

CHROMATOGRAPHY

Multiple Choice Type Questions

1. In gas chromatography, Capacity factor K_C can be represented as

[WBUT 2009, 2011]

- a) $t_M - t_R / t_R$
- b) t_R / t_M
- c) $(t_R - t_M) / t_M$
- d) $t_M - t_R$

where t_R is retention time and t_M is dead time.

Answer: (c)

2. In an inhomogeneous magnetic field, paramagnetic gases tend to

[WBUT 2011, 2015, 2016]

- a) move from weaker to stronger field
- b) move from stronger to weaker field
- c) move in any direction
- d) none of these

Answer: (a)

3. In gas chromatography, the FID response only to substances that can be ionized in

[WBUT 2012, 2015, 2016]

- a) air-acetylene flame
- b) air-hydrogen flame
- c) air-nutritious oxide flame
- d) none of these

Answer: (a)

4. A chromatography process time vs signal curve is known as

[WBUT 2014]

- a) chromatography
- b) chromatogram
- c) chromatographic
- d) none of these

Answer: (b)

5. If the length of a GC column is doubled, resolution will improve by

[WBUT 2015]

- a) $\sqrt{2}$
- b) 2
- c) $2\sqrt{2}$
- d) 2^2

Answer: (a)

6. Absorptivity is a property of a substance for a given wavelength that will vary with

[WBUT 2016]

- a) concentration
- b) concentration and viscosity
- c) length of the light path
- d) concentration and length of the light path

Answer: (d)

Short Answer Type Questions

1. From two component chromatogram, determine the expressions of capacity factor, selective factor & resolution. [WBUT 2009]

Answer:

In two component chromatography, the typical chromatogram diagram is given below. From diagram $L/t_R = u_s$ is the average linear velocity of the solute in cm/sec. The peak at t_m in the figure is for a constituent that is not retained by the column packing and hence $L/t_m = u_m$ is the velocity of the mobile phase.

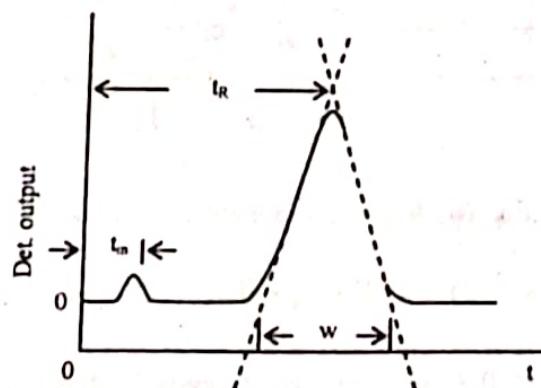


Fig: (i) A typical chromatogram peak

Now u_s and u_m are related by the equation

$$u_s = u_m (\text{No. of moles of the solute in the mobile phase} / \text{Total No. of moles of the solute})$$

This is because a solute migrates only when it is in the mobile phase. If v_s and v_m are the total volumes of the stationary and mobile phases, then

$$u_s = u_m (\chi_m v_m) (\chi_m v_m + \chi_s v_s) = u_m / (1 + K v_s / v_m) = u_m / (1 + K_c)$$

where K_c is known as the capacity factor and is obviously a measure of the migration of the solute. K_c can be found out from the chromatogram.

Substituting the values of u_s and u_m in the last equation $K_c = (t_R - t_m) / t_m$

Both t_R and t_m are obtained from the chromatogram.

Selectivity factor: The ratio of the partition coefficients of two components *A* and *B* is called the selectivity factor F_s , thus, $F_s = K_B / K_{cA}$

Component *B* is considered to be more strongly retained here, so that $t_{RB} > t_{RA}$. In terms of K_c 's one can, thus, write $F_s = K_{cB} / K_{cA}$

so that from the chromatogram $F_s = (t_{RB} - t_m) / (t_{RA} - t_m)$

Answer:

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

4. Find the expression for the resolution of a chromatography column separating a gas mixture of two components A and B. [WBUT 2011]

Answer:

Let A and B are two components. The separation of these components can be expressed in terms of the resolution, R, can be given by as below.

$$R = \frac{2[(t_r)_A - (t_r)_B]}{w_A + w_B} \quad \dots \text{(i)}$$

$$R = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k'_B}{1 + k'_B} \quad \dots \text{(ii)}$$

From the above two equations we can say that separation of the two target components can be optimized by manipulation of the k' , α , and N terms. The first two terms are known as thermodynamic effects, and the third terms is associated with kinetic features of the column. The k' term is optimized by increasing the temperature of the column (as in GC) and by changing the mobile-phase composition (as in LC). The options available to optimize α are a change in the mobile-phase composition, column temperature, and stationary-phase composition. N can be improved by increasing the length of the column and reducing H, which is accomplished by changing the mobile-phase flow rate and reducing the size of the solid support, the thickness of the liquid stationary phase, the viscosity of the mobile-phase solvent, the temperature of the column, and the diameter of the column. The use of capillary columns and columns packed with small particles is a common practice in GC/MS and LC/MS applications.

5. On a 1000 cm long chromatography column, the helium carrier gas velocity is 40 cm/sec. The elution time for dicane is 1.27 sec and the peak width at the base is 1.5 sec. Calculate [WBUT 2011]

- i) The capacity factor
- ii) The effective number of theoretical plates, N and
- iii) The height equivalent of theoretical plates, H.

Answer:

- i) Capacity factor = $\frac{t_r - t_m}{t_m}$, data given in the problem seem to be incorrect. Because t_r should me greater than t_m . Capacity factor is always positive and greater than one.

Resolution: Resolution of a column is another important factor to be considered. Considering a two-component chromatogram as shown in Fig. (ii), the resolution R_s is defined as

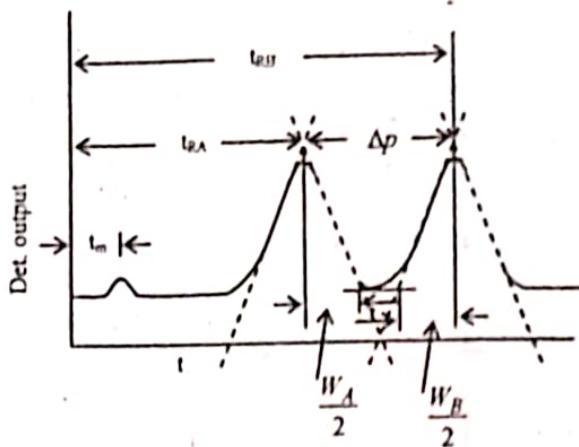


Fig: (ii) A two-component chromatogram

$$R_s = \Delta t / (W_A/2 + W_B/2) = 2\Delta t / (W_A + W_B) = 2(t_{RB} - t_{RA})(W_A + W_B)$$

If R_s is 1.5, there is about 0.3 per cent overlapping and for $R_s = 1$, about 4 per cent overlapping. By increasing L, R_s can be increased. Resolution R_s can be expressed in terms of N, F and K_c for a paired components. In fact, using the equations above, it is possible to write $R_s = (N^{1/2}/4)(K_{cB} - K_{cA})/(1 + K_{cB}) = (N^{1/2}/4\{F_s - 1\}/F_s)K_{cB}/(1 + K_{cA})$ and the elution time is obtained as $(t_R)_A = (16R_s^2 H/u_m)(F_s/(F_s - 1))^2(1 + K_{cA})^3/K_{cA}^2$.

2. Describe continuous and stepwise elusion process of solvent programming in HPLC. [WBUT 2009]

Answer:

Basically, the analysis of mixtures of widely varying composition frequently leads to a very wide spread in retention times. The longer the retention time, the broader the peak, so for those components which take a long time to elute, detector sensitivity is diminished and analysis times can be very long.. With solvent programming, successively eluted substances experience stronger solute-mobile phase interactions and so emerge from a column more rapidly than they would under conditions in which the solvent was not varied.. So long as one does not experience peak overlap (i.e. resolution remains tolerable), solvent programming gives a superior separation.

3. What is the principle used in nuclear magnetic resonance? [WBUT 2010]

Answer:

The principle behind NMR is that many nuclei have spin and all nuclei are electrically charged. If an external magnetic field is applied, an energy transfer is possible between the base energy to a higher energy level (generally a single energy gap). The energy transfer takes place at a wavelength that corresponds to radio frequencies and when the spin returns to its base level, energy is emitted at the same frequency. The signal that matches this transfer is measured in many ways and processed in order to yield an NMR spectrum for the nucleus concerned.

- 4. Find the expression for the resolution of a chromatography column separating a gas mixture of two components A and B.** [WBUT 2011]

Answer:

Let A and B are two components. The separation of these components can be expressed in terms of the resolution, R, can be given by as below.

$$R = \frac{2[(t_r)_B - (t_r)_A]}{w_A + w_B} \quad \dots \text{(i)}$$

$$R = \frac{\sqrt{N}}{4} \frac{\alpha - 1}{\alpha} \frac{k'_B}{1 + k'_B} \quad \dots \text{(ii)}$$

From the above two equations we can say that separation of the two target components can be optimized by manipulation of the k' , α , and N terms. The first two terms are known as thermodynamic effects, and the third terms is associated with kinetic features of the column. The k' term is optimized by increasing the temperature of the column (as in GC) and by changing the mobile-phase composition (as in LC). The options available to optimize α are a change in the mobile-phase composition, column temperature, and stationary-phase composition. N can be improved by increasing the length of the column and reducing H, which is accomplished by changing the mobile-phase flow rate and reducing the size of the solid support, the thickness of the liquid stationary phase, the viscosity of the mobile-phase solvent, the temperature of the column, and the diameter of the column. The use of capillary columns and columns packed with small particles is a common practice in GC/MS and LC/MS applications.

- 5. On a 1000 cm long chromatography column, the helium carrier gas velocity is 40 cm/sec. The elution time for dicane is 1.27 sec and the peak width at the base is 1.5 sec. Calculate** [WBUT 2011]

- i) The capacity factor
- ii) The effective number of theoretical plates, N and
- iii) The height equivalent of theoretical plates, H.

Answer:

- i) Capacity factor = $\frac{t_r - t_m}{t_m}$, data given in the problem seem to be incorrect. Because t_r should me greater than t_m . Capacity factor is always positive and greater than one.

$$\text{ii) } N = 16 \left[\frac{t_R^2}{W^2} \right] = 16 \times \left[\frac{1.27}{1.5} \right]^2 = 11.46 = 12$$

$$\text{iii) } H = \frac{L}{N} = \frac{1000}{12} \approx 8.33 \text{ cm}$$

6. On a 122 cm long chromatography column, the retention times for the mobile phase, heptane and octane were 0.9 min, 1.22 min and 1.43 min respectively. The base widths of the bands in the chromatogram were 0.14 min for heptanes and 0.20 min for octane. Find out the relative R of the heptanes/octane band. [WBUT 2013]

Answer:

$$\text{Mobile phase } t_{RM} = 0.9 \text{ min}$$

$$\text{Heptane } t_{R \text{ heptane}} = 1.22 \text{ min}$$

$$\text{Octane } t_{R \text{ octane}} = 1.43 \text{ min}$$

$$W_{\text{heptane}} = 0.14 \text{ min}$$

$$W_{\text{octane}} = 0.20 \text{ min}$$

$$\text{Resolution } R = \frac{2(t_{R \text{ octane}} - t_{R \text{ heptane}})}{W_{\text{octane}} - W_{\text{heptane}}} = \frac{2(1.43 - 1.22)}{0.20 - 0.14} = \frac{2 \times 0.21}{0.06} = 7.0$$

Long Answer Type Questions

1. a) Explain with a block diagram the components used in a gas chromatograph for composition analysis of a gas mixture?

b) A packed column of 25 cm when used in chromatograph, three species A, B, C show retention times as 5, 14 and 20 min respectively and peak base widths as 0.4, 1.1 and 1.7 respectively. An uncertained species passes in 3 min. Calculate the column resolution, the plate in the column and the plate height. [WBUT 2010]

Answer:

a) **Gas Chromatograph**

The basic components of a gas chromatographic system are:

- Carrier gas supply along with pressure regulator and a flow monitor, sample injection system, chromatographic column, thermal compartment or thermostat, the detection system, the recorder.

The figure below shows a GC arrangement

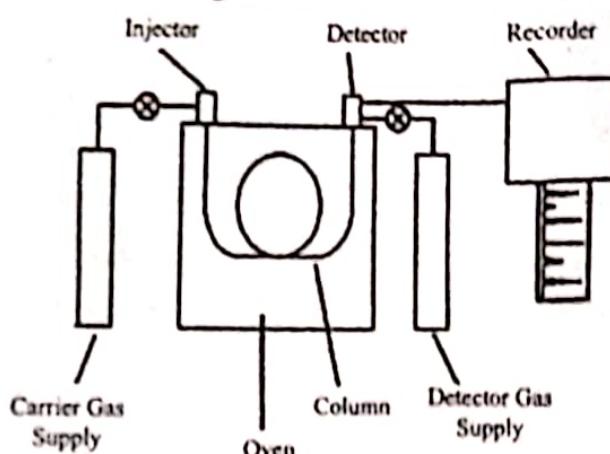


Fig: 1

In the above arrangement, firstly the sample components are vaporized and the gas flowing through the injector sweeps them into the column which is located inside the oven. The temperature of the oven precisely and reproducibly controlled. Each compound in the sample interacts with the column to a different degree and so travels through the column at a different rate. The rate of travel through the column for each individual compound depends on the type of column, the gas flow rate, and the temperature of the oven. As each compound exits the column it is detected and an electrical signal is sent to a recording device (integrator or computer data system, etc.). The recorded signal will appear as a series of peaks plotted versus time. This is called a chromatogram.

Normally, each peak represents an individual compound in the sample. In reality, however, it is not unusual for a peak represent more than one compound when two peaks overlap. Using the proper column and operating conditions, this problem can be resolved or at least minimized. The time at which a compound exits from the column after introduction of the sample upon injection is called retention time. Most compounds have a unique retention time under particular conditions. This unique property is used for compound identification by GC.

b)

$$t_{RA} = 5 \text{ min} \quad W_A = .4$$

$$t_{RB} = 14 \text{ min} \quad W_B = 1.1$$

$$t_{RC} = 20 \text{ min} \quad W_C = 1.7$$

$$N_A = 16 \left(\frac{5}{.4} \right)^2 = 2500$$

$$N_B = 16 \left(\frac{14}{1.1} \right)^2 = 2591.7355$$

$$N_C = 16 \left(\frac{20}{1.7} \right)^2 = 2214.5328$$

$$H_1 = \frac{L}{N_A} = \frac{25}{2500} = 0.01$$

$$H_2 = \frac{L}{N_B} = \frac{25}{2591.7355} = .00964$$

$$H_3 = \frac{L}{N_C} = \frac{25}{2214.5328} = 0.11$$

$$R_{S_1} = 2(14 - 5)/(1.1 - .4) = \frac{2 \times 9}{.7} = \frac{180}{7} = 25.71$$

$$R_{S_2} = 2(20 - 14)/(1.7 - 1.1) = \frac{2 \times 6}{.6} = \frac{2 \times 60}{6} = 20$$

2. What is gas chromatography? Explain with a block diagram, the components used in a gas chromatograph for composition analysis of a gas mixture.

[WBUT 2012, 2018]

OR,

Draw a diagram of the gas chromatography set-up and explain the functions of the components.

[WBUT 2013]

Answer:

Gas chromatography (GC) is an analytical technique used for separating compounds based primarily on their volatilities. Gas chromatography provides both qualitative and quantitative information for individual compounds present in a sample. Compounds move through a GC column as gases, either because the compounds are normally gases or they can be heated and vaporized into a gaseous state.

Basic parts of a Gas Chromatograph

The basic parts of a gas chromatograph are shown in the figure below. It consists of the following parts:

- Carrier gas supply along with pressure regulator and flow monitor
- Sample injection system
- Chromatographic column
- Thermal compartment or thermostat
- The detection system and
- Recorder.

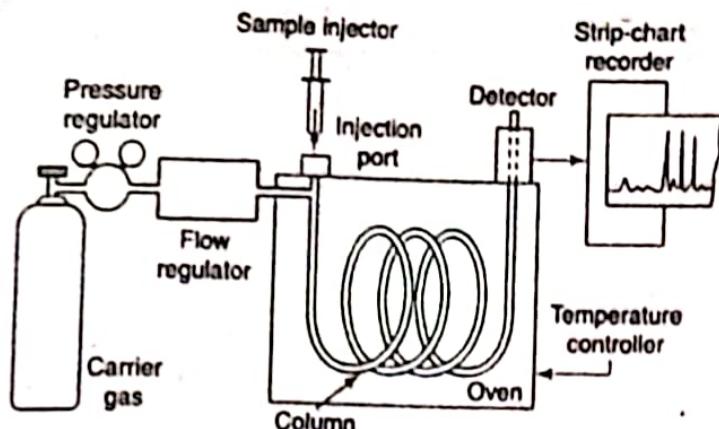


Fig: Block diagram of a gas chromatograph

The carrier gas, normally N₂, Ar or He, is usually available in a compressed form in a cylinder fitted with a suitable pressure regulator. The gas is conducted from the cylinder through a flow regulator, to a sample injection port maintained at a certain temperature T_1 , which is such that it ensures rapid vaporization, but not thermal degradation of the solute. Gas and liquid samples are almost always injected by syringe through a self-sealing silicon rubber diaphragm in the injection port. The solute vapour mixes almost instantaneously with the flowing carrier gas and is swept into the chromatograph column, which is the heart of the chromatograph. It is there that the different solutes in the vaporized sample are separated from each other, by virtue of their different interaction with the column packing. The column is maintained at another temperature T_2 . This temperature determines the time for the passage of the solutes and to some extent, the resolution and efficiency obtained with a particular column. At the end of the column, the solutes emerging individually enter the detector, which produces an electrical signal corresponding to the quantity of solute leaving the column. The detector signal is supplied to a recorder and a plot of the time – signal amplitude called chromatogram is obtained. This record is used to determine the identity of the components in the mixture and their respective concentrations.

3. Describe the working principle of ESR.

[WBUT 2012]

Answer:

When an atomic or molecular system with unpaired electrons is subjected to a magnetic field, the electronic energy levels of the atom or molecule will split into different levels. The magnitude of the splitting is dependent on the strength of the applied magnetic field. The atom or molecule can be excited from one split level to another in the presence of an external radiation of frequency corresponding to the frequency obtained from the difference in energy between the split levels. Such an excitation is called a magnetic resonance absorption. The atom or molecule under investigation may be in different environments in an actual sample. The magnetic resonance frequency will hence be

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influenced by the local environment of the atom or molecule. The electron spin resonance technique is therefore, a probe for a detailed identification of the various atomic and molecular systems and their environments and all associated parameters.

4. What is the principle behind separation, identification and quantification of the constituent components of a gas mixture using gas chromatography?

[WBUT 2012, 2014]

Answer:

Chromatography is a very important analytical tool because it allows the chemist to separate components in a mixture for subsequent use or quantification. Chromatograph makes it possible to separate the volatile components of a very small sample and to determine the amount of each component present. The essentials required for the method are an injection port through which samples are loaded, a "column" on which the components are separated, a regulated flow of a carrier gas (often helium) which carries the sample through the instrument, a detector, and a data processor. In gas chromatography, the temperature of the injection port, column, and detector are controlled by thermostatted heaters.

The sample to be analyzed is loaded at the injection port via a hypodermic syringe. The injection port is heated in order to volatilize the sample. Once in the gas phase, the sample is carried onto the column by the carrier gas, typically helium. The carrier gas is also called the mobile phase.

The column is where the components of the sample are separated. The column contains the stationary phase. Gas chromatography columns are of two types—packed and capillary. Capillary columns are those in which the stationary phase is coated on the interior walls of a tubular column with a small inner diameter. We will use a capillary column in this experiment. The components in the sample get separated on the column because they take different amounts of time to travel through the column depending on how strongly they interact with the stationary phase.

If the column conditions are chosen correctly, the components in the sample will exit the column and flow past the detector one at a time. The choice of detector is determined by the general class of compounds being analyzed and the sensitivity required. Flame ionization detectors (FIDs) are the most widely used detectors for organic samples. Thermal conductivity analyzers may also be used for identification and quantization of the gas constituents in the mixture.

5. a) Draw a schematic diagram of a binary system HPLC and explain its working principle.

b) How bands spread in GC column?

c) How many theoretical plates produce a chromatography peak eluting at 12.83 min with a width at half height of 8.7s? Find the plate height when the length of the column = 15.8 cm.

[WBUT 2015]

Answer:

a) High performance liquid chromatography (HPLC) also known as high pressure liquid chromatography is a versatile and sophisticated instrument used for a wide variety of analytical and preparative applications. The instrument consists of the main components

- i) solvent or eluent reservoir(s) which is simply a glass bottle to hold the liquid
- ii) a microprocessor or computer-controlled, high-pressure pump to deliver the mobile phase from the reservoir to the head of the column at a constant flow rate
- iii) sample injection port through which a pulse of the sample mixture is delivered onto the guard column
- iv) guard column and analytical column containing the packed stationary phase
- v) a suitable detector (e.g., UV or UV-Visible spectral detector or fluorescence detector)
- vi) a data processor with display or recorder provision
- vii) fraction collector to collect separated samples, as shown the Fig. 1.

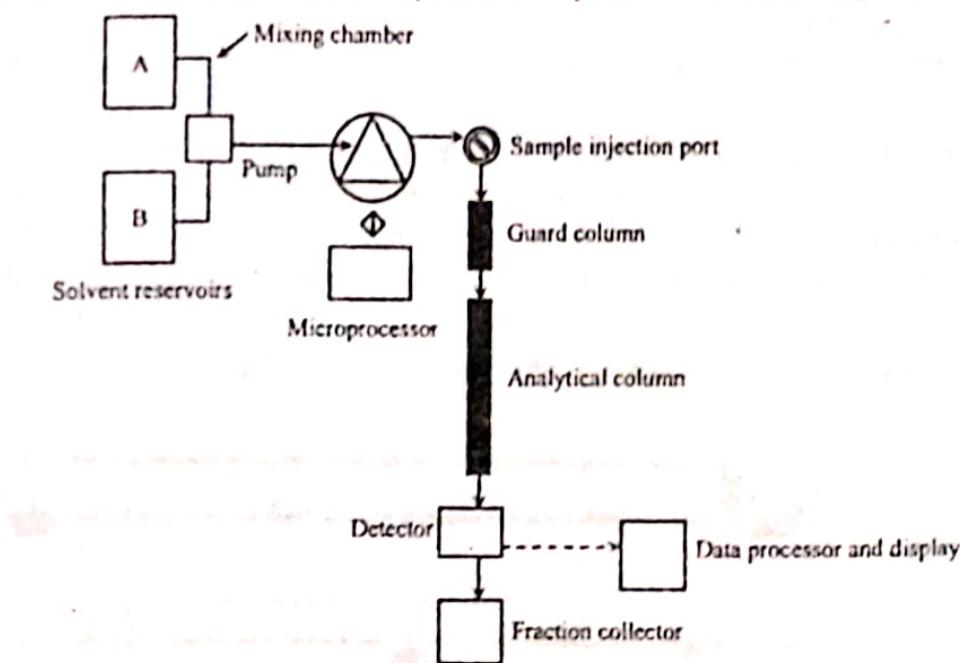


Fig: 1 A schematic diagram of a binary HPLC system

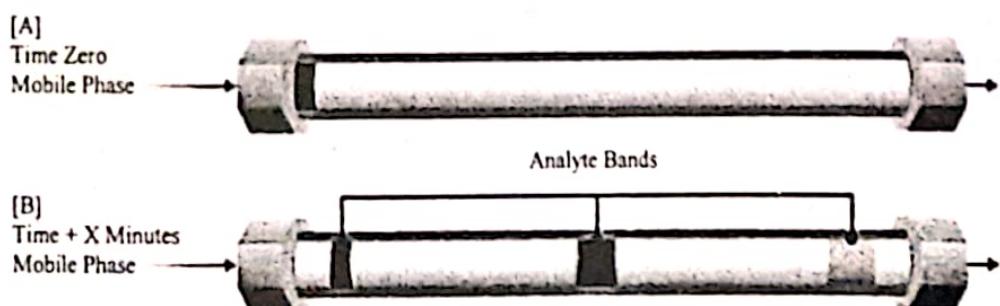
The solvent reservoir contains highly purified eluent which has been filtered through a microfilter to remove any suspended and particulate impurities and degassed to remove any dissolved gases. A single solvent is used for isocratic elution. A mixture of two, three or four solvents (binary, ternary, quaternary) for gradient elution when the composition of the mobile phase is altered during the chromatographic run to optimise the separation. The mixture of solvents is blended in the mixing chamber under microprocessor or computer control the eluent is pumped by the microprocessor or computer controlled high pressure pump, one of the most important components of HPLC since its performance directly affects the detector sensitivity and reproducibility of chromatographic separations. The pump is capable of operating at pressures up to 500 atmospheres.

delivers the eluent in a precise and pulse-free manner at flow rates in the range 0.02 to 10 ml/min. The sample injection device is a valve capable of introducing a sharp pulse or plug of sample of 10 to 20 μl (sometimes up to 100 μl) with the help of a micro syringe. The column is made of SS tubing 25 cm long and 3 to 4 mm diameter for analytical purposes. A pre-column or guard column is usually included between the sample injector and the column to protect the main column from damage. The columns contain the packed bed of stationary phase particles.

Support materials for column packing are small rigid particles mostly of silica or alumina having a narrow particle size distribution. Rigid, porous polymeric beads based on synthetic polystyrene crosslinked with devinyl benzene or crosslinked natural polymers such as dextran or agarose are used for size exclusion chromatographic mode of HPLC. Bonded phases prepared by reacting organochlorosilanes or organo alkoxy silanes with porous silica particles are used in the reverse phase mode. Ion exchange chromatography, uses ion-exchange resins based on synthetic or natural polymeric gels. For affinity, pseudo-affinity, covalent and hydrophobic interaction chromatographic techniques the stationary phase consists of specific ligands coupled or immobilized on agarose beads.

b) The band spread is shown through the following figure [flow direction is represented by green arrows]. Figure A represents the column at time zero [the moment of injection], when the sample enters the column and begins to form a band. The sample shown here, a mixture of yellow, red, and blue dyes, appears at the inlet of the column as a single black band.

Injected Sample Band (blue, red & yellow mixture appears black)



After a few minutes, during which mobile phase flows continuously and steadily through the packing material particles, we can see that the individual dyes have moved in separate bands at different speeds [Figure B]. This is because there is a competition between the mobile phase and the stationary phase for attracting each of the dyes or analytes. Notice that the yellow dye band moves the fastest and is about to exit the column. The yellow dye has a greater affinity for [is attracted to] the mobile phase more than the other dyes. Therefore, it moves at a faster speed, closer to that of the mobile phase. The blue dye band has a greater affinity for the packing material more than the mobile phase. Its stronger attraction to the particles causes it to move significantly slower. In other words, it is the most retained compound in this sample mixture. The red dye band has an

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intermediate attraction for the mobile phase and therefore moves at an intermediate speed through the column. Since each dye band moves at a different speed, we are able to separate the mixture chromatographically.

c) Given data

Chromatography peak width $(t_R + t_M) = 12.83 \text{ min} = 12.83 \times 60 \text{ sec}$

Width at half height $W_h = 8.7 \text{ sec}$

Let number of theoretical plates be N

From gas-chromatography formula

$$N = 5.54 \left(\frac{t_p + t_M}{W_h} \right)^2 = 5.54 \left(\frac{12.83 \times 60}{8.7} \right)^2 = 43,373.76 = 4.373 \times 10^4 \quad (\text{Ans.})$$

Let the plate height be H

Given column length $L = 15.8 \text{ cm}$

We know

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

After inserting the value we get

$$H = \frac{15.8}{4.373 \times 10^4} = 3.61 \times 10^{-4} \text{ cm} \quad (\text{Ans.})$$

6. Draw and describe the operation of the Electron Capture Detector (ECD).

[WBUT 2015]

Answer:

In the electron capture detector (ECD), the column effluent passes between two electrodes of the PID, one of which has been treated with a radioactive source (tritium or nickel-63), with the latter being preferred because of extended detector stability) that emits high-energy electrons. These electrons produce large quantities of low-energy thermal electrons in the carrier gas, which are in turn collected by the other electrode to produce a steady-state current in the presence of pure carrier gas. Compounds eluting from the column that have an affinity for thermal electrons reduce this steady-state current thereby producing the chromatogram. The detector is thus highly selective, with halogenated compounds being the most responsive (detection at the picogram level). Other groups exhibiting good selectivity include anhydrides, peroxides, conjugated carbonyl, nitriles and nitrates, and sulphur-containing compounds (Fig. 1).

Maintenance is of critical importance with this detector more so than with any other GC detector (except the helium ionization detector, considered next). It responds exceptionally well to oxygen, necessitating leak-free systems and oxygen-free carrier gases. Also, response to water vapour can cause unstable baselines so that molecular sieve traps (which require periodic maintenance) are required in the carrier gas lines. Finally, care must be taken to use this detector only with columns of very low bleed as

the condensed stationary phase in the detector can easily be polymerised (by radiation and electron bombardment) to a hard insoluble deposit, which is almost impossible to eliminate and which interferes with the proper functioning of the detector.

Although a pulsed mode of operation has improved the linear range of the ECD, it has not improved its robustness, and all of the above-outlined concerns still apply.

Schematic of an electron capture detector arranged for constant voltage operation. Also shown is the chromatogram produced by the reduction of the standing current when compounds of a higher electron affinity than the carrier gas are eluted from the column and pass through the detector.

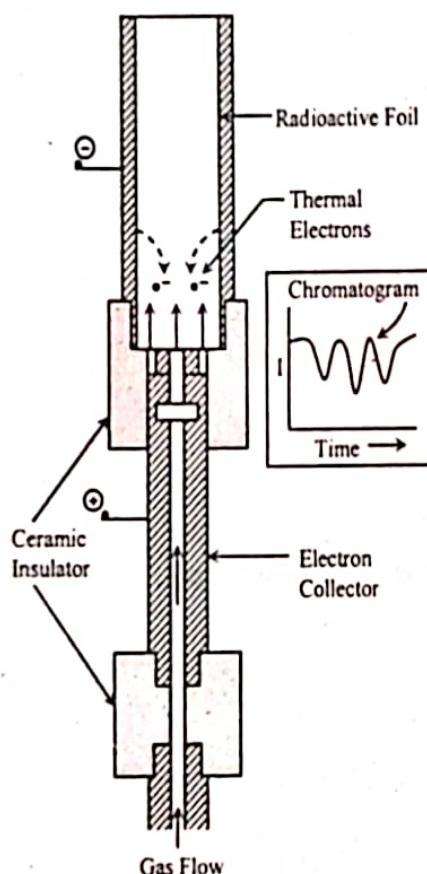


Fig: 1

**7. What do you mean by temperature programming in gas chromatography?
[WBUT 2016]**

Answer:

Temperature programming in gas chromatography is an approach to resolve components completely. During a sample run, if the column is maintained at a low temperature, the first peaks to elute will likely be well-spaced, but the components staying on the column longer will find themselves bound to the stationary phase for longer periods of time; this results in large band-broadening, and long run times. At higher temperatures, these components spend more time in the mobile (gas) phase, helping them elute faster and

minimizing band-broadening; the faster peaks also elute faster however, pressing the peaks so close together that they may not be resolved.

In temperature programming, this effect is overcome by maintaining a low temperature for a short period of time, and increasing the temperature to help force out the longer-'sticking' compounds. This changes the retention time compared to a isothermal run, but if the same temperature 'ramp' is used, the elution times will remain constant for each component. Since retention time generally cannot be used to gain any real molecular information, this situation is acceptable, and can be readily used to speed up assays while giving better-resolved peaks.

8. a) Find the expression for the resolution of a chromatography column for separating a gas mixture of two components A and B.
b) Draw and describe the operation of the electron capture detector (ECD).
c) The substances A and B were found to have retention times of 16.40 min and 17.63 min, respectively, on a 30.0 cm column. An unretained species passed through the column in 1.30 min. The peak widths (at base) for A and B were 1.11 min and 1.21 min respectively.

Calculate the following:

- The column resolution
- The average number of theoretical plates in the column
- The height equivalent to theoretical plates (HETP)_H
- The length of column required to achieve a resolution of 1.5. [WBUT 2016]

Answer:

a) Refer to Question No. 4 of Short Answer Type Questions.

b) The electron capture detector (Fig. 1) consists of a cell containing a β -emitting radioactive source, purged with an inert gas. Electrons emitted by the radioactive source are slowed to thermal velocities by collision with the gas molecules and are eventually collected by a suitable electrode, giving rise to a standing current in the cell. If a gas with greater electron affinity is introduced to the cell, some of the electrons are "captured," forming negative ions, and the current in the cell is reduced. This effect is the basis of the electron capture detector. The reduction in current is due both to the difference in mobility between electrons and negative ions and to differences in the rates of recombination of the ionic species and electrons.

The radioactive source may be tritium or ^{63}Ni , with ^{63}Ni usually being preferred, since it allows the detector to be operated at higher temperatures, thus lessening the effects of contamination. A potential is applied between the electrodes that is just great enough to collect the free electrons. Originally, the detector was operated under DC conditions, potentials up to 5 volts being used, but under some conditions space charge effects produced anomalous results. Present detectors use a pulsed supply, typically 25 to 50 volts, 1 microsecond pulses at intervals of 5 to 500 microseconds. Either the pulse

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interval is selected and the change in detector current monitored, or a feedback system maintains a constant current and the pulse interval is monitored.

The electron capture detector is extremely sensitive to electronegative species, particularly halogenated compounds and oxygen. To obtain maximum sensitivity for a given compound, the choice of carrier gas, pulse interval, or detector current and detector temperature must be optimized.

- A - Inlet for carrier gas and anode
- B - Diffuser - made of 100 mesh brass gauze
- C - Source of ionizing radiation
- D - Gas outlet and cathode

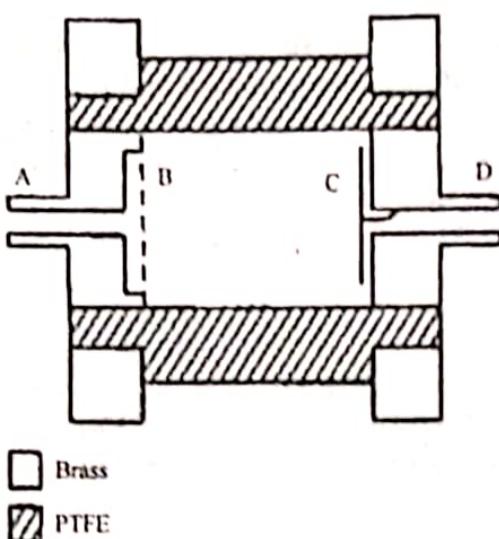


Fig: 1 Electron capture detector

The electron caputure detector is most often used in gas chromatography, with argon, argonmethane mixture, or nitrogen as carrier gas, but it is also used in leak or tracer detectors. The extreme sensitivity of the ECD to halogenated compounds is useful, but high-purity carrier gas and high-stability columns are required to prevent contamination. Under optimum conditions, 1 part in 10^{12} of halogenated compounds, such as Freons, can be determined.

$$\text{c) } R_s = \frac{2[(t_r)_y - (t_r)_x]}{W_x + W_y}$$
$$R_s = \frac{2(17.63 - 16.40)}{1.11 + 1.21} = 1.06$$
$$N = 16 \left(\frac{t_r}{W} \right)^2$$

ANALYTICAL INSTRUMENTATION

$$N_A = 16 \left(\frac{16.40}{1.11} \right)^2 = 3493 \quad N_B = 16 \left(\frac{17.63}{1.21} \right)^2 = 3397$$

$$N_{avg} = \frac{(3493 + 3397)}{2} = 3345$$

$$H = \frac{L}{N} = \frac{30.0}{3345} = 8.7 \times 10^{-3} \text{ cm}$$

k' and α do not change with increasing N and L , so by using

$$R_s = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \frac{k'}{(1 + k')} \text{ we can solve for } N \text{ at a given } R_s.$$

$$\frac{R_{S_1}}{R_{S_2}} = \frac{\sqrt{N_1}}{\sqrt{N_2}} \text{ where 1 and 2 are the initial and longer columns, respectively}$$

$$N_2 = N_1 \left(\frac{R_{S_2}}{R_{S_1}} \right)^2 = 3445 \left(\frac{1.5}{1.06} \right)^2 = 6.9 \times 10^3$$

$$L = N \times H = (6.9 \times 10^3) (8.7 \times 10^{-3} \text{ cm}) = 60 \text{ cm}$$

$$\text{Since } (t_r)_r = \frac{16R_s^2 H}{u} \left(\frac{\alpha}{\alpha - 1} \right)^2 \frac{(1 + k')^3}{(k')^2} \text{ we get}$$

$$\frac{t_{r_1}}{t_{r_2}} = \frac{R_{S_1}^2}{R_{S_2}^2}$$

$$\text{or, } t_{r_2} = t_{r_1} \left(\frac{R_{S_2}^2}{R_{S_1}^2} \right)$$

$$t_{r_2} = (17.63 \text{ min}) \times \left(\frac{1.5}{1.06} \right)^2 = 35 \text{ min.}$$

9. Write short notes on the following:

- a) Solvent programming in HPLC
- b) High Pressure Liquid Chromatography (HPLC)
- c) Mass Spectrometer Detector in HPLC
- d) Electron Capture Detector in GC

[WBUT 2012]

[WBUT 2013, 2014]

[WBUT 2015]

[WBUT 2015]

OR,

Electron Capture Detector (ECD)

[WBUT 2017]

Answer:

a) Solvent programming in HPLC:

HPLC is a type of liquid chromatography where the column is specially packed to provide greater surface area. For a useful flow rate through this rigidly packed column, a greater pressure is to be maintained across the column which is actually done by a pump.

The HPLC instrument physically separates the components of a sample, typically in solution and provides information about the concentration of each separated component. For this purpose, the instruments allow the injection of a measured small volume of sample in a mobile phase. This mobile phase flows through a chromatographic column where the separation takes place and further through a detector (or detectors) capable of generating a signal proportional with the analyte concentration. Using calibrations, the concentration of the analytes can be determined. This process can be achieved using a large number of models of HPLC systems. The construction of these instruments depends significantly on the intended function and size of the HPLC separation. Modern HPLC instrumentation is sophisticated and is in continuous development. For this reason, this section is intended only to give a basic and simplified view regarding the HPLC equipment.

The solvent supply provides the solvent(s) necessary as a mobile phase for the HPLC.

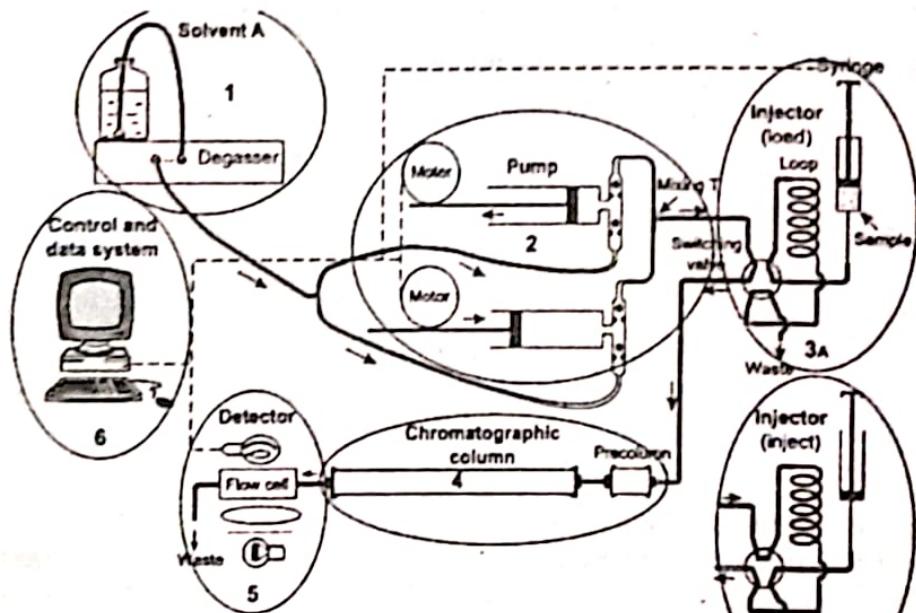


Fig: Schematic description of a simple HPLC system

- 1) A solvent supply system (solvent container and degasser),
- 2) a high pressure pumping system (a dual mechanical pump is pictured),
- 3) an injector (a syringe with the sample and a switching valve in two positions A load loop, B inject),
- 4) a chromatographic column (possibly with a precolumn or guard column),
- 5) one or more detectors (a spectrophotometric detector is schematized),
- 6) a controller/data processing unit

Some solvent supply systems may also have the capability to remove the gasses dissolved in solvents (the degassing capability). The solvent(s) are transferred through low pressure tubing to the pumping system. The tubes used for passing the mobile phase through the system need to fulfill mainly the requirement of being inert to be utilized solvents and to stand pressures up to about 50 psi (1 psi = 6.89476 kPa = 6.89476×10^3 bar = 6.89460×10^3 atm; 1 bar = 14.5037738 psi). Fluorocarbon polymers such as Teflon are common materials used for this type of tubing, but polypropylene is also sometimes used. The solvent supply system of an HPLC has one or more reservoirs for the solvents used as the mobile phase. For HPLC performed in isocratic conditions and using pure or a premixed solvent, only one reservoir is necessary. However, it is common in HPLC to use gradient separations, or to use an isocratic separation but to generate the mixture of solvents using the pumps. In this case, two (or more) solvents that are mixed with the pumping system in variable proportions are required. For this reason, two or more solvent reservoirs are common in modern HPLC instruments. The reservoirs must be clean and inert to the solvents they contain. The solvents from the reservoirs must be free of particles and they are either purchased as HPLC grade or/and filtered through $0.45\mu\text{m}$ filters before use. The filter selected for the filtration must be inert to the solvent. In the case of solvent mixtures containing a buffer, the general rule indicates that the buffer solution is made in water, then filtered and only then mixed with the organic solvent (assuming correct concentrations and no precipitation after organic solvent addition).

b) High Pressure Liquid Chromatography (HPLC):

Principle: High performance liquid chromatography (HPLC) is a form of column chromatography with lower resolution but wider applicability than GC. The acronym HPLC is often shortened to LC. The analyte is carried through a stationary phase in a packed column by a liquid mobile phase at high pressure, during which time the components separate from each other on the column. Traditionally, HPLC columns were polar, e.g., silica and the mobile phases used were relatively nonpolar in nature. This mode of HPLC was called normal phase chromatography.

More common today is 'reversed' phase HPLC where the columns are hydrophobic, e.g., C-18 bonded phase. Solvents used in reversed phase HPLC include any miscible combination of water and various organic modifiers (the most common are methanol or acetonitrile). On a reversed phase column, hydrophobic analytes are better retained, eluting more readily as the proportion of the hydrophobic component of the stationary phase is increased. Often, a gradient of solvent composition passing through the column is used to separate mixtures, e.g., a water/methanol gradient. Following separation, the analytes are detected by the detector as they elute from the column. Trace organic and inorganic materials can be determined at concentrations of 10^{-6} – 10^{-12} g depending on the detector chosen. Further information on HPLC is available elsewhere.

Instrument: A HPLC instrument consists of the components shown in Fig. 1.

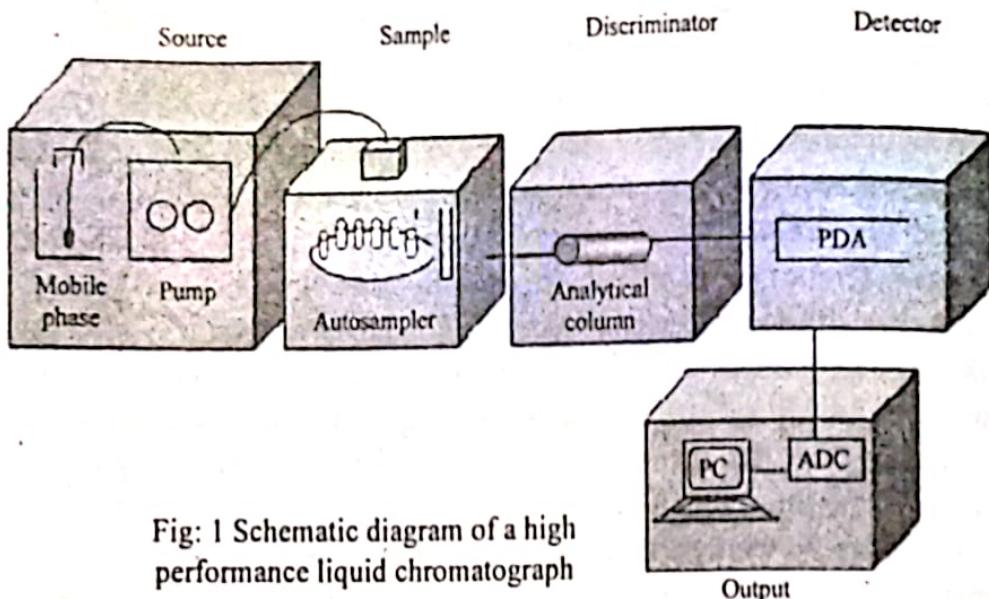


Fig: 1 Schematic diagram of a high performance liquid chromatograph

Source: The mobile phase and pump comprise the source. The mobile phase is chosen such that it has the optimal eluting power for the HPLC mode being used, low viscosity, high purity and stability and such that it is compatible with the detection system. Solvents usually need to be degassed prior to use either by filtering under vacuum, use of an ultrasonic bath or degassing online. An optional solvent gradient mixer can precede the pump to enable mixing of solvents.

Liquid chromatography pumps can deliver a range of flow rates from nL/min to 10mL/min depending on the type of pump purchased, e.g., standard or microflow. A standard pump used at a flowrate of 1mL/min with a standard column of 250×4.6mm dimensions and 3-10 μ m particle size would tolerate a pressure of up to 3000psi. The most commonly used pumps in HPLC are constant flow pumps, which can be subdivided into syringe pumps and reciprocating piston types. Syringe pumps deliver a smooth, pulse-less flow but are limited by the volume they can hold; also during the refill of the syringe barrel, there will be no flow. Hence reciprocating piston pumps are the most prevalent type in use today. However, because pistons having a fill stroke and a delivery stroke, pulsed flow results; this can be reduced with more than one piston, e.g., dual piston or triple piston pumps. In the dual head pump, each of the pistons is 180° out of phase with the other so that while one is filling, the other is delivering. This balances the pulsing somewhat and

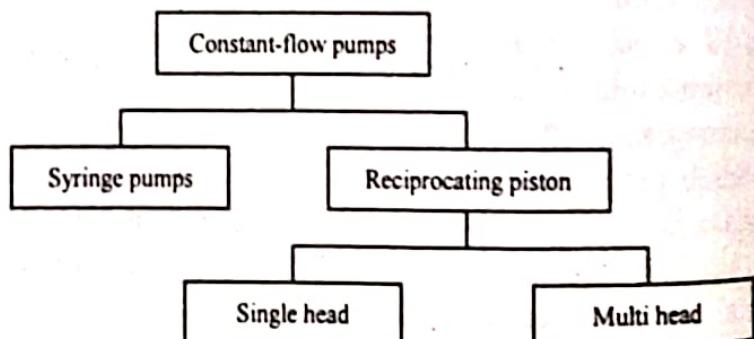


Fig: 2 Types of constant flow pumps in HPLC

smoothes the flow. With more piston heads, the flow is smoothed even more. Nearly all new pumps also have pulse damping included.

c) **Mass Spectrometer Detector in HPLC:**

The detector senses the presence of individual components as they leave the column. The detectors convert a change of effluents into an electrical signal that is recorded by the recorder.

The detectors should have the following properties such as high sensitivity, a linear response to the solute, good reproducibility and stability, negligible base line noise, short response time and independent of flow rate, ease of operation, inexpensive and non-destructive.

Common HPLC detectors are UV-Visible, Refractive Index, Fluorescence, Conductivity (for ion chromatography). HPLC most commonly uses a UV-VIS absorbance detector. Refractive index detectors provide readings by measuring the changes in the refractive index of the effluent as it moves through the flow cell.

d) **Electron Capture Detector in GC:**

Refer to Question No. 6 of Long Answer Type Questions.