WINTER – 19EXAMINATIONS

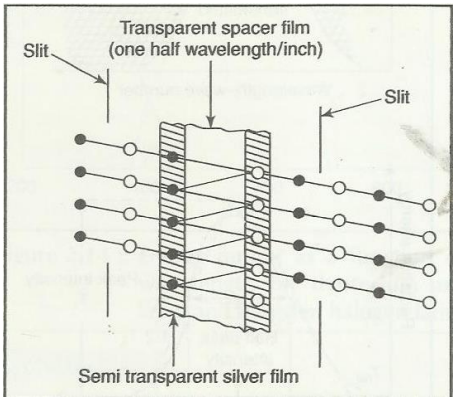
Subject Name: Analytical Instrumentation Model Answer Subject Code: 22543

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 tryto assess the understanding level of the candidate.
3) The language errors such as grammatical, spelling errors should not be given moreImportance (Not applicable for subject English and Communication Skills.
4) While assessing figures, examiner may give credit for principal components indicated in thefigure. The figures drawn by candidate and model answer may vary. The examiner may give credit for anyequivalent figure drawn.
5) Credits may be given step wise for numerical problems. In some cases, the assumed constantvalues 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		**Attempt any fiveof the following:**	**10-Total Marks**
	a)	**State the role of radiation source in analytical Instrumentation.**	**2M**
	Ans:	**Role of radiation source:** **i. It provides sufficient intensity of light for making measurement.** **ii. It converts electrical energy into radiation**	**1M Each**
	b)	**List any two properties of analytes used in analytical Instrumentation.**	**2M**
	Ans:	**Two properties of analytes: (Any two)** **i.** Conductivity **ii.** Electrode potential **iii.** Light absorption, or emission **iv.** Mass to charge ratio **v.** Fluorescence,	**2M**
	c)	**State Beer Lambert's law.**	**2M**
	Ans:	**Statement:** 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. Absorbance A = εcb Where, A = absorbance ε = molar absorptivity c = molar concentration	**2M**

		b = path length Mathematically, absorbance is related to percentage transmittance T by the expression: A=log10(*I*0*I*) =log10(100*T*) = εbc	
	d)	**Name any two applications of flame photometer.**	**2M**
	Ans:	**Applications of flame photometer:** **i. To determine the availibility of alkali and alkaline earth metals which are critical for soil cultivation.** **ii. In ariculture** **iii. In clinical field** **iv. Analysis of soft drinks,fruit juices and alcoholic beverages.** **Note:Any other relevent applications are to be considered**	**2M-1 Each**
	e)	**State the effect of column length in chromatography.**	**2M**
	Ans:	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. 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%. Longer Column length →Retention time is more→Good separation→Chromatogram will be separated.	**2M**
	f)	**State the principle of chromatography.**	**2M**
	Ans:	**Principle of Chromatography:** i. Chromatography is a physical method of seperation in which the components to be seperated are distributed between two phases,mobile phase and stationary phese. ii. When the analytes are introduced into column,the molecules distribute between two phases.	**2M**
	g)	**State the need of pollutant monitoring.**	**2M**
	Ans:	**Pollutant Monitoring** helps in assessing the level of pollution in relation to the ambient air quality standards. Standards are a regulatory measure to set the target for pollution reduction and achieve clean air. Pollutant Monitoring keeps us informed, helps us to maintain our health, and alerts us to future problems that may arise.	**2M**

Q.2		Attempt any THREE of the following:	12- Total Marks
	a)	**List the different filters used in analytical Instruments. Explain any one filter in detail.**	4M
	Ans:	Filters can be broadly classified as i. Absorption filters ii. Interference filter	1M
		Absorption filter: The absorption type optical filter usually consists of color media including colour glasses,coloured films such as gelatin and solutions of the coloured substances.This type of filter has a wide spectral bandwidth,which may be 40 to 50μ in width at one-half the maximum transmittanse.Their efficiency of transmission is very poor and is of the order of 5 to 25 percent. It is possible to obtain more selective light filters from coloured media by increasing their thickness by two,three or more times.Here the transmission of the filter for the light of the wavelength isolated is decresed,but there is a simultaneous increase in the selectivity,but fall in transmission efficiency would have to be componsated by suitable amplification of the photocurrent. As absorption type filters do not provide a high degree of monochromaticity required for isolating complex systems,their use is restricted to only the very simple type of photometers.	3M
		OR	
		Interference filter:  Fig: Path of light rays through an interference filter Interference filters usually consist of two semi-transparent layers of silver,deposited on glass by evaporation in vaccum and separated by a layerof dielectric(ZnS or MGF_2) In this arrangement,the semi-transparent layers are held very close together.The spacer layer is made of a substance which is of a low refractive index.The thickness of the dielectric layer determines the wavelength transmitted.Figure shows the path of light rays through an interference filter.Some part of light that is transmitted by the first film is reflected by the second film and is again reflected on the inner face of the first film, as the thickness of the intermediate layer is one-half the wavelength of a desired peak wavelength.Only light which is reflected twice will be in-phase and will come out of the filter,while other wavelengths with phase differences would cause distructive interference.Constructive interference	

between different pairs in superposed light rays occurs only when the path differene is exactly one wavelength or some miltiple thereof.The relationship expressing a maximum for the transmission of a spectral band is given by

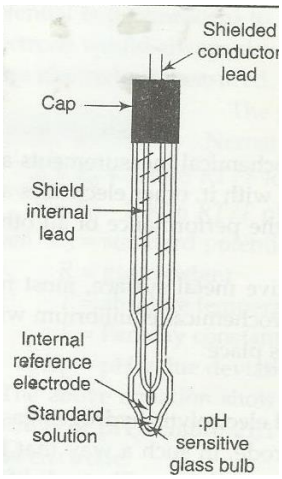
$$m\lambda = 2\ d(n)\ \sin\ \theta,$$

When light is incident normally, $\sin\ \theta = 1$

$$\mathrm{m}\lambda = 2d(n),$$

Where d is the thickness of the dielectric spacer, whose refractive index is n.
Interference filters allow a much narrower band of wavelengths to pass and are similar to monochromators in selectivity.

b)	**Draw a neat labelled diagram of glass eletrode and explain its working.**	**4M**
Ans:	**Diagram:**	**2M**



Explanation:
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. This glass is chemically doped with lithium ions,which rmakes it to react electrochemically to hydrogen ions.
On the inside of the membrane is a system of effectively constant pH.It is composed of a silver-silver chloride or calomel electrode dipped in hydrochloric acid.Changes in electrical potential of the outer membrane surface are measured by means of the rxternal reference electrode and its associated salt bridge.The complete pH cell is represented as follows.

Internal Reference Electrode	Internal Electrolyte	Glass Membrane	Test Solution	External Reference Electrode

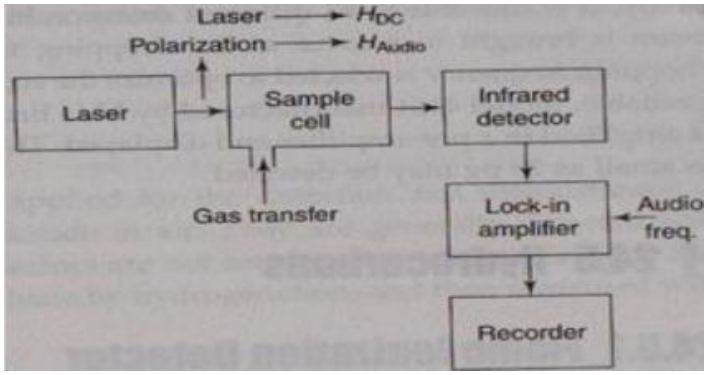
The ideal pH response of glass electrode behaving exactly in the same manneras a hydrogen electrode is given by

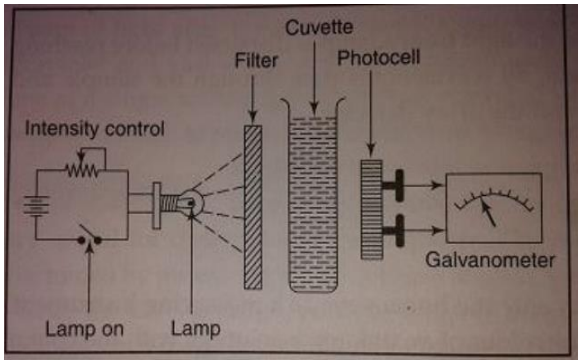
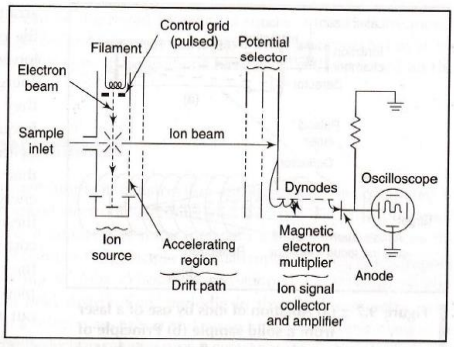
$$E_2 - E_1 = 2.3026\ RT/F(\mathrm{pH}_2 - \mathrm{pH}_1)$$

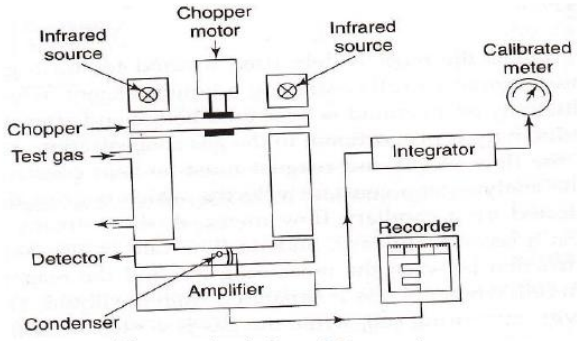
Where E2,E1are the values of electromotive force of cell 1 of test solutions of pH equal pH1 and pH2 respectively

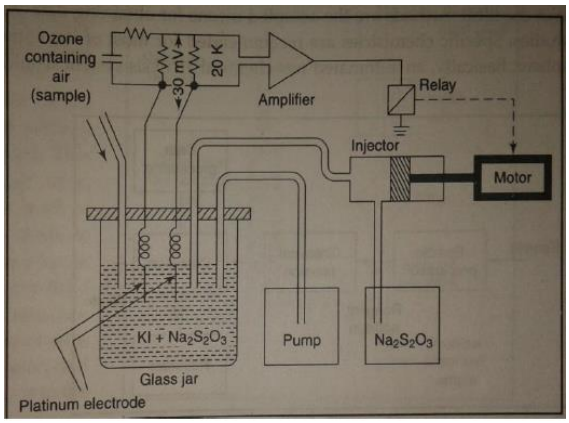
2M

c)	**List any four applications of GCMS.**	**4M**

	Ans:	Applications of GCMS (Any four) **Note: Any other relevant applications to be considered** i. Petrochemical and hydrocarbon analysis ii. In bio chemical analysis in medicine and other field. iii. Studying respiratory physiology in routine clinical investigation of patients breathing cycle. iv. Analysis of lighter hydrocarbon. v. Geochemical research vi. Sports anti-doping analysis vii. Pharmaceutical and drug analysis viii. Environmental and forensic applications ix. Analysis of pesticides in foodstuffs x. Analysis of biologically important aromatic amines xi. Identification of volatile components xii. For the determination of parathyroid residues in vegetable samples	**1Meach**
	d)	**Explain nitrogen oxide measurement using CO laser.**	**4M**
	Ans:	**Diagram:**  Nitrogenoxides measurement using CO laser: i. Figure shows the block diagram of detecting nitric oxide in 0.25 ppm concentration. ii. Apparatus consists of CO Laser, which emits radiation that is absorbed by the NO in the mixture. iii. The amount of absorption being proportional to the concentration of NO present. iv. 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. v. A diffraction grating is used at one end of the cavity as a selector. vi. The absorption cell is made of pyrene and is of 15 mm diameter and 90cm length. vii. It is evacuated to a press of 10- 6to 10-5. viii. The DC Magnetic field produces a field up to 2.5 KG by a solenoid. ix. The detector is a liquid nitrogen cooled Ge-Au element. x. The signal is amplified and locks in an amplifier before given to recorder. xi. The signal amplitude varies linearly with the concentration of NO in the sample.	**2M** **2M**

Q.3		Attempt any FHREE of the following:	12- Total Marks
	a)	**Draw a neat labeled diagram of single beam filter photometer and explain its working.**	4M
	Ans:	**Diagram:** 	2M
		Single beam filter photometer: 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 (i) With photocell darkened the meter is adjusted mechanically to read zero. (ii) The blank or pure solvent or reference solution is inserted in the path of light beam and incident light intensity is regulated. 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
	b)	**Explain the working of time of flight type mass spectrometer.**	4M
	Ans:	**Diagram:** 	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. 2. This requires the starting time to be well defined. Therefore, ions are either formed by	2M

		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 sensitizing 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.	
	c)	**Draw a neat labelled bock diagram of infrared gas analyser.**	**4M**
	Ans:	**Diagram:**  **Diagram for infrared Gas analyzer** **Function of Block of Infrared analyzer:** **1.Infrared Source** The source of infrared radiation is hot wire spiral. It absorbs specific wavelengths of infrared radiation. One of the most commonly measured gases using infrared radiation absorption method is the carbon dioxide. **2.Measuring &Reference Cell** 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. One beam passes through the sample cell, other beam through a reference cell. And both beam through a reference cell towards detection chamber. **3.Chopper motor** The radiation emitted by infrared source is pulsed by motor driven chopper. The rotating chopping disc occludes each beam twice per rotation. The chopping frequency is 2-10 Hz for industrial and 2-50Hz for medical application. **4.Detector** Detection chamber is permanently sealed unit divided into two compartments by thin metallic diaphragm. Both compartments are charged to same pressure with the gas being measured and both beams from both cells enter opposite ends of the detection chamber **5.Amplifier** As an arrangement capacitor is supplied with constant charge and resulting voltage changes at chopping frequency are amplified in three stage tuned amplifier with high input	**2M** **2M**

		impedance in input stage. **6.Intergrator &Recorder** The signal from amplifier is rectified, integrated and smoothed and output signal is displayed on meter or recorder.	
	d)	**Explain in brief ozone measurement using conductivity meter.**	**4M**
	Ans:	**Diagram:**  **Explanation:** 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 O3levels 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, whereas the outside is let in through a Tygon tube which is inert to ozone. When ozone enters the solution the following reaction takes place $O_3+2I+H_2O \rightarrow I_2+O2+2OH^-$ The iodine then reacts with thiosulphate $I_2+2(S_2O3)^- \rightarrow 2(I^-)+S_4O_6^-$ Reaction continues so long as there is thiosulphate in the solution. When all the thiosulphate has been reacted, free iodine appears and reacts at the electrodes. The electrical resistance is high as long as there is an excess of thiosulphate. 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 0.5cm3thiosulphate 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** **2M**
Q.4	**(A)**	**Attempt any THREE of the following :**	**12- Total Marks**

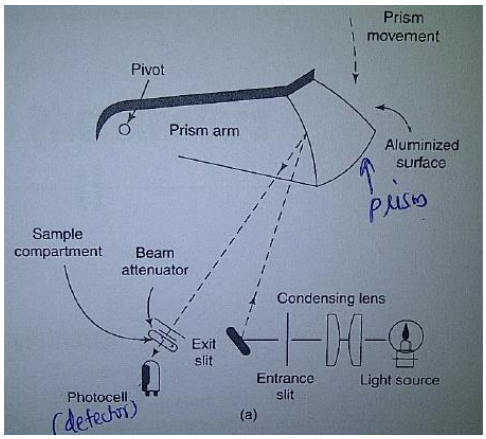
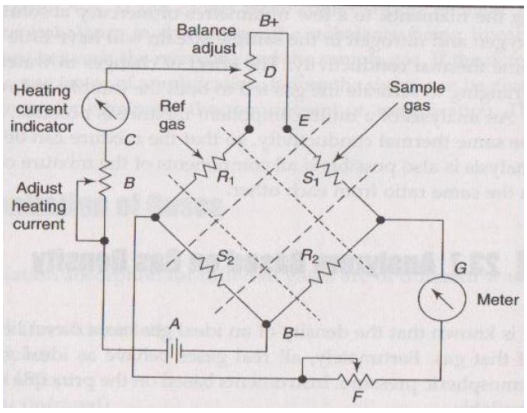
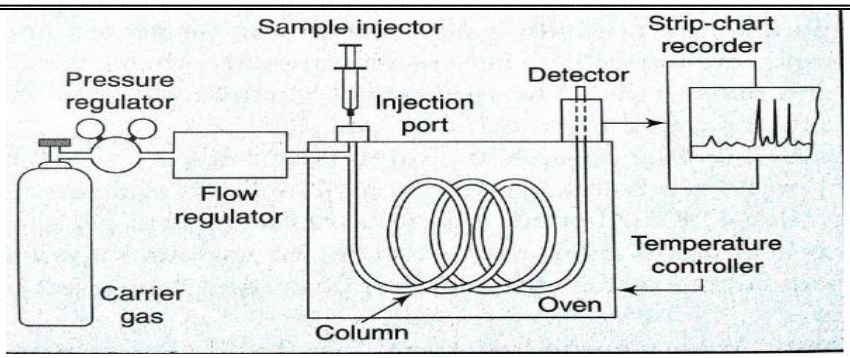
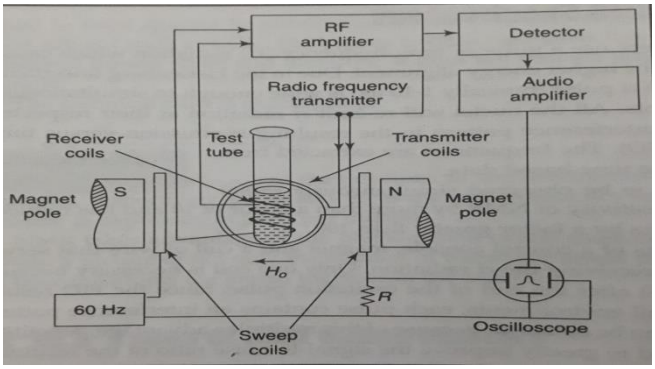
	a)	**Explain the working principle of spectrophotometer using prism.**	**4M**
	Ans:	**Diagram:**  **Explanation:** The isolation of different wavelengths in prism monochromator is based upon the fact the refractive index of material is different for radiation of different wavelengths. If parallel beam of radiation falls on prism the radiation of two different will bent through different angles. 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. Prism is an optical component to disperse the light or modify the direction of light. The wavelength selection is done by rotating the prism about a pivot. 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.	**2M** **2M**
	b)	**In chromatography if the temperature of oven decreases, what will be its effect on retention time in chromatogram.**	**4M**
	Ans:	In chromatographic techniques, the sample is introduced into the column with the help of a sample injection system. Various components of the sample are fractionated during their passage through the column which is placed in Oven. If we lower the temperature of oven the retention of a column increases	**4M**
	c)	**State the principle of thermal conductivity analyzer and explain its working.**	**4M**
	Ans:	**Diagram:**  	**2M**

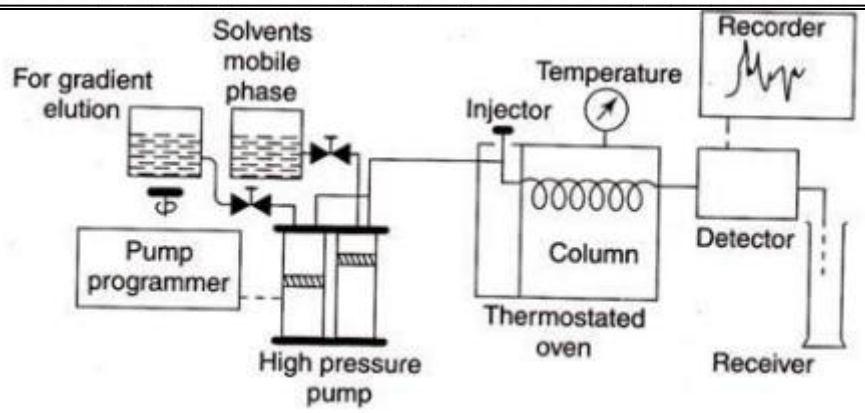
		Diagram of thermal conductivity gas analyzer **Working:** In a typical hot-wire cell thermal conductivity analyzer; four platinum filaments are employed as heat sensing elements. They are arranged in a constant current bridge circuit and each of them is placed in a separate cavity in a brass or stainless steel block. The block acts as a heat sink. The material used for construction of filaments must have a high temperature-coefficient of resistance. The materials generally used for the purpose include tungsten, Kovar (alloy of CO,Ni and Fe) or platinum. Two filaments connected in opposite arms of the Wheatstone bridge act as reference arms, whereas the other two filaments are connected in the gas stream, which act as measuring arms. The use of a four-cell arrangement serves to compensate for temperature and power supply variations. Initially, reference gas is made to flow through all the cells and the bridge is balanced precisely with the help of potentiometer D. When the gas stream passes through the measuring pair of filaments, the wires are cooled and there is a corresponding change in the resistance of the filaments. The higher the thermal conductivity of the gas, the lower would be the resistance of the wire and vice versa. Consequently, the greater the difference in thermal conductivities of the reference and sample gas, the greater would be the unbalance of the Wheatstone bridge. **(Note: thermal conductivity analyzer using thermistor can be considered)**	**2M**
	d)	**List the types of gas pollutant and their concentration.**	**4M**
	Ans:	a)Carbon monoxide: Its average concentration is below 200 ppm. b)Sulphur dioxide: Its concentration in urban areas is 0.024 ppm. c)Nitrogen oxides: Its level ranges from 0.5 to 0.12 ppm. d)Hydrocarbon: Its average concentration is below 80 ppm. e)Oxidants: Its average concentration is below 500 ppb.	**Any four** **1M Each**
	e)	**Explain carbon monoxide measurement using gas chromatography.**	**4M**
	Ans:	**Explanation:** 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. 5. The accuracy is about +-2%.peak heights of CO and CH4 would gives sensitivity of 50 ppb. **Diagram:**	**2M** **2M**



OR

Note-Any other relevant diagram shall be considered

Q.5		**Attempt any TWO of the following:**	**12Total Marks**
	a)	**Describe the procedure to troubleshoot NMR.**	**6M**
	Ans:	**Diagram:**  **Procedure to troubleshoot NMR:** i. Acquire the background information ii. Identify the problem iii. Cross check the break wires of NMR CIRCUITS. iv. Checkout each components of NMR ie RF transmitter and receiver,RF Amplifier,Detector,coil,audio amplifier,oscillator in proper conditions. v. Conduct simple diagnostic test,if NMR is working properly.It should give proper reading for known sample. vi. Finally recalibration is necessary if it gives error in readings.	**2M** **4M**
	b)	**Draw block diagram of LC and explain role of each block.**	**6M**
	Ans:	**Block diagram:**	**2M**



4M

Explanation:

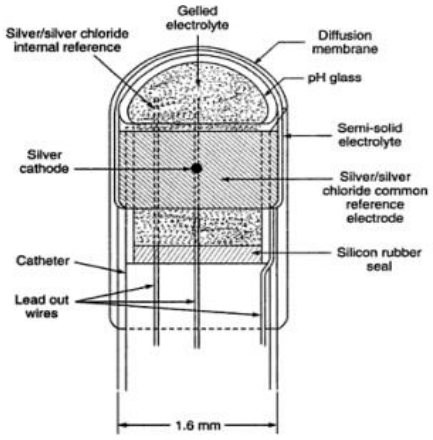
It basically consist of :

(a) A high pressure pump system to force the liquid mobile phase through the column
(b) Gradient elution or solvent programmer
(c) The sample injection system
(d) column
(e) The detection system including display or recording devices (f) Computer for data processing and storage.

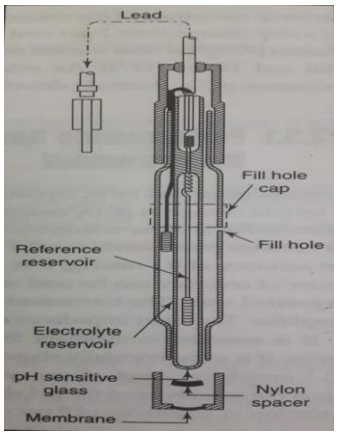
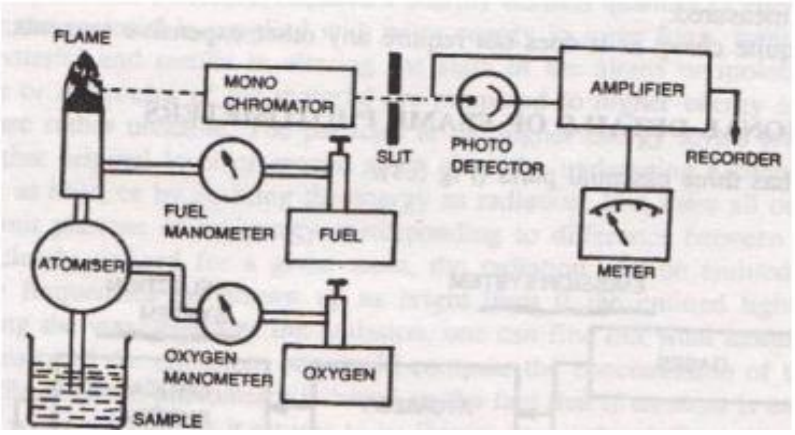
Liquid Chromatography (LC) is an analytical chromatographic technique which is useful for separation of ions or molecules that are dissolved in a solvent. If the sample solution is in contact with a second solid or liquid phase, the different solute will interact with other phase to differing degree 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.

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.

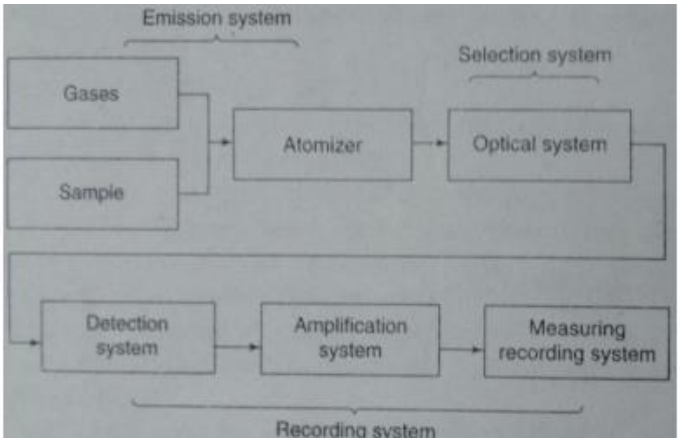
	(c)	**Explain construction and working of pCO_2 electrode.**	**3M**
	Ans:	**Diagram:**	**2M**



OR

		 **Construction:** i. These are the miniature electrodes for in vivo transcutaneous measurement of blood PCO2 The electrodes are small enough to be mounted on the catheter tip and able to perform more than one parameter i.e measurement of blood PCO2. ii. The electrode consists of pH sensitive glass bulb at the tip of catheter for measurement of silver cathode electrode for measurement of blood PCO2. Silver/ silver chloride is the common electrode. A semisolid electrolyte is common for both PCO2 electrodes. iii. The electrodes are dip-coated with a thin polystyrene diffusion membrane. When the device is placed in blood, water vapours diffuse through the membrane. This, together with NaHCO3 & NaCl crystals deposited in the hydgrogel film, constitute the electrolyte for PCO2 electrode. Under these conditions, the output signal from both PCO2 electrodes is thus obtained.	**4M**
Q.6		**Attempt any TWO of the following:**	**12Total Marks**
	(a)	**Draw a neat labelled diagram of flame photometer. Explain the role of each element of flame photometer.**	**6M**
	Ans:	**Diagram:** 	**2M**

OR



Explanation of each block is as follows: **4M**

The flame photometer consists of three essential parts

i. Emission system.
ii. Optical system.
iii. Recording system.

1. Emission system:
It has other parts.
i. Fuel Gases and Regulator.
ii. Atomizer
iii. Burner
iv. Flame.

a) Fuel Gases and their Regulation Pressure Regulators:
In order to obtain a steady emission reading, it is needed to have steady flame. In order to achieve this, the air or oxygen and fuel pressure has to be maintained constant during the operation of the instrument. Suitable pressure gauges are therefore provided in the instrument to indicate the pressure that isactually present in the line. The fuel gas normally used in flame photometry is acetylene gas, which is commercially available in cylinders of various sizes.

b) Atomizer: It is a system used to form aerosol (drop of liquid) by breaking atoms of liquid into small drops. This little device is responsible for introducing the liquid sample into the flame at a stable and reproducible rate. The atomizer must not be attacked by corrosive solutions.

c) Burner: The burner brings the fuel, oxidant and sample aerosol together so that they may react safely and produce a good flame.

d) Flames: It forms the source in which the light radiations characteristics of sample elements are produced.

2. **Optical system**: the emitted light by flame is passed through filters, monochromators and focused on photo detector. This detector converts light energy into electrical signal.

3. **Recording system**: electrical signals o/p of detector is recorded or indicated using suitable photometer recorders using amplifier.

	(b)	**Draw labelled diagram of complete blood gas analyzer. Explain measurement of HCO_3using same.**	**6M**
	Ans:	**Diagram:**	**4M**

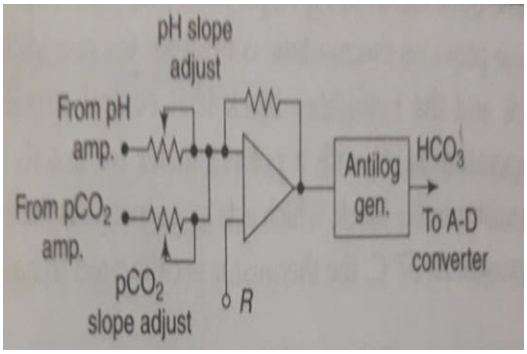
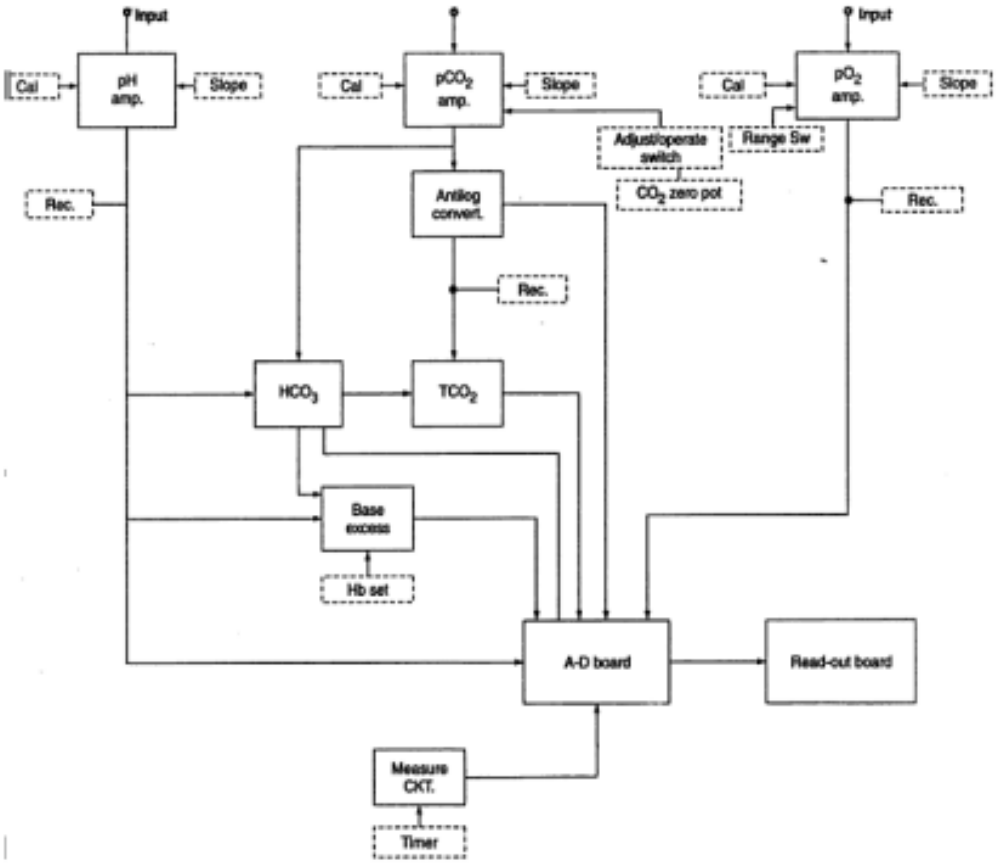


Figure : Circuit diagram for computation Bicarbonate(HCO3)

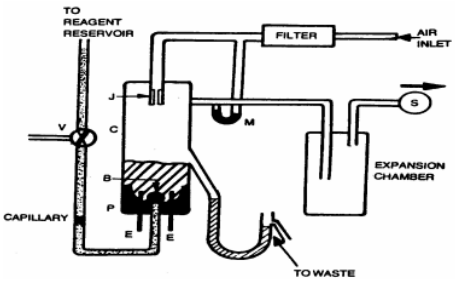


2M

Figure : Total Blood Gas Analyzer

Measurement of HCO_3:

1. Blood gas analyzers are designed to measure pH, pCO_2 and pO_2 from a single sample

		of whole blood. With built in calculators they also calculate total CO2 , (HCO3 -) and base excess. **2.** The input signal to (HCO_3 -) calculator comes from the outputs of pH and pCO_2 amplifiers. **3.** The outputs are adjusted by multiplying each signal by a constant and given to an adder. The next stage is an antilog-generator and output of this circuit is given to A-D converter for display.	
	c)	**Explain SO_2 measurement using conductivity method.**	**6M**
	Ans.	**Diagram:**  **Explanation:** 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. $H_2O_2+SO_2 \rightarrow H_2SO_4 \rightarrow H^+ + (HSO_4)^-$ Conductivity cell is used for continuous measurement of SO_2 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 SO_2 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 SO_2 at any instant is proportional to slope of saw-tooth at that instant.	**2M** **4M**