MODEL ANSWER

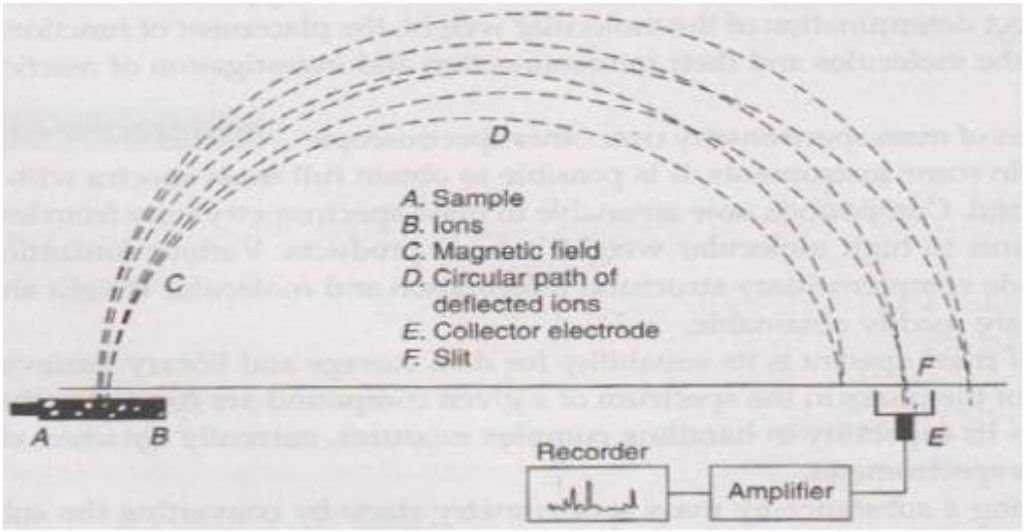
WINTER– 17 EXAMINATION

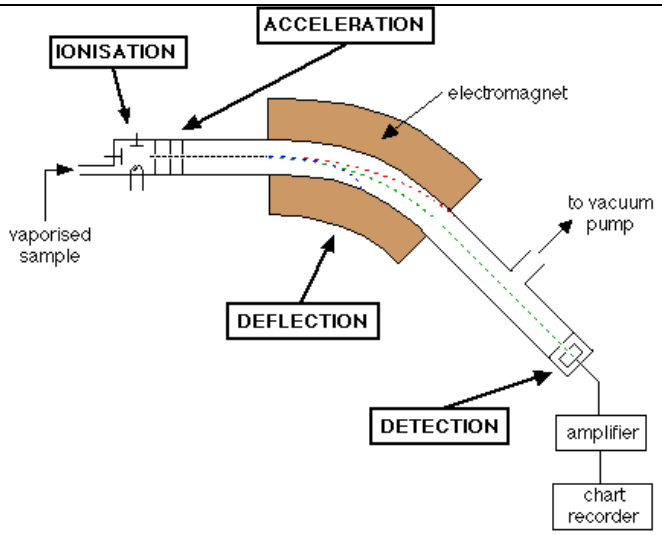
Subject Title: Analytical Instrumentation Subject Code: 17539

Important Instructions to examiners:

1) The answers should be examined by key words and not as word-to-word as given in the model answer scheme.
2) The model answer and the answer written by candidate may vary but the examiner may try to assess the understanding level of the candidate.
3) The language errors such as grammatical, spelling errors should not be given more Importance (Not applicable for subject English and Communication Skills.
4) While assessing figures, examiner may give credit for principal components indicated in the figure. The figures drawn by candidate and model answer may vary. The examiner may give credit for any equivalent figure drawn.
5) Credits may be given step wise for numerical problems. In some cases, the assumed constant values may vary and there may be some difference in the candidate's answers and model answer.
6) In case of some questions credit may be given by judgement on part of examiner of relevant answer based on candidate's understanding.
7) For programming language papers, credit may be given to any other program based on equivalent concept.

Q. No.	Sub Q.N.	Answer	Marking Scheme
Q.1	(a)	**Attempt any THREE:**	12M
	(i)	**What is Electrophoresis? List the parts of electrophoresis apparatus.**	4M
	Ans:	**Electrophoresis:** • Electrophoresis is a separation technique of individual components of the colloidal solution that is based on the mobility of ions in an electric field. • Ions have different migration rates depending upon their total charge, size and shape, and can therefore be separated. • If E is a strength of electric field is a charge on molecule and F is the frictional force on the molecules then V the velocity of migration is given by $V=EZ/F$ E-Strength Of Electrical field. Z-Charge On Molecule F-frictional force on molecule The frictional force can be defined as $F=6\pi\eta r$ η- viscosity of medium r-stroke radius of molecule Where η is viscosity of medium and r is stoke radius of molecules therefore $V=EZ/6\pi\eta r$ This implies that the electrophoretic mobility is proportional to the charge on molecules and inversely proportional to the radius of molecules. **The parts of electrophoresis apparatus:** 1)Electrophoresis cabinet 1.1. Plastic cabinet 1.2. Gable cover	2M 2M

		1.3. Carrier rack 2)Power supply 3)Densitometer	
	(ii)	**State and explain the principle of mass spectrometer.**	**4M**
	Ans:	 **<u>Principle of operation of a mass spectrometer</u>**: The molecules in the gas sample (A) to be analyzed are bombarded with electrons to produce ions (B). These ions are accelerated in a high vacuum into a magnetic field (C), which deflects them into circular paths (D). Since the deflection for light ions is greater than that for heavy ions, the ion stream separates into beams of different molecular weight. A suitably placed slit (F) allows a beam of a selected molecular weight to pass through to a collector electrode (E). As the accelerating voltage on the ion source is gradually reduced, ion beams of successively greater mass pass through the slit. When these ions fall on the collector electrodes, they produce minute electric currents, which may be measured after suitable amplification. Their amplitude will indicate the number of ions in each beam. The proportion of molecules of different masses in the gas sample may thus be found and a complete analysis of the gas sample may be made; provided all the constituent gases have a different molecular weight. This is usually the case in respiratory gas analysis work. **<u>OR</u>**	**2M** **2M**



Mass spectrometry works in five stages.

1. Vaporization
2. Ionization
3. Acceleration
4. Deflection
5. Detection

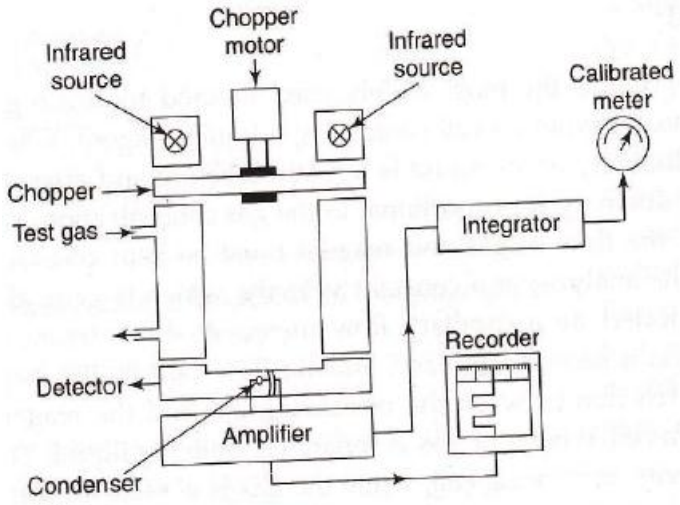
Vaporization: The sample is heated to very high temperature by current passing through the coil. The sample gets vaporized.

Ionization: The sample gets ionized by bombarding with electrons. Cations (positive ions)are formed by loosing electrons.

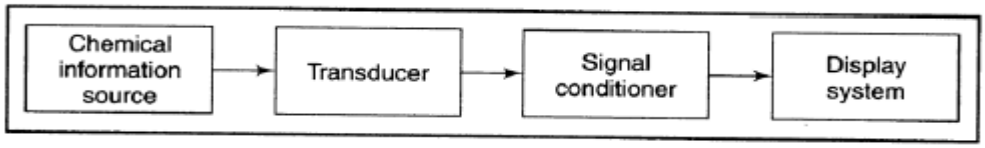
Acceleration: Ions get accelerated by applying strong electric field.

Deflection: The ions get deflected at different angles due to magnetic field. Due to different mass to charge ratio, ions are deflected at different angles.

Detection: Deflected ion are detected by detection system. It determine mass-charge ratio.

(iii)	**Explain the principle of working of Infrared gas analyzer with block diagram.**	**4M**
Ans:		**2M**

		Working : • Infrared gas analyzers based on principle that some gases and vapours absorb specific wavelengths of infrared radiation. • One of the most commonly measured gases using infrared radiation absorption method is the carbon dioxide. This technique used for this purpose is the conventional double-beam. • One cell is filled with a reference gas, which is a non absorbing gas like nitrogen, whereas the measuring cell contains the sample. The difference is optical absorption detected between the two cells is a measure of absorption of the sample at the particular wavelength. Since the vibration excitation occurs only if we have hetero-atomic molecules. • Infrared analyzers are used for the determination of a large no of components like CO, CO2, SO2, NH3, H2O, Nitric oxide as well as most gaseous hydro carbons. • One beam passes through the sample cell, and the other beam through a reference cell, and the both beam through a reference cell, and both beams enter opposite ends of the detection chamber. The detection chamber is permanently sealed unit divided into two compartments by a thin, metal diaphragm. Both compartments are charged to the same pressure, with the gas being measured. • When the gas being measured enters the sample cell, it absorbs infrared radiation at the same wavelength as gas in the detection chamber. This reduces the amount of radiation reaching the gas in the sample side of the detection chamber and produces a lower pressure in that side .the diaphragm bends toward the sides of lower, and this movement is converted into electrical impulses.	**2M**
	(iv)	**Write the general equation of representation of concentration of gases. State the importance of each term on equation.**	**4M**
	Ans:	Gas concentrations in atmosphere are generally represented as parts per million by volume, i.e. ppm / v or simply ppm per hundred million (pphm), i.e. parts per billion (ppb). On the other hand, toxicological data is generally represented on a gravimetric basis, e.g. micrograms per cubic meter or milligrams per litre. Conversion from volumetric to gravimetric concentration can be obtained by applying gas laws , the general equation from this being: $\mu g/ m3 = ppm \times PM / RT \times 10^3$, Where ; P= Total pressure (atm) M= Molecular weight of gas of interest R= Gas constant = 0.0821 1-atm /(mole) (K^0) T=absolute temperature , K^0	**2M** **2M**
	b)	**Attempt any ONE:**	**6M**
	i)	**Draw the block diagram of analytical instruments and explain the function of each block.**	**6M**
	Ans:	**Basic block diagram of Analytical instrumentation system:**	**2M**



Elements of an analytical instrument

1.Chemical information source:
It generates a set of signals containing necessary information. The source may be in the sample itself. For example, yellow radiation emitted by heated sodium atoms constitutes the source of the signal in a flame photometer.

2. Transducer:
Transducer converts signal from one form to another. Because of the advantages of electric and electronic methods of measurement, it is the usual practice to convert all non-electrical quantities associated with the analysis of a sample into electrical form.

3. Signal conditioner:
Signal conditioner converts the output of the transducer into electrical quantity suitable for operation of display system. Signal conditioner may be varying in complexity from a simple resistance network or impedance matching device to multi-stage amplifiers and other electronic circuit.

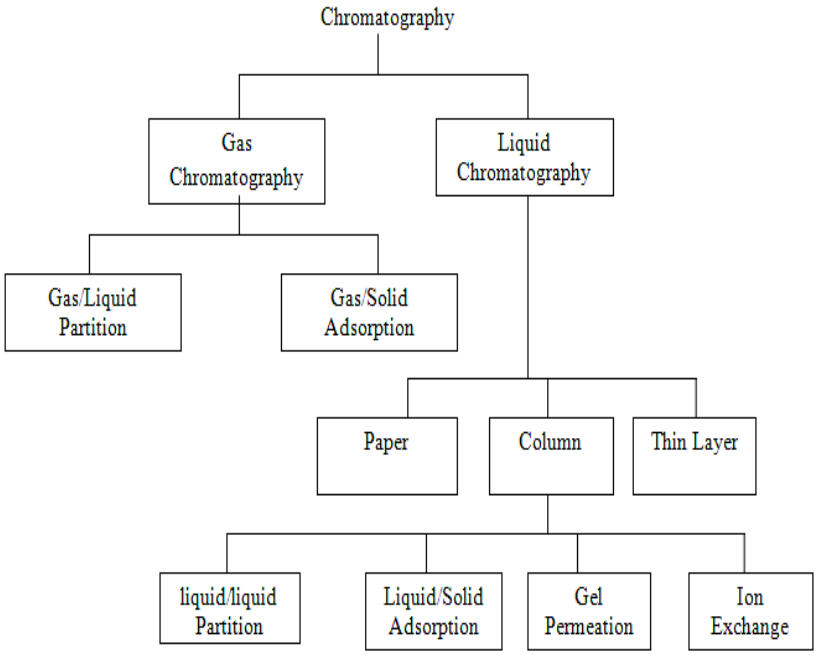
4. Display system:
It provides a visible representation of the quantity as a displacement on a scale or on the chart of a recorder or on the screen of a CRT or in numerical form.

4M(Each 1M)

ii) State the principle of chromatography. Classify the chromatography in detail. 6M

Ans: **3M**

Classification of Chromatography



Principle of chromatography:

- When analytes are introduced into the column, the molecules distribute between stationary and mobile phase. **3M**

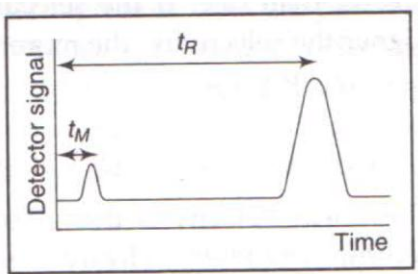
		• Molecules in the mobile phase re-enter the stationary phase when they collide with the stationary phase. • At the same time, molecules leave the stationary phase and enter the mobile phase. • This process is repeated many times and separation of components takes place. • The molecules which have less interaction between stationary and mobile phase, they move faster. • The molecules which have more interaction between stationary and mobile phase, they move slower. • The position of the distribution equilibrium determines the migration velocity. It reflects the Intermolecular interaction of analyte with stationary and mobile phase.	
Q 2		**Attempt any FOUR:**	**16M**
	a)	**State and explain Beer-Lambert's laws.**	**4M**
	Ans:	**<u>Statement</u>:** Beer Lambert Law, states the relationship between absorbance (A) and transmittance (T). It states that the concentration of a substance in solution is directly proportional to the 'absorbance', A, of the solution. **<u>Absorbance :</u>** $A = \varepsilon cb$ Where, A = absorbance (no unit of measurement) ε = molar absorptivity (dm3 mol -1 cm-1) C = molar concentration (mol dm -3) B = path length (cm). It may be noted that ε is a function of wavelength. So, the Beer Lambert Law is true only for light of a single wavelength or monochromatic light. Absorptivity is a constant, depending upon the radiation and nature of the absorbing material. Absorptivity is also sometimes referred to as specific extinction and absorbance as 'Optical Density'. Absorbance is the property of a sample, whereas absorptivity is the property of substance and is a constant. Mathematically, absorbance is related to percentage transmittance T by the expression: $A = \log_{10}(\frac{10}{I})$ $= \log_{10}(\frac{100}{T})$ $= \varepsilon cb$ The relationship between energy absorption and concentration is of great importance for the purpose of analysis.	**2M** **2M**
	b)	**Define chromatography. What is the significance of column length on chromatogram?**	**4M**
	Ans:	**<u>Definition of Chromatography:</u>** Chromatography is a physical method of separation of the components of a mixture by distribution between two phases, of which one is a stationary bed of large surface area and other a mobile phase that percolates(spreads slowly) through or along the	**1M**

stationary phase.

significance of column length on chromatogram: **3M**

A chromatographic column is the heart of a chromatograph where the fundamental process of separation takes place. When a sample of gas or vapour is introduced into the column, it spreads by molecular diffusion to yield a concentration profile. As the sample moves up the shape of the band is detected and recorded as chromatographic peak.

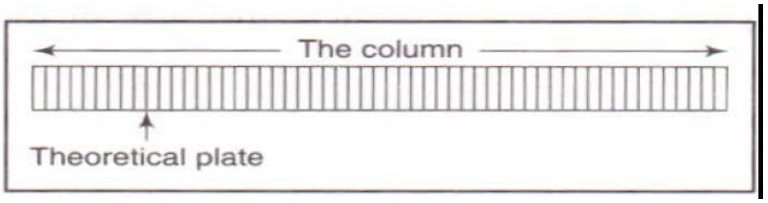
1. A longer column length improves the process of separation. The column efficiency is a function of column length. Longer the column, greater is the efficiency. Doubling the length increases the resolution by 40%.
2. A longer column generally improves the separation of components of the sample.
3. Longer column increases the retention time, significant peak broadening will be observed because of increased longitudinal diffusion(spread over a long area) inside the column.



t_R-retention time
$t_{M\text{-}Dead}$ time

4.One has to keep in mind that the gas molecules are not only travelling in one direction but also sideways and backwards. Because of longer column length, diffusion is more, peak broadening happens.

5.This is drawback of increasing length, which increases peak width.
So we have to compromise somewhere. This can be done by increasing theoretical plate(N)

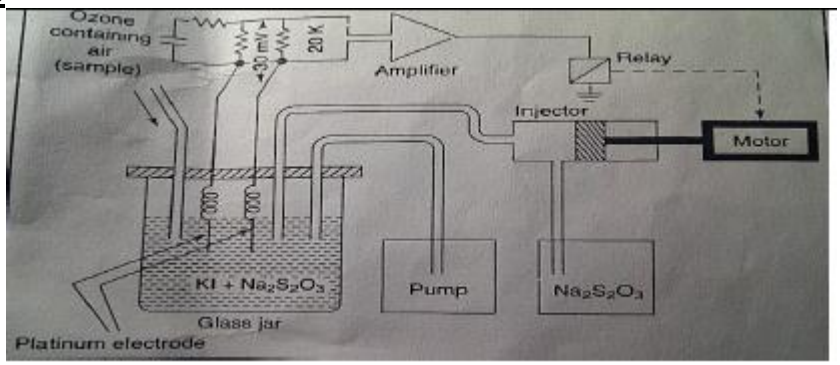


6.These plates do not really exist, these plates provide separate equilibrations of the sample between stationary and mobile phases.

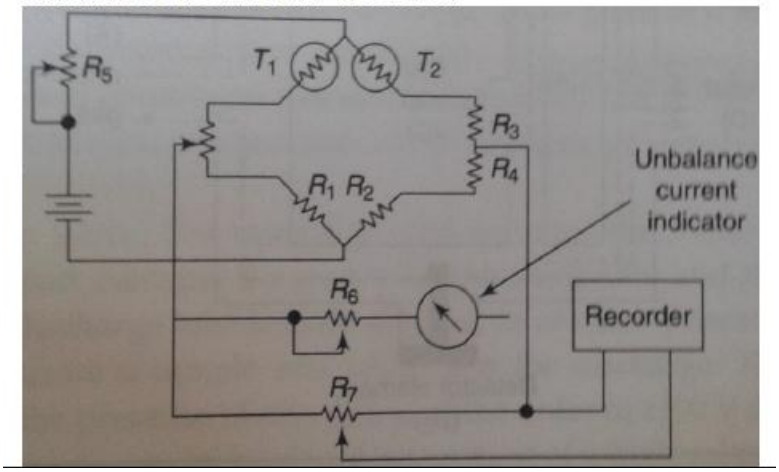
7.The drawback of increasing column can be done by another way by decreasing the Highest equivalent theoretical plate(HETP).The relationship between N and HETP is

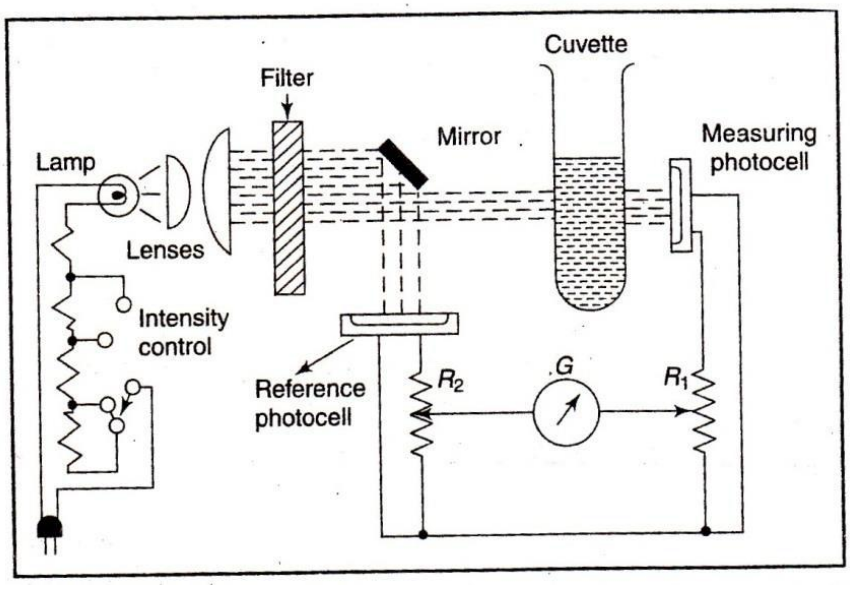
$$H=\frac{L}{N}$$

So to get proper separation, column length is more and drawback of increasing peak

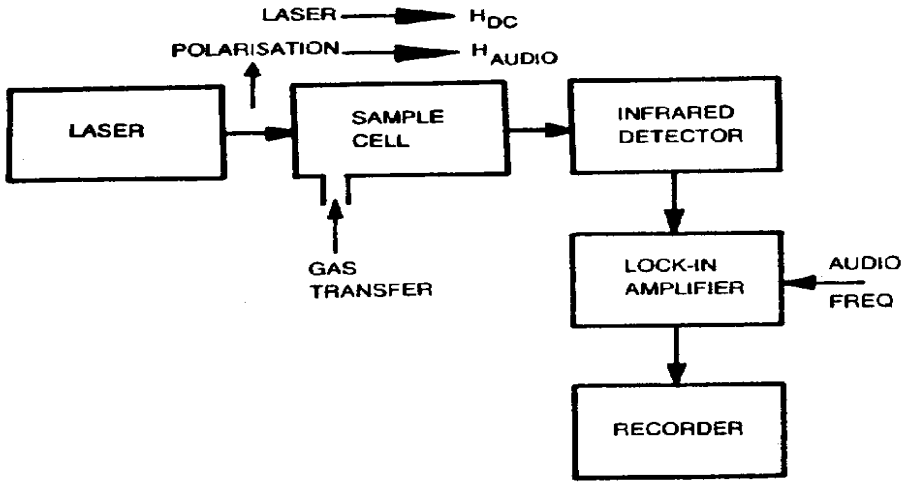
		broadening is done by increasing theoretical plates and decreasing HETP.	
	c)	**List the types and concentration of various gas pollutants.**	**4M**
	Ans:	The major gas pollutants are carbon monoxide, sulphur oxides, hydrocarbons, nitrogen oxides and particulates. **Carbon monoxide:** Its average concentration is below 200 ppm. **Hydrocarbon:** Its average concentration is below 80 ppm **Oxidants:** Its average concentration is below 500 ppb **Sulphur dioxide:** Its concentration in urban areas is 0.024 ppm. **Nitrogen oxides:** Its level ranges from 0.5 to 0.12 ppm.	**2M** **2M**
	d)	**Explain in brief the term Chemical shift with its mathematical expression.**	**4M**
	Ans:	**Chemical shift:** 1.This is the phenomenon that occurs in some atoms like Carbon or hydrogen in a given molecule which resonate at slightly different frequency based on its local chemical environment so this difference in resonance frequency is called as chemical shift. 2.The difference between the field necessary for resonance in the sample and in some arbitrarily chosen reference compound(tetramethyl-silane TMS)is called chemical shift. TMS is most common reference compound in NMR for TMS $\delta = 0$ (Chemical Shift) Chemical shift is expressed as $\delta = \frac{FREQUENCY\ OF\ SIGNAL - FREQUENCY\ OF\ REFERENCE}{SPECTROMETER\ FREQUENCY} * 10^6$ $= \frac{HS - HTMS}{H1} * 10^6$ H_S--Absorption radio frequency by the sample H_{TMS}-Absorption radio frequency by reference by ref compound H_1-Applied radio frequency	**2M** **2M(Mathematical expression)**
	e)	**Draw and explain ozone measurement using conductivity meter.**	**4M**
	Ans:	**Diagram:** 	**2M**

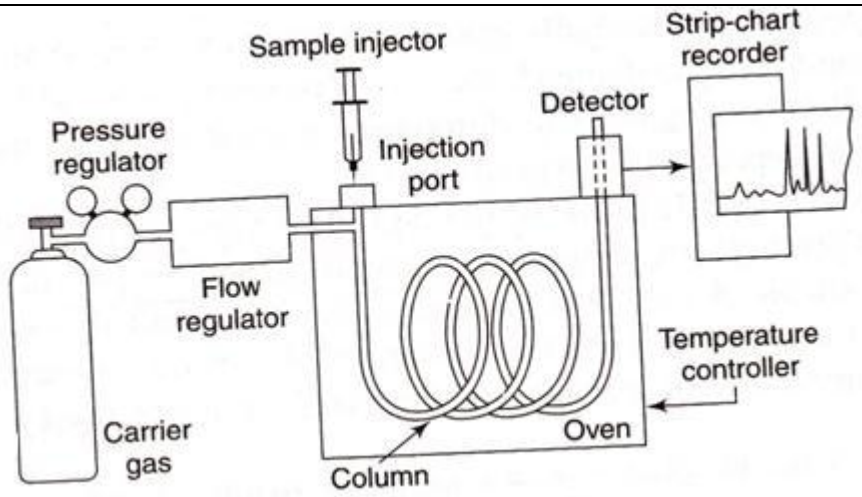
		Explaination: A wet chemical method which uses the oxidizing properties of O3 is employed to sensitive meter for continuous sampling of contaminating oxidants in atmosphere. The ozone containing air is bubbled into potassium iodide solution and resulting iodine determined by measuring current through the cell. The current is related to ambient O3 levels by previous calibration with known ozone source. Thus construct air-ozone meter which measures and records instantaneous ozone concentrations. The arrangement is as shown in figure. • It consist of an hermetically sealed glass jar containing 150 cm3 of buffered 10 % KI solution and 0.5 cm3 sodium thiosulphate of known concentration. • Two spiral platinum electrodes dip into the solution and bias voltage of 30mV is applied across them. The air above the solution is evacuated. When ozone enters the solution the following reaction takes place • $O_3+2I+H_2O \rightarrow I_2+O_2+2OH^-$ iodine then reacts with thiosulphate $I_2+2(S_2O_3)^- \rightarrow 2(I^-)+S_4O_6^{--}$ • Reaction continues so long as there is this sulphate in the solution. When all the this sulphate has been reacted, free iodine appears and reacts at the electrodes. • The electrical resistance is high as long as there is an excess of this sulphate. The resistance decreases when it is used up. This change is used to control the operation of instrument. • The voltage drop across 20kohm resistance which is in series with the electrode is used to operate the recorder as well as relay which controls a motorized injector which injects 0.5cm3 this sulphate in each operation. The recorder serves mainly to indicate as to when the injection was made and thus the average ozone concentration between any two injections can be calculated. Since the pumping rate is known, knowledge of the time intervals gives the total volumes of air sample	**2M**
	f)	**Draw the block diagram of thermal conductivity analyzer using thermistor and explain in brief its working**	**4M**
		Diagram:	**2M**

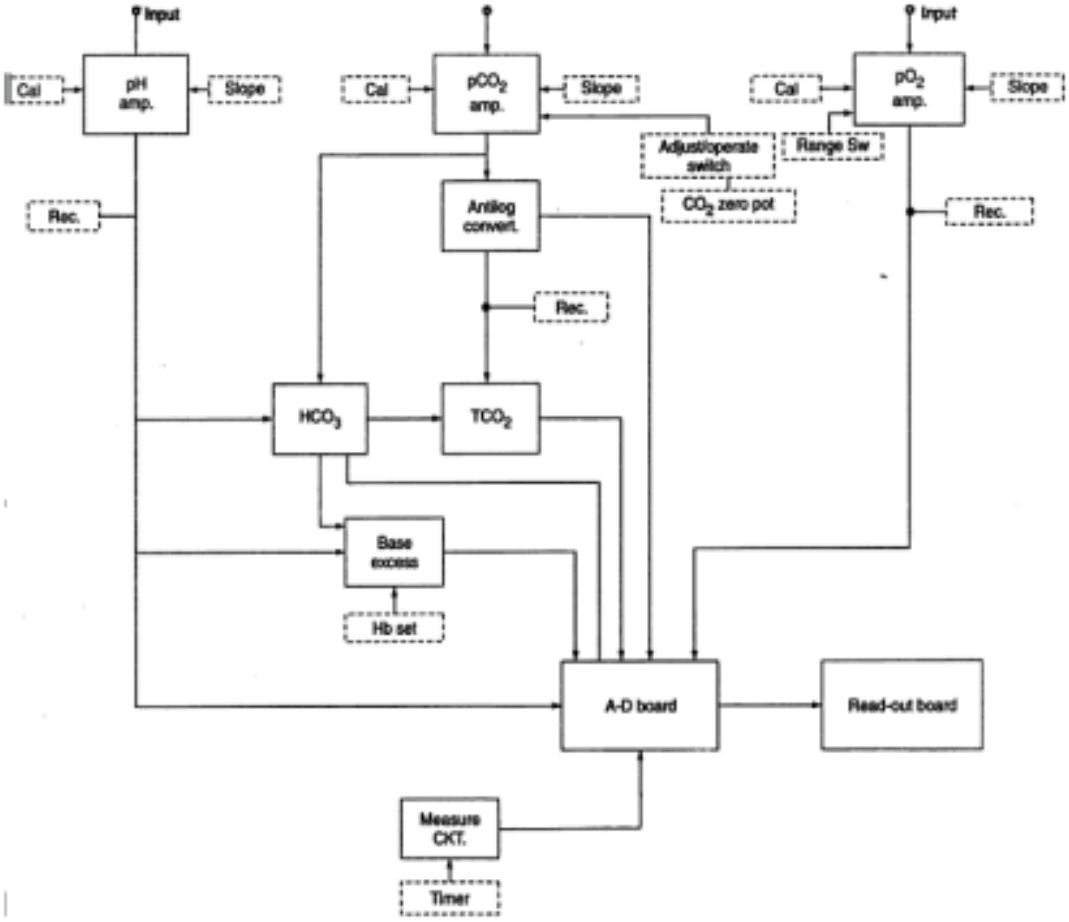
		 **Explanation:** • In thermal conductivity analyzer by using thermistor, two thermistors are employed as heat sensing elements and two platinum filaments are used in four arms of Wheatstone bridge. • They are arranged in a constant current bridge circuit and each of them is placed in a separate cavity in a brass or stainless steel block (thermistor is encapsulated in glass). • The materials generally use for the purpose include tungsten, platinum. • Two filaments connected in opposite arms of the Wheatstone bridge act as reference arms, whereas the other two thermistors are connected in the gas stream, which act as measuring arms. • Initially, reference gas is made to flow through all the cells and the bridge is balanced precisely with the help of potentiometer. • When the gas stream passes through the measuring pair of thermistor, there is a corresponding change in the resistance of the thermistor. • The higher the thermal conductivity of the gas, the lower would be the resistance of the wire and vice versa.	**2M**
Q. 3		**Attempt any FOUR:**	**16M**
	a)	**Draw the schematic of double beam filter photometer and explain its working in brief.**	**4M**
	Ans:	**Diagram-**	**2M**

		 **Working:** The light from the lamp source is made to pass through the filter F and then is divided into two parts. One part will pass through the sample solution in cuvette and then fall on the measuring photocell. The part of light directly falls on the reference photocell. Galvanometer G which is kept in between the photocells receives opposing currents through it. Potentiometer P1 is graduated in transmittance and absorbance limits. • With lamp off, the galvanometer zero is adjusted mechanically. The potentiometer R_1 is set to T=1or A=0 • Then with the lamp on the blank solution is placed in the light path of the measuring cell. • Potentiometer R_2 is adjusted until galvanometer G reads to zero. • The solution to be analyzed is then substituted for the blank and R1 is adjusted until the current through galvanometer is zero, with setting R_2 remaining unchanged. The absorbance or transmittance can then be read directly on the scale of potentiometerR_1.	**2M**
	b)	**State the applications of NMR spectroscopy.(any 4)**	**4M**
	Ans:	**Applications of NMR:** 1) NMR is used in magnetic resonance imaging in medical diagnosis 2) By studying peak of nuclear magnetic spectra, chemist can determine structure of chemical compound. 3) NMR is extremely used for analysis of sample non-destructively. 4) NMR is used for data acquisition in petroleum industry and natural gas exploration and recovery. 5) NMR is used in process control and process optimization in oil refineries and petrochemical plant. **OR** ***Note-Any relevant application.***	**1M for each application (any four)**

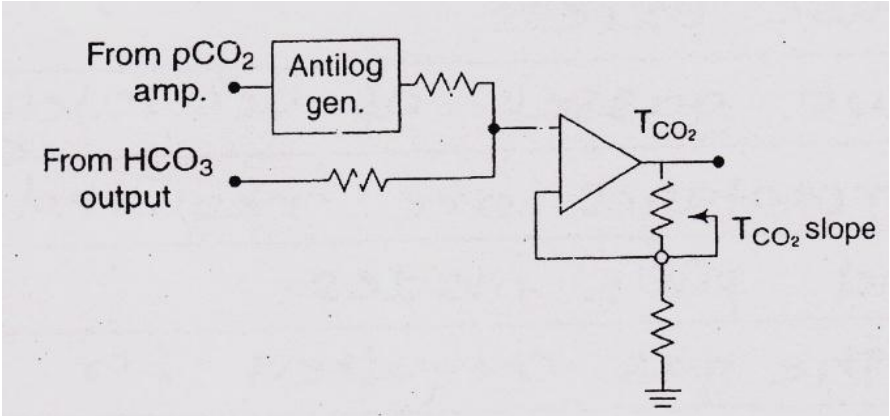
	c)	**What is pH? Explain in brief the principle of pH measurement.**	**4M**
	Ans:	**pH:** Hydrogen ion concentration, as distinct from total acidities in a chemical solution is represented by a symbol pH. It is defined by following equation pH= -log10CH where CH is the hydrogen ion concentration The lower case letter "p" in pH stand for negative common (base ten) logarithm, while the upper case letter "H" stands for the element Hydrogen. Thus pH is a logarithmic measurement of the number of moles of hydrogen ion (H+) per liter of solution. OR pH is the negative logarithm of the hydrogen ion concentration (more exactly the activity), algebraically $pH = -\log_{10}[H^+]$ or $pH = \log_{10} 1/[H^+]$. **Principle of pH measurement:** The measurement of the pH of a sample can be done by measuring the cell potential of that sample by measurement electrode in reference to a standard reference electrode. The measurement electrode is designed to allow hydrogen ions in the solution to migrate through a selective barrier, producing a measurable potential (voltage) difference proportional to the solution's pH. The circuit will be completed by another electrode called reference electrode. These two electrodes generate a voltage directly proportional to the pH of the solution. At a pH of 7 (neutral), the electrodes will produce 0 volts between them. At a low pH (acid) a voltage will be developed of one polarity, and at a high pH (alkaline) a voltage will be developed of the opposite polarity.	**2M** **2M**
	d)	**State the two applications of each:** **i)Gas Chromatography and ii)Liquid chromatography.**	**4M**
	Ans:	**Applications of Chromatography (Any two)** 1) In bio chemical analysis in medicine and other field. 2) In studying respiratory physiology in routine clinical investigation of patients breathing cycle. 3) Analysis of lighter hydrocarbon in petrochemical industries. 4) To analyse recyclable solvent in chemical industries. **Applications of liquid chromatography (Any two)** 1. Purity of final pharmaceutical drug 2. Analysis of biological fluids, 3. Therapeutic Drug Monitoring and Toxicology, 4. R&D in pharmaceutical industries, 5. Vitamins and Related Metabolites, 6. Steroid Hormones **OR** ***Note-Any relevant application.***	**1M for each application**
	e)	**Draw and explain the measurement of Nitrogen oxide using CO Laser.**	**4M**
	Ans:	**Diagram-**	**2M**

		 **Explanation-** **Nitrogen oxides measurement using CO laser:** 1. Figure shows the block diagram of detecting nitric oxide in 0.25 ppm concentration. 2. Apparatus consists of CO Laser, which emits radiation that is absorbed by the NO in the mixture. 3. The amount of absorption being proportional to the concentration of NO present. 4. The CO laser used is a dc excited continuous working laser, which operates on a single wavelength of 5.307 μ and at liquid nitrogen temperature. 5. A diffraction grating is used at one end of the cavity as a selector. 6. The absorption cell is made of pyrex and is of 15 mm diameter and 90cm length. 7. It is evacuated to a pressure of 10^{-6} to 10^{-5} torr. 8. The DC Magnetic field produces a field up to 2.5 KG by a solenoid. 9. The detector is a liquid nitrogen cooled Ge-Au element. 10. The signal is amplified and locks in an amplifier before given to recorder. 11. The signal amplitude varies linearly with the concentration of NO in the sample.	**2M**
Q. 4	**a)**	**Attempt any THREE:**	**12M**
	i)	**Draw and explain the schematic of gas chromatography(GC)**	**4M**
	Ans:	**Diagram-**	**2M**

		 **Working Principle-** Gas Chromatography is an analytical technique used for compound separation based primarily on their volatilities. It provides qualitative and quantitative information for individually present compounds. Compounds moves through column in gaseous phase and they are portioned between a stationary phases. The carrier gas (N2, Ar or He) is available in compressed form in a cylinder fitted with pressure regulator. The gas is conducted through flow regulator to sample injection port maintained at temperature T1. Gases and liquids samples are injected by syringe. The solute vapour mixes instantaneously with the flowing carrier gas and swept through chromatographic column. It is there the actual separation takes place. Column is maintained at temperature T2. At the end of the column, solutes emerging individually enter the detectors which produce an electrical signal corresponding to the quantity of solute leaving the column. The detector signal is supplied to recorder and plot of time – signal amplitude called chromatograph is obtained.	**2M**
	ii)	**Draw the labelled diagram complete -blood gas anlyzer**	**4M**
	Ans:	**Diagram-**	**4M**

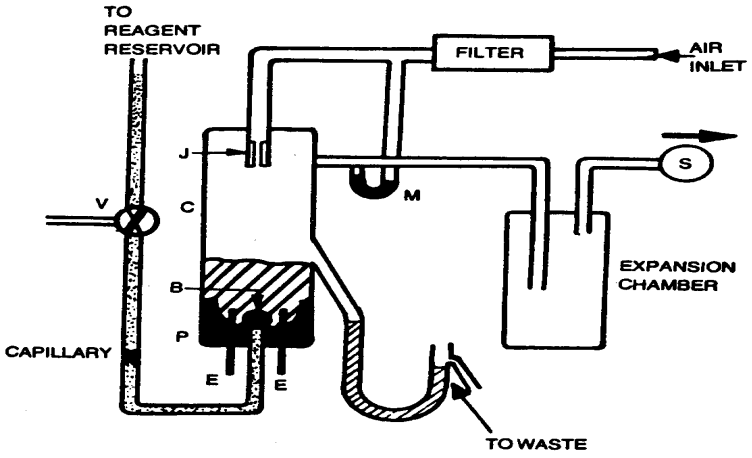


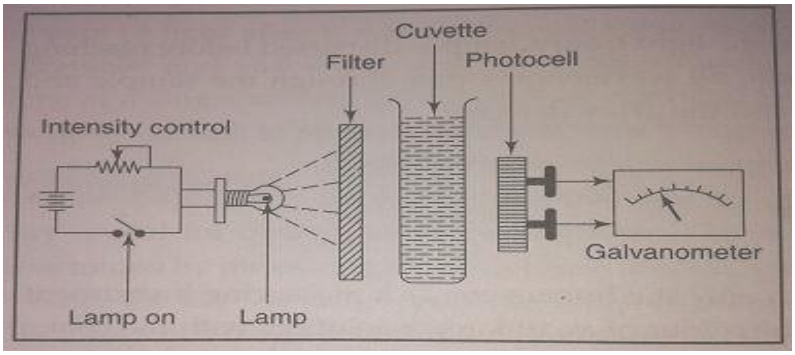
	iii)	**Draw and describe circuit diagram for calculation of total CO_2.**	**4M**
	Ans:	**Diagram-**	**2M**



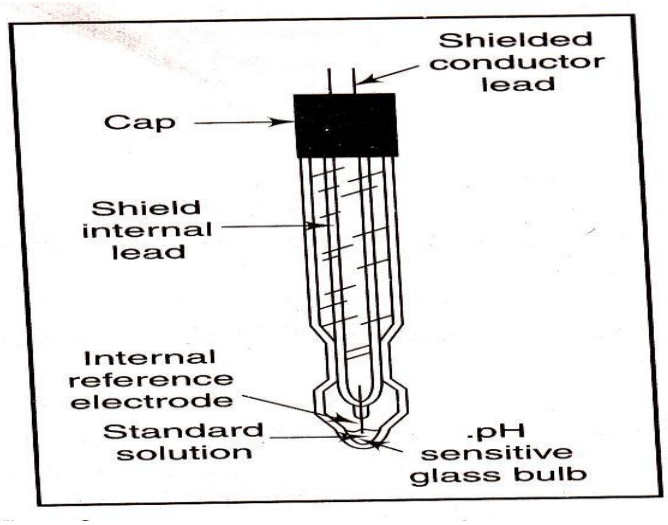
Explaination- **2M**

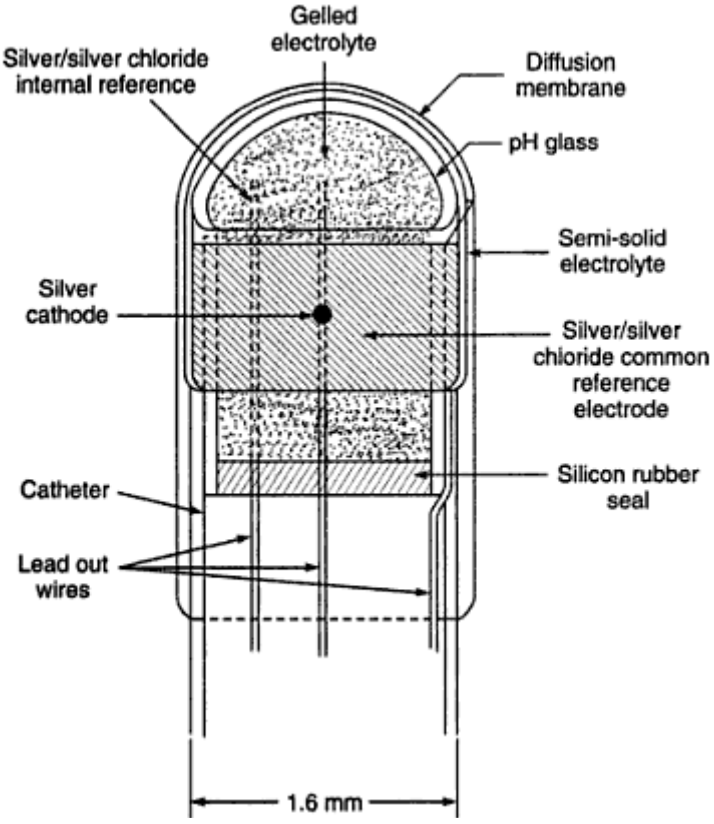
i) Blood gas analyzers are designed to measure pH, pCO_2, and pO_2 from single sample of blood.

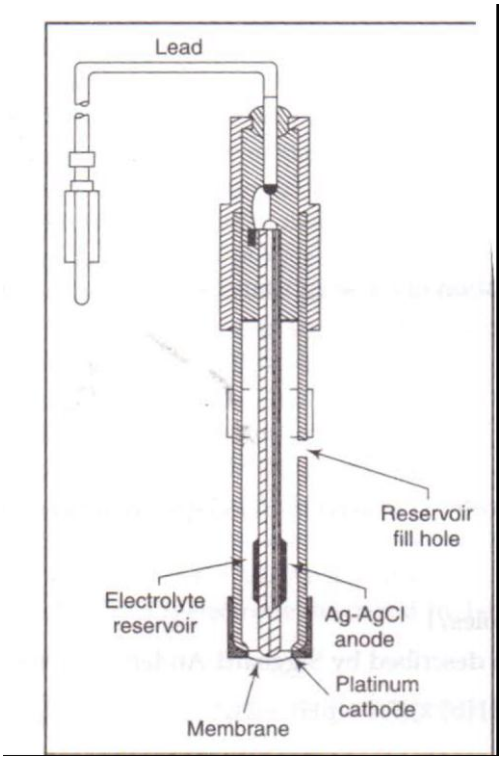
		ii)The input signal to HCO_3 calculator comes from the outputs of pH amplifier and pCO_2 amplifier. The outputs are suitably adjusted and given to the adder. The next stage is an antilog generator. iii)The total CO_2 (TCO_2) is calculated by summing the output signals of HCO_3 and antilog output of pCO_2 amplifier . iv)The output of this circuit goes to ADC for display.	
	iv)	**State and explain the principle of NMR.**	**4M**
	Ans:	**<u>Principle of NMR:</u>** **<u>Nuclear Spin:</u>** Elementary particles such as electrons or a nucleus behaves as if they rotate about an axis possesses the property of spin known as nuclear spin. The angular momentum is associated with the spin of particle would be an integral or half integral multiple of $h/2\pi$ where, h is planck's constant. **<u>Nuclear energy level</u> :** Since a nucleus possesses a charge, its spin gives rise to a magnetic field that is analogues to the field produced when an electric current is passed through a coil of wire. The resulting magnetic dipole or nuclear magnetic moment μ is oriented along the axis of spin and has a value that is characteristic for each kind of particle. When spinning nucleus is placed in a strong uniform magnetic field (H), the field exerts a torque upon the nuclear magnet. This would make the nucleus to assume a definite orientation with respect to the external field. The torque is a vector with its direction at right angles to the plane of μ and H. This results in a rotation of the nuclear axis around the direction of the external field. This is called processional motion.	**4M**
	b)	**Attempt any ONE:**	**6M**
	i)	**Draw and explain SO_2 measurement using conducting method.**	**6M**
	Ans:	When air sample containing SO_2 (sulphur dioxide) is passed through a solution consisting of sulphuric acid and hydrogen peroxide, its electrical conductivity changes due to formation of sulphuric acid by oxidation of SO_2. **<u>Diagram-</u>**  	**Diagram-2M**

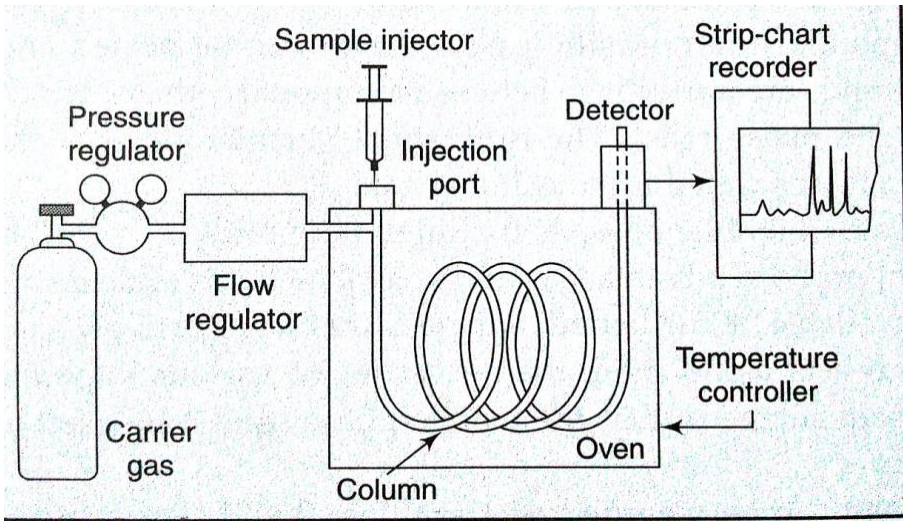
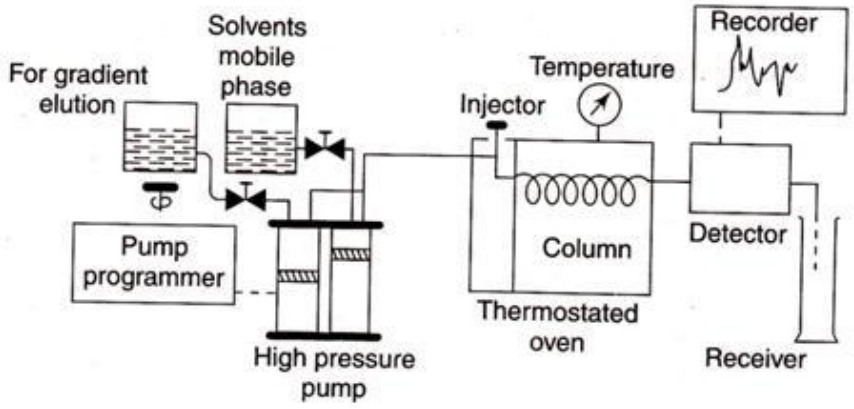
		$H_2O_2+SO_2 \rightarrow H_2SO_4 \rightarrow H^+ + (HSO_4)^-$ Conductivity cell is used for continuous measurement of SO2 in the air. It is made of glass, consists of a jet **J**, and orifice near the jet. It consists of 2 electrodes **E**, made of stainless steel wire. It is inserted through a cap **P**. The cap is sealed to the base of the cell. Reagent enters the cell from a central feed tube inserted in the cap. A small glass bead **B** in the cell acts as a non return valve on the entry of the central feed tube and prevents sulphuric acid diffusing from the cell.The end of the jet is made from a piece of capillary tube. A filter is placed before the jet to prevent blocking due to solid material. Since the cell is of small size, its capacity to absorb SO2 is limited. Therefore electrolyte is discharged and replaced at regular intervals.5 V AC is applied to the cell to measure the conductivity. AC avoids polarization. The resulting output current is recorded as saw-tooth waveform. Concentration of SO2 at any instant is proportional to slope of saw-tooth at that instant.	**1M for chemical reaction** **3M for explanation**
	b)	**State the principle of colorimeter. Draw the schematic and explain the working the single beam filter photometer.**	**6M**
	Ans:	**<u>Working Principle:</u>** Colorimetric methods are more precise and eliminate the necessity of preparing a series of standards every time a series of unknowns is run. Colorimetric determination involves visual measurement of colour and this method employ photoelectric measurement hence called as photometric or spectrophotometric method. As this method involves measurement of color in electromagnetic spectrum (400-700mμ) thus is referred as colorimetric method. In a colorimeter sample is normally a liquid. The sample compartment of colorimeter is provided with a holder to contain the cuvette in which liquid is examined. The holder is mounted on the slide with positions for at least two cuvettes so that sample and reference cuvettes are measured first and a shutter is moved in to or out of light beam until the micro ammeter gives full scale deflection. The sample is then moved into the beam and the light passing through it is measured as a percentage of reference value. Sample concentration = Standard concentration * (Sample reading /reference reading) **<u>Diagram-</u>**  	**2M** **2M**

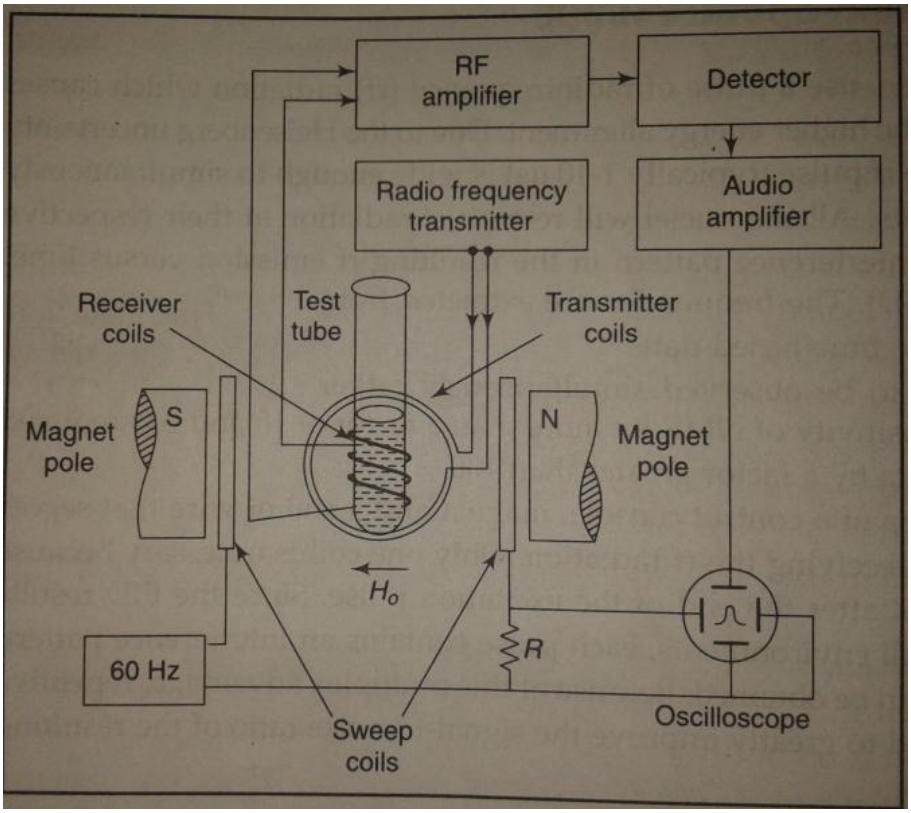
		Explaination- The figure shows basic component of filter photometer. 1. The source of light is tungsten filament lamp which is held in a reflector and which through light on the sample holder through a filter. 2. The filter may be either of absorption or interference type. 3. The sample holder is a cuvette with parallel walls or may be a test tube. 4. The light after passing through the sample holder falls on the surface of photocell. The output of cell is measured on micro ammeter. In order to operate instrument following steps are taken 1. With photocell darkened the meter is adjusted mechanically to read zero. 2. The blank or pure solvent or reference solution is inserted in the path of light beam and incident light intensity is regulated. 3. Solution of both standard and unknown are inserted in the place of blank and the reading of specimen relative to blank is recorded. The meter scale is calibrated in linear transmittance unit.	**2M**
Q.5		**Attempt any FOUR:**	**16M**
	a)	**Explain in brief the significance of prism and gratings in spectrophotometer.**	**4M**
	Ans:	**Significance of prism :** 1. The isolation of different wavelengths in prism monochromator is based upon the fact the refractive index of material is different for radiation of different wavelengths. 2. If parallel beam of radiation falls on prism the radiation of two different will bent through different angles. 3. In spectrophotometer, Light is made to fall on the prism. The monochromatic light is obtained by allowing the light beam to pass through a prism monochromator. 4. Prism is an optical component to disperse the light or modify the direction of light. 5. The wavelength selection is done by rotating the prism about a pivot. 6. The prism has an aluminized rear surface. Due to the prism, shorter wavelength is dispersed more. Light, after getting dispersed by prism, passes through the sample and then detected by the photocell. **Significance of Grating:** 1. In spectrophotometer diffraction grating is used as dispersion media. 2. It consists of a series of parallel grooves ruled on a highly polished reflecting surface. 3. When the grating is put into a parallel radiation beam so that one surface of grating is illuminated, this surface acts as a very narrow mirror. 4. The reflected radiation from grooved mirror overlaps the radiation from neighboring grooves. 5. White light shining onto a diffraction grating reflects back in rainbow colors. 6. White light reflecting in rainbow colors from the surface is a result of the same dispersion phenomenon of the diffraction grating. 7. In the same way as a prism, the diffraction grating can be rotated to change the color of the light extracted through the slit of spectrophotometer.	**2M** **2M**

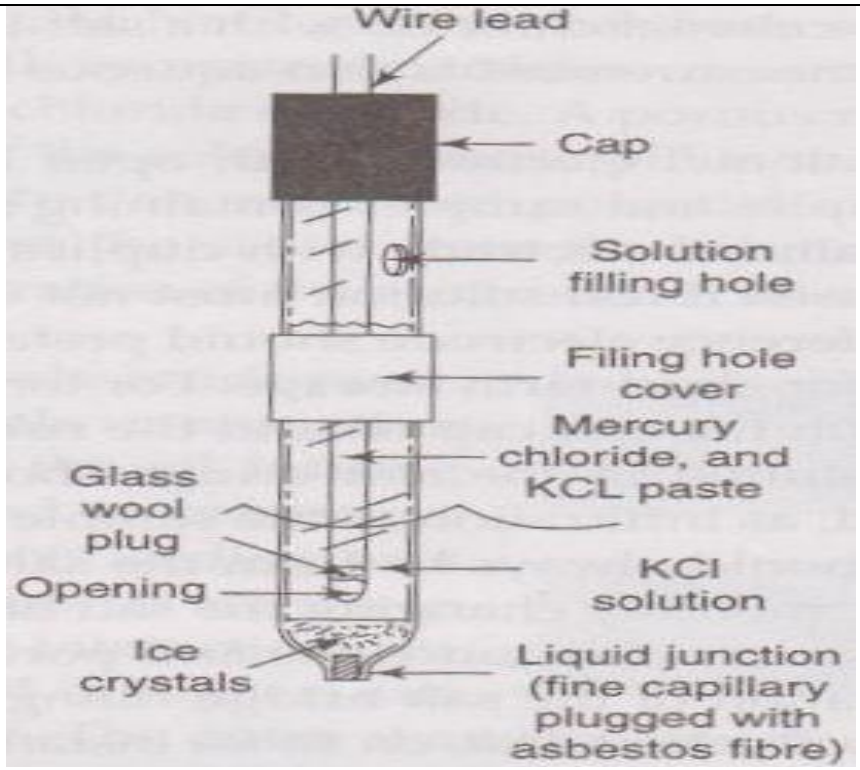
	b)	**Draw and explain the constructional details of glass electrode used for pH measurement.**	**4M**
	Ans:	**Diagram-**  **Constructional Feature:** 1. Glass electrode consists of thin walled bulb of PH sensitive glass sealed to a stem of non ph sensitive high resistance glass. 2. The PH response is limited to the area of special glass membrane, thus making response independent of depth of immersion. 3. The membrane has thickness of order of 0.05-0.15mm and bulb is of order of 10mm in diameter. 4. The glass pH electrodes are constructed of special glass to create the ion selective barrier needed to screen out hydrogen ions from all the other ions floating around in the solution. 5. This glass is chemically doped with lithium ions, which is what makes it react electrochemically to hydrogen ions. 6. Since glass is an insulator, this presents a major problem if it is intended to measure voltage between two electrodes. 7. The circuit path from one electrode contact, through the glass barrier, through the solution, to another electrode and back through the other electrode's contact is therefore one of the extremely high resistance. 8. On the inside of membrane is a system of effectively constant pH .It is composed of a silver silver chloride or calomel electrode dipped in hydrochloric acid. 9. Changes in electrical potential on the outer membrane surface are measured by means of an external reference electrode such as calomel and its associated salt bridge.	**2M** **2M**
	c)	**Define Chemiluminescence .How measurement of Nitrogen oxide is done using Chemiluminescence**	**4M**
	Ans:	**Definition:** The phenomenon of emission of radiation from chemi-excited species is known as Chemiluminescence. It results due to the formation of new chemical bonds. The species in the excited state possess higher energy levels than the ground state and	**1M**

		usually have a very short life. **<u>Nitrogen oxide measurement using Chemiluminescence:</u>** 1. Chemiluminescence phenomenon is very useful for measurement of air pollutants, particularly NO and NO2. 2. Instruments based on the measurement of chemiluminescent emission, based on the following reaction have been developed: $NO + O3 \rightarrow NO_2 + O_2$ $NO_2 \rightarrow NO_2 + h\upsilon$ (λ max =6300 Å) 3. Since NO_2 reacts only slowly with ozone and the reaction which produces NO_3 is not accompanied by Chemiluminescence, it is necessary to reduce to NO_2 to NO before admission into the reactor $NO_2 \rightarrow NO + ½ O_2$ 4. Nitric oxide and ozone containing gas steam are mixed in a vessel at a sub atmospheric pressure of about 2 mm of Hg. 5. Light emission is measured with a photomultiplier. 6. With the use of high gain, low dark current photomultiplier tubes, extremely low levels of radiation can be measured. 7. The response of the instruments based on Chemiluminescence is linear from 1 ppb to 1000ppm of NO. 8. This technique is extremely useful for measurement of NO in automotive exhaust gases.	**3M**
	d)	**Draw and explain the catheter tip electrode for measurement of pO_2**	**4M**
	Ans:	**<u>Catheter Tip Electrode:</u>**  	**2M**

		OR  1. The partial pressure of oxygen in blood or plasma indicates the extent of oxygen between lungs and blood. 2. Catheter tip electrode is small enough to be mounted on Catheter tip and preferred for measurement of more than one parameter. 3. Catheter tip electrode is used for continuous measurement PO_2 and PCO_2. 4. A180Um diameter silver cathode constitutes a PO_2 measuring electrode. 5. The common electrode used is silver/silver chloride electrode. A semisolid electrolyte is common for both PO_2 and Pco2. 6. The electrodes are dip coated with thin polystyrene diffusion membrane. 7. When device is placed in blood, water vapour diffuses through the membrane and together with NHCO3 and NACL crystal deposited in hydrogel film constitutes the electrolyte normally used with PCO2 electrode. 8. Under these conditions output signal from PO_2 and PCO_2 is obtained.	**2M**
	e)	**Explain in brief CO2 measurement using gas chromatography.**	**4M**
	Ans:	1. When an air sample containing CO is passed through a stripper column, the heavy hydrocarbons are retained and CO and methane are passed into chromatographic column and then into a catalytic reducing chamber. 2. The methane would pass through the reducing chamber unaffected while CO is reduced to methane. 3. By using hydrogen flame ionization detector both methane peaks can be detected. 4. The first peak is due to methane while the second peak would correspond to CO.	**2M**

		5. The accuracy is about +-2%.peak heights of CO and CH_4 would gives sensitivity of 50 ppb. **Diagram-**  **OR** ***Note-Any other relevant diagram shall be considered***	**2M**
	f)	**State the principle of Liquid chromatography.**	**4M**
	Ans:	**Block Diagram:**  **Principle of Operation:** Liquid Chromatography (LC) is an analytical chromatographic technique which is useful for separation of ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase, the different solute will interact with other phase to differing degree due to differences in adsorption, ion exchange, partitioning or size. These differences allow the mixture components to be separated from each other by using these differences to determine the transit time of solutes through a column.	**2M** **2M**

		Simple LC consists of a column with a fritted bottom that holds a stationary phase in equilibrium with a solvent. Typical stationary phase is solid, ionic groups on a resin, liquids on an inert solid support, and porous inert particles. The mixture to be separated is loaded on top of column followed by more solvent, the different component in the sample mixture pass through the column at different rate due to differences in their portioning behavior between the mobile liquid phase and the stationary phase. The compounds are separated by collecting aliquots of column effluent as a function of time.	
Q.6		**Attempt any FOUR:**	**16M**
	a)	**Draw the block diagram of NMR spectrometer.**	**4M**
	Ans:	**<u>NMR Spectrometer:</u>**  	**4M for Suitable diagram**
	b)	**Draw and explain the calomel electrode's construction details.**	**4M**
	Ans:	**<u>Diagram of Calomel Electrode:</u>**	**2M**

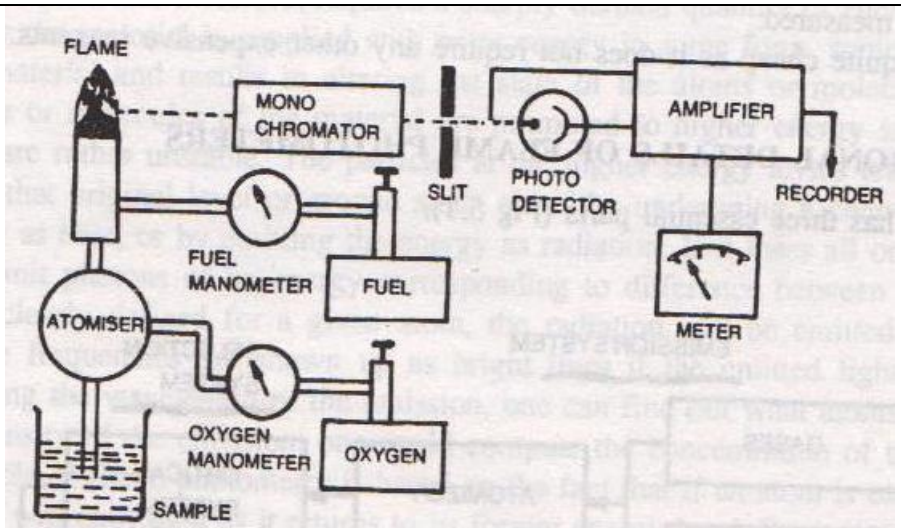


Constructional details of Calomel Electrode: **2M**

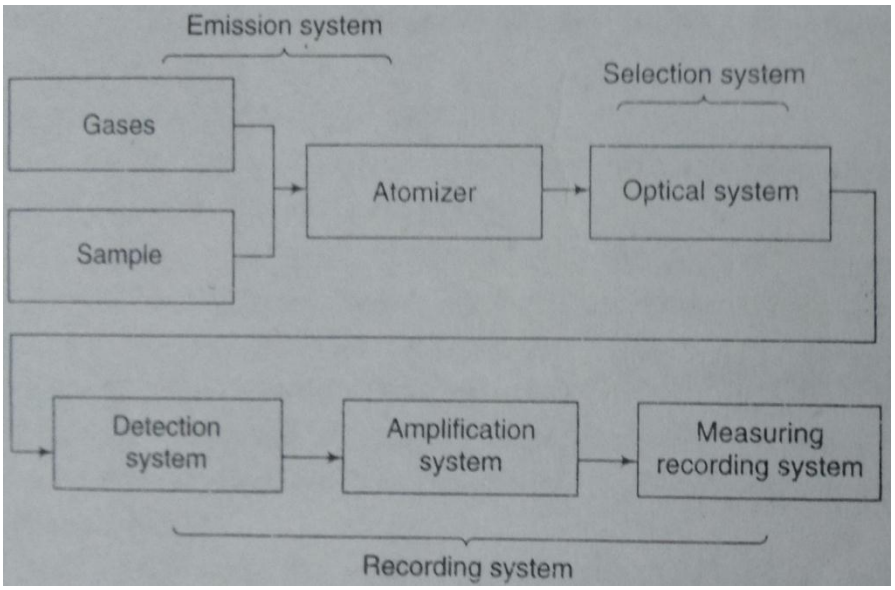
As shown in the figure

1. Calomel electrode consist of, a metallic internal element, typically of mercury-mercurous chloride (calomel), immersed in an electrolyte, which is usually a saturated solution of potassium chloride.
2. The electrolyte solution forms a conductive salt bridge between the metallic element and the sample solution, in which the measuring and reference electrodes are placed.
3. For stable electrical conductivity between the internal metallic element and sample solution, a small but constant flow of electrolyte solution is maintained through a liquid junction in the tip of outer body of reference electrode.
4. ½ Hg_2Cl_2 + e < -- > □Hg + Cl^-
5. Since at given temperature, the activity of mercurous chloride is constant and that of the mercury is unity by definition, it is chloride ion activity which is potential determining. The most common source of chloride ion is potassium chloride saturated at 3.8, 3.5 or 0.1 M concentrations.

c)	**State and explain the principle of flame photometer with neat diagram.**	**4M**
Ans:	**Statement:** It is based on the fact that if an atom is excited in a flame to high energy level, it will emit light as it returns to its former energy level. By measuring the amount of light emitted we can measure the no of atoms excited by the flame.	**1M**
	Diagram-	**2M**

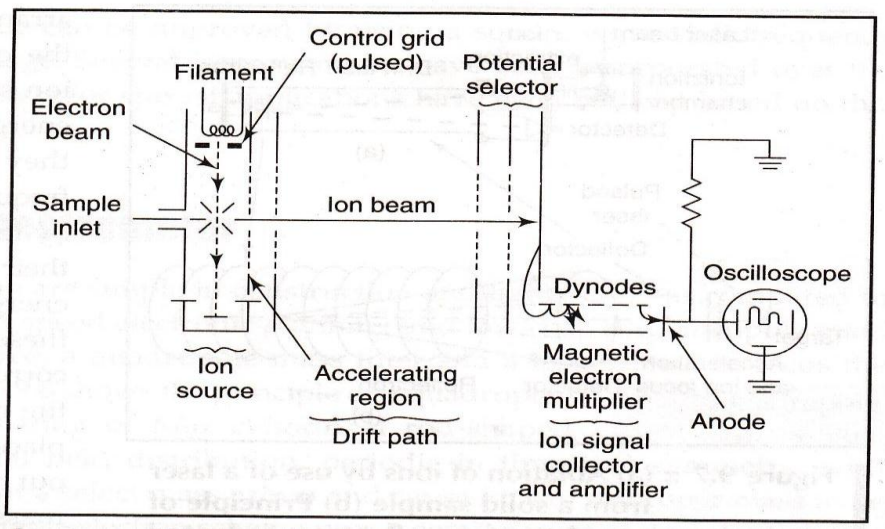


OR



Principle -A solution of sample is prepared. A special sprayer operated by compressed air or oxygen is used to introduce this solution in the form of fine spray (aerosol) into flame of burner operating on some fuel gases like acetylene or hydrogen. The heat of flame vaporize sample constitutes, molecules and ions of the sample species are decomposed and reduced to give atom. Also the heat of flame causes excitation of some atom into higher electronic states. Excited atoms revert to ground state by emission of high energy of characteristic wavelength which can be detected for individual composition analysis. **1M**

d)	**Draw and explain the operation of time of flight mass spectrometer.**	**4M**
Ans:	**Diagram of time of flight mass spectrometer -**	**2M**



Operation:

1. In the time-of flight spectrometer, ions of different mass/ charge ratio are separated by the difference in time they take time to travel over an identical path from the ion source to the collector. **2M**
2. This requires the starting time to be well defined. Therefore, ions are either formed by pulsed ionization method or various kinds of rapid electric field switching are used as gates to release the ions from the ion source in a very short time.
3. In pulse mass spectrometer, ion packets of a few microseconds duration are emitted at intervals of a few milliseconds from a voltage source.
4. The ions transverse an evacuated tube called the drift tube to reach the detector. The detector is sensitized for a brief instant to register their arrival.
5. Since ions of different masses arrive at the detector at different times, the accurate measurement of the time between activating the source and sensitising the detector gives information concerning the mass of the ions.
6. The signal from the ions reaching the detector is amplified and applied to the vertical deflection plates of an oscilloscope.
7. The horizontal axis deflection of the oscilloscope commences as the ion packet start out. This produces a mass spectrum on the screen of the oscilloscope.

e) List the basic elements of Liquid chromatography and explain their functions. 4M

Ans:

Basic Elements liquid chromatography: **List of any 4 element 2M**

It basically consist of :

1. A high pressure pump system
2. Gradient elution or solvent programmer
3. The sample injection system
4. The column
5. The detection system including display or recording devices
6. Computer for data processing and storage.

Functions of Elements:

1. **A high pressure pump system:** A high pressure pump system is used to **2M**

		force the liquid mobile phase through the column. Narrow bore columns packed with fine mesh particles needs high inlet pressure to yield required flow rate. For these various pump system incorporating piston pumps, peristaltic pumps, diaphragm pumps and syringe pumps are used. 2. **Gradient elution or solvent programmer:** In LC a single substance may be used as mobile phase during an analysis of the mixture of two or more substances to properly adjust the characteristic of the phase. Also one may maintain a constant mobile composition during analysis or change it. Gradient elution is required to resolve complex mixtures, those containing components with significantly different chromatographic behavior. A solvent programmer helps to control the composition of the mobile phase according to a predetermined program as the analysis proceeds 3. **The sample injection system:** The sample is introduced into the column with the help of a sample injection system. There could be several methods for introduction of sample on the top of a liquid chromatographic column. One method is to disconnect the solvent supply, to add sample in solution and reconnect the solvent supply to column. This mechanism is simple but method is tedious to operate. Newly added syring injection and injection valve method enable sample to be introduce directly into column packing, without interrupting the solvent flow. 4. **Column:** The ultimate performance of the chromatograph is determined by column. Various components of the sample are fractionated during their passage through the column. The separation largely depends on column diameter and length. The reduction in sample size and column diameter result in improved separation efficiency. 5. **The detection system including display or recording devices:** The detector is the component that emits a response due to eluting sample compound and subsequently produce peak on chromatogram. High sensitive detection system gives optimum column separating performance by use of small sample. Several detection system depend on measurement of physical property of column elute. This physical property could be change in UV absorption, IR absorption, heat of absorption, refractive index or electrical conductivity. The detection system senses these components as they elute from the column and produces a signal proportional to the amount of solutes passing through the detection system. The detector determines what separation has taken place and provides data permitting qualitative and quantitative evaluation of results. 6. **Computer for data processing and storage:** LC is finding online application for monitoring chemical processes and providing information on desired adjustments. Thus LC operates automatically. Computer can control and adjust analysis time, inject the sample, select and measure the peak, and present the result for display & storage.	