinate and concentration of chromium on abscissa.
- Read the absorbance of the given sample in the same manner and report its concentration in mg/L.
- Calculate the slope of the curve/straight line

Calculation

Concentration of $K_2Cr_2O_7$ (mg/L) = $\frac{\textbf{weight of K2Cr2O7 (mg)}}{\textbf{Volume (L)}}$

Conc. of Cr(VI) in stock solution(mg/L)

$$= \textbf{\textit{Con. of } } K_2Cr_2O_7 \times \frac{\textbf{\textit{Mol.weight of Cr(VI)}}}{\textbf{\textit{Mol.Wt.of }} \textbf{K2Cr2O7}}$$

Conc. Of Cr (VI) in standard solution (mg/L)

$$= \frac{\textbf{\textit{con.of Cr(VI) in stock}} \times \textbf{\textit{Volume of sample taken(ml)}}}{\textbf{\textit{Total volume(ml)}}}$$

Q4. Write down the method for the determination of Fe(III) in given sample of water.

Ans. Methods for analysis:

A. Instrumental method (ICPMS/AAS)

B. Colorimetric method

B. Colorimetric method

Apparatus and equipment:

- UV –visible spectrophotometer with an operation range of 400-700 nm.
- Volumetric flask

Reagents:

- **Ammonium Acetate Buffer Solution**

  Dissolve 250 gm Ammonium acetate ($NH_4C_2H_3O_2$) in 150 ml D.W. and add 700 ml of glacial acetic acid. Make up the volume 1.0 liter with D.W.

- **1, 10-Phenanthrolin Solution**

  Dissolve 100 mg of 1, 10-Phenanthrolin monohydrate ($C_{12}H_8N_2.H_2O$) in 100 ml D.W. Add 2 drop of Conc. HCl.

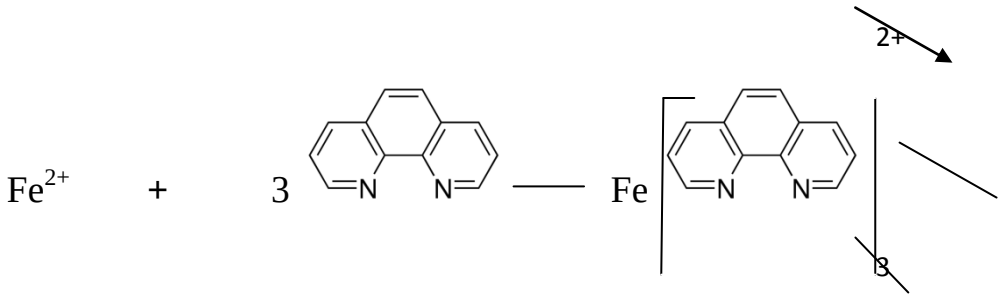
- **Stock Iron Solution**

  Dissolve 561.6 mg Ferrous Ammonium Sulphate (Mol. wt. 392.14, Fe=56.0) in 50 ml D.W. Add 8 ml of Conc. H_2SO_4 and make up the volume to 100 ml in volumetric flask with D.W

Principle:

1,10-phenanthroline is a tricyclic nitrogen heterocyclic compound that reacts with metals such as iron, nickel, ruthenium, and silver to form strongly colored complexes. This property provides an excellent and sensitive method for determining these metal ions in aqueous solution. For example, o-Phen reacts with ferrous ion to produce a deeply colored red complex.

Three molecules of Phenanthroline chelate each atom of ferrous iron to form an orange-red complex. The coloured solution obeys Beer's law; its intensity is independent of pH from 3 to 9. A pH between 2.9 and 3.5 insures rapid colour development in the presence of an excess of Phenanthroline. Colour standards are stable for at least 6 months.



Phenanthrolin Fe- Phenanthrolin complex

Procedure

- **Standard Iron Solution**
  Dilute 6.25 ml of stock solution to 1.0 liter with D.W. in volumetric flask.
- Take 5, 10, 15, 20, 25, 30 ml of standard Iron solution in separate 100 ml volumetric flasks.
- Add 10.0 ml acetate buffer followed by 2.0 ml Phenanthrolin reagent.
  Make up the volume to 100 ml with D.W.
- Prepare a reagent blank solution taking D.W. in place of standard Iron solution.
- Plot the absorbance at 510 nm as ordinate and concentration in mg/L as abscissa.
- Calculate the slope of the calibration curve.
- Measure the amount of soluble iron in given sample

Calculation:

Concentration of stock (mg/L) = $\frac{\textbf{weight of ferous amonium sulfate (mg)}}{\textbf{Volume (L)}}$

Conc. of Fe in stock solution (mg/L)

= Conc. of stock $\times \frac{\boldsymbol{Volume\ of\ stock}}{\boldsymbol{Total\ \ make\ up\ volume}} \times \frac{\boldsymbol{Mol.weight\ of\ Fe}}{\boldsymbol{Mol.Wt.of}\ \textbf{ferous amonium sulfate}}$

Conc. of Fe in standard solution (mg/L)

$$= \frac{\boldsymbol{con.of\ Fe\ in\ stock} \times \boldsymbol{Volume\ of\ sample\ taken(ml)}}{\boldsymbol{Total\ make\ up\ volume(ml)}}$$

Q5. Write down the principle of flame photometry and discuss the different parts of this instrument with diagram.

Ans. **Introduction**

During 1980s Bowling Barnes, David Richardson, John Berry and Robert Hood developed an instrument to measure the low concentrations of sodium and potassium in a solution. They named this instrument as Flame photometer. The principle of flame photometer is based on the measurement of the emitted light intensity when a metal is introduced into the flame. The wavelength of the colour gives information about the element and the colour of the flame gives information about the amount of the element present in the sample.

Flame photometry is one of the branches of atomic absorption spectroscopy. It is also known as flame emission spectroscopy. Currently, it has become a necessary tool in the field of analytical chemistry. Flame photometer can be used to determine the concentration of certain metal ions like sodium, potassium, lithium, calcium and cesium etc. In flame photometer spectra the metal ions are used in the form of atoms. The International Union of Pure and Applied Chemistry (IUPAC) Committee on Spectroscopic Nomenclature has named this technique as flame atomic emission spectrometry (FAES).

Principle of Flame photometer

The compounds of the alkali and alkaline earth metals (Group II) dissociate into atoms when introduced into the flame. Some of these atoms further get excited to even higher levels. But these atoms are not stable at higher levels.

Hence, these atoms emit radiations when returning back to the ground state. These radiations generally lie in the visible region of the spectrum. Each of the alkali and alkaline earth metals has a specific wavelength.

Element	Emitted wavelength	Flame color
Sodium	589 nm	Yellow
Potassium	766 nm	Violet
Barium	554 nm	Lime green
Calcium	622 nm	Orange
Lithium	670 nm	Red

For certain concentration ranges,

The intensity of the emission is directly proportional to the number of atoms returning to the ground state. And the light emitted is in turn proportional to the concentration of the sample.

Parts of flame photometer

A simple flame photometer consists of the following basic components:

Source of flame: A Burner in the flame photometer is the source of flame. It can be maintained in at a constant temperature. The temperature of the flame is one of the critical factors in flame photometry.

Fuel-Oxidant mixture	Temperature (°C)
Natural gas-Air	1700
Propane-Air	1800

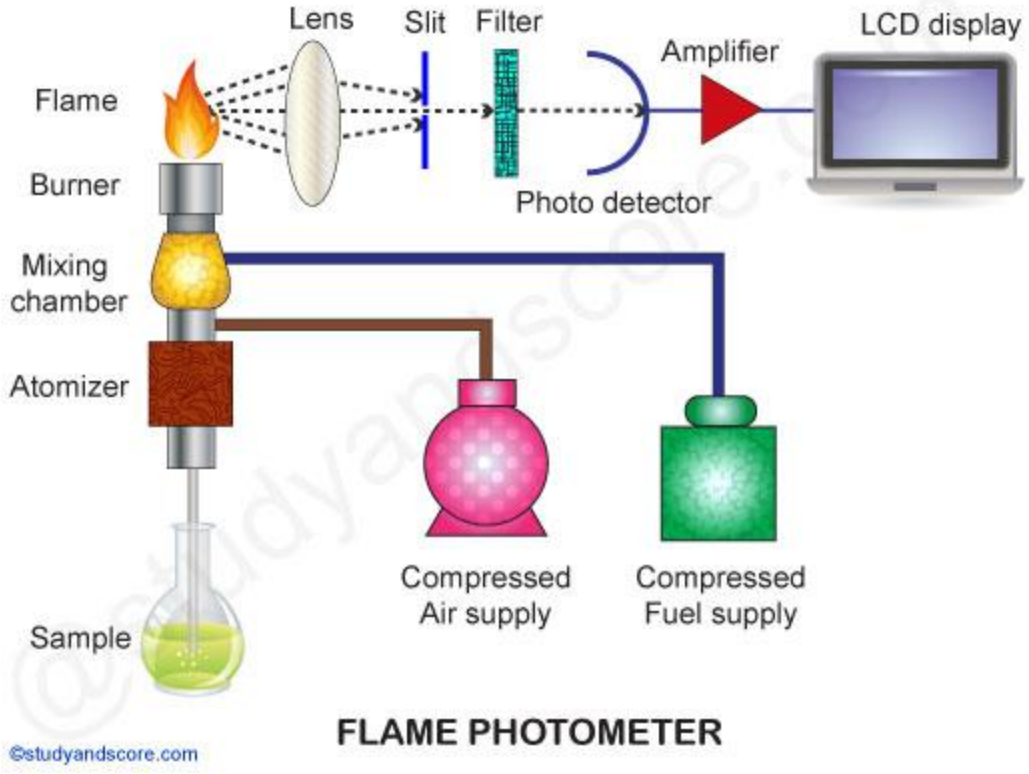
Fuel-Oxidant mixture	Temperature (°C)
Hydrogen-Air	2000
Hydrogen-Oxygen	2650
Acetylene-Air	2300
Acetylene-Oxyen	3200
Acetylene-Nitrous oxide	2700
Cyanogen-Oxygen	4800

Nebuliser: Nebuliser is used to send homogeneous solution into the flame at a balanced rate.

Optical system: The optical system consists of convex mirror and convex lens. The convex mirror transmits the light emitted from the atoms. Convex mirror also helps to focus the emissions to the lens. The lens helps to focus the light on a point or slit.

Simple colour filters: The reflections from the mirror pass through the slit and reach the filters. Filters will isolate the wavelength to be measured from that of irrelevant emissions.

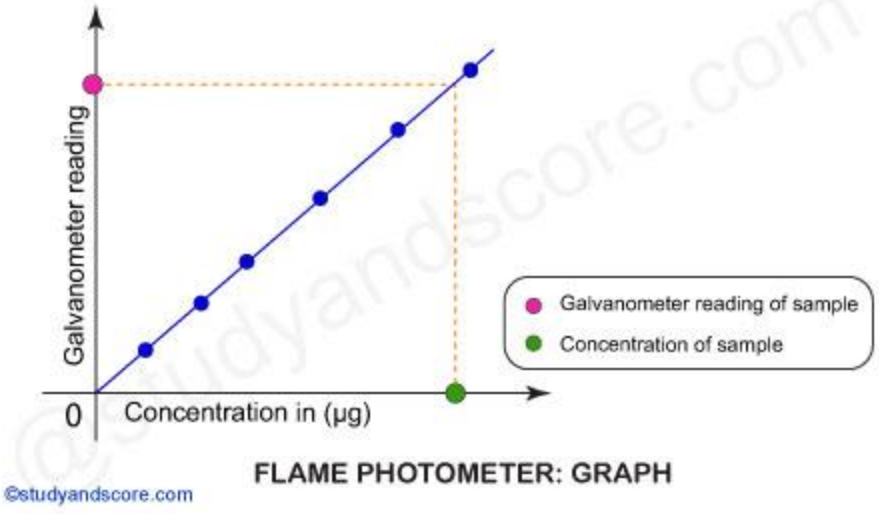
Photo-detector: The intensity of radiation emitted by the flame is measured by photo detector. Here the emitted radiation is converted to an electrical signal with the help of photo detector. These electrical signals are directly proportional to the intensity of light.



FLAME PHOTOMETER

- Both the standard stock solution and sample solution are prepared in fresh distilled water.
- The flame of the photometer is calibrated by adjusting the air and gas. Then the flame is allowed to stabilize for about 5 min.
- Now the instrument is switched on and the lids of the filter chamber are opened to insert appropriate colour filters.
- The readings of the galvanometer are adjusted to zero by spraying distilled water into the flame.
- The sensitivity is adjusted by spraying the most concentrated standard working solution into the flame. Now the full scale deflection of the galvanometer is recorded.

- Again distilled water is sprayed into the flame to attain constant readings of galvanometer. Then the galvanometer is readjusted to zero.
- Now each of the standard working solutions is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- Finally sample solution is sprayed into the flame for three times and the readings of galvanometer are recorded. After each spray, the apparatus must be thoroughly washed.
- Calculate the mean of the galvanometer reading.
- Plot the graph of concentration against the galvanometer reading to find out the concentration of the element in the sample.



FLAME PHOTOMETER: GRAPH

Q6. Explain the working principle and use of gas chromatography.

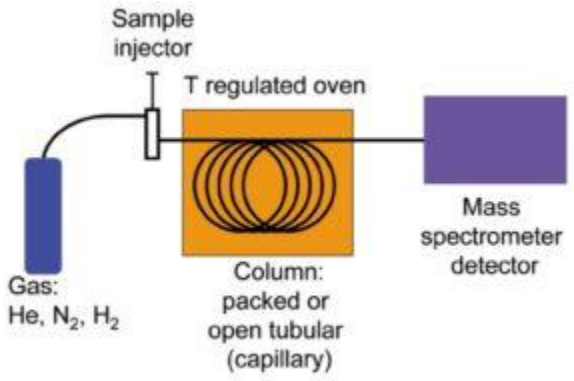
Ans. Gas Chromatography

Gas chromatography is one of the sophisticated models of chromatography. As the name indicates the system operates with the flow of gas in the procedure. This type of chromatography was primarily designed to evaluate volatile compounds like fatty acid compounds, essential oils, etc. The system is cumbersome and also with delicate instrumentation. It is quite expensive regarding instrumentation, maintenance, and even the operating costs. Further, an expert handling is recommended in its operation, unlike other easy chromatography techniques. But still, gas chromatography is an important tool in analytical chemistry, especially in the medicinal field.

Gas Chromatography & principle

The principle in gas chromatography principle involves separation of components of the sample under test due to partition in between gaseous mobile phase and stationary liquid phase. The elements partitioned into gas come out first while other come later.

Gas chromatography runs on the **principle of partition chromatography** for separation of components. Based on the stationary and mobile phases it is categorized under *the gas-liquid type of chromatography*, i.e., the stationary phase is a liquid layer supported over a stationary phase while the mobile phase is an inert and stable gas. Hence the perfect name as Gas-Liquid chromatography (GLC).



By: K. Murray https://commons.wikimedia.org

How gas chromatography works:

The gas is set to flow at a constant rate from the cylinder on to the liquid layer impregnated on a solid support in a column. The sample is injected into the injection point and is carried by the mobile gas into the column. Inside the column, the components get separated by the differential partition in between the mobile phase gas and stationary phase liquid. The component that partitioned into gascomes out of the column first and is detected by the detector. The one partitioned into liquid phase comes out later and is also detected. The recordings are displayed onto a computer software. From these peaks, one can identify the components and also their concentration.

Must read article Gas Chromatography Theory for details on other important aspects of GC.

Gas chromatography method: Below is the video of the instrumentation and method simultaneously.

The gas chromatography apparatus can be listed as

1. The mobile phase gas in a cylinder: The mobile phase is an **inert gas (**monoatomic element **gases or non-reactive gases** like **nitrogen, helium & hydrogen**. The carrier gas is kept in a metallic cylinder and outflow is controlled by a regulator. From gas carrier cylinder, the gas is passed under fixed rate through a pressure gauge which indicates the speed of flow of gas into the column. Most **commonly used gas is helium**.

2. The injection system: This is present before column yet inside the thermal chamber to load sample under analysis into the system.

3. The column for gas chromatography. The gas chromatography column is a usually long (few meters like 3 to 6 meters) and coiled for accommodation into a small thermal chamber. The column is mostly made of steel or glass.

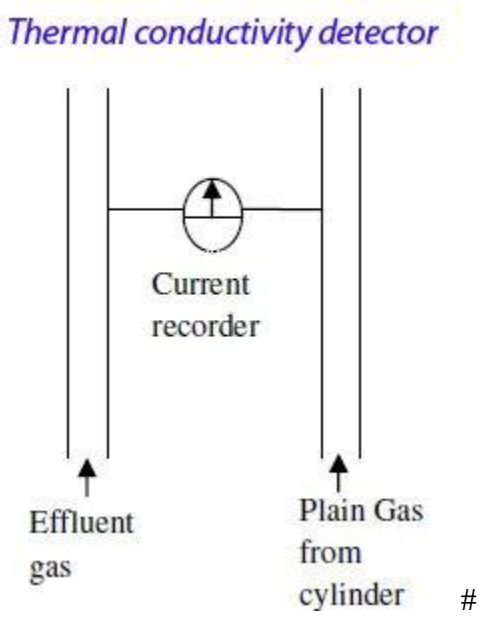
Packed column. This is a column into which solid beads are packed. This column has advantages like efficient separation and precise readings.

♠ Tubular column. Here into a stainless steel hollow tube a thin layer of liquid is coated to act as a stationary phase. This column offers least resistance to flow of gas.

♠ Support coated tubular column**.** Here into stainless steel column a thin solid layer is coated on to which a thin layer of liquid stationary phase is present.

4. <u>The Detector:</u> is another vital component of the gas chromatography apparatus.
GC detectors detect the isolated components and helps in identification and quantification of the sample. They are of 4 types of GC detectors like

♦ Thermal conductivity detector: Here there are two columns which have a conducting wire in between. The gas is allowed to pass through the two columns of detectors i.e to one the effluent from gas chromatography column and to other gas from the gas cylinder directly. Since the temperature of both gases is same, the thermal conduction is constant.

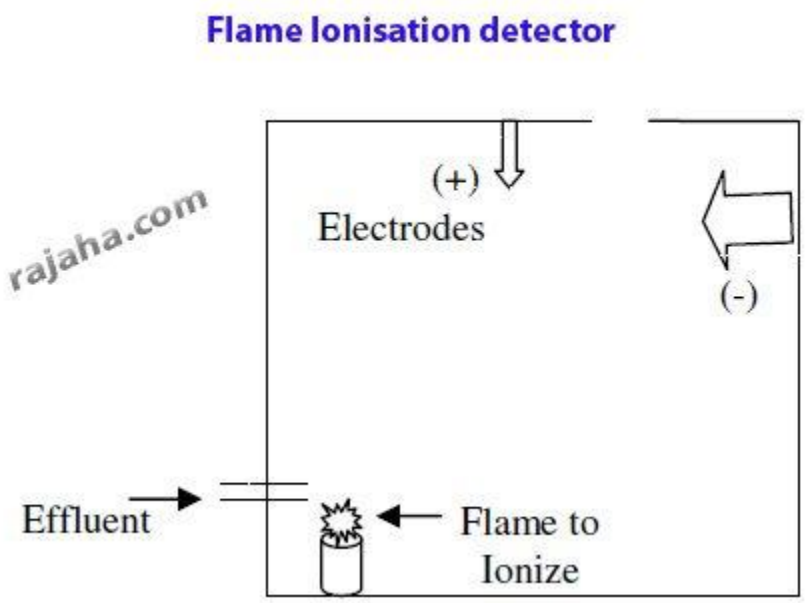


Thermal conductivity detector

When the sample is injected into gas chromatography column. The effluent gas carries the sample components into the detector column. Since effluent gas is mixed with sample components there results in difference in thermal conductivity from prior one recording. This

difference in conductivity is specific for the component analyzed. This is recorded for further comparison and identification of the components and their quantity.

♦ Flame ionization detectors: Here the sample components from effluent are ionized by subjecting to flame in a chamber. These ions raise upwards and are attracted towards anode or cathode based on charge on them. When they impinge on the electrodes, current is passed which is recorded. The strength and intensity of current depends on the sample and is specific.



flame-ionisation-detector

♦ Argon ionization detector; These detectors are similar to flame ionization detectors with only difference that argon ion gas is used to ionize the sample molecules. The argon ions are obtained by reacting argon gas with radioactive elements. Once argon ionizes they try to get back to stable state by either taking or giving electron from the sample components thus making sample molecules to ions for detection.

♦ Electron capture detector, etc.

5. The **computer to record** the analysed readings. This is connected with the detector and hence records the detector changes in reference to the flow of separated components from the exit of the column. The record is called gas chromatograph.

5. The **thermal chamber** to fix or maintain fixed temperature.

* Precolumn and post column treatment of sample (if necessary). This is done to modify the sample. The sample should be stable on heating and also be separated properly. For this precolumn derivatization is used and for the sample to be detected properly, post-column derivatization is done by making a suitable chemical change.

As a further improvement in GC, the gas chromatography apparatus is fixed with Mass spectroscopy system (GC-ms) for better analysis of components regarding their mass.