SUMMER– 15 EXAMINATION

Subject Code: 17544 **Model Answer** Page No: _1/13

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.

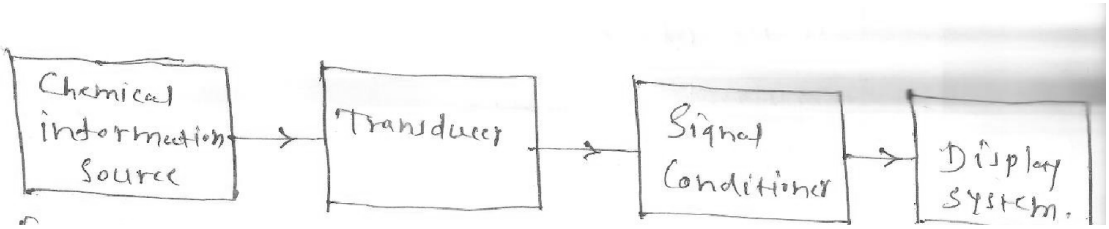
3) The language errors such as grammatical, spelling errors should not be given more Importance (Not applicable for subject English and Communication Skills.

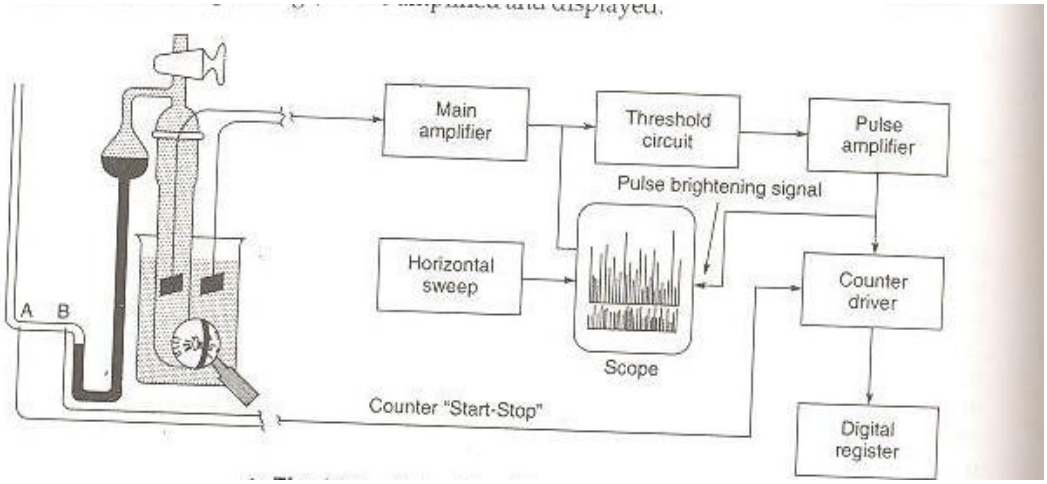
4) While assessing figures, examiner may give credit for principal components indicated in the figure. The figures drawn by candidate and model answer may vary. The examiner may give credit for any equivalent figure drawn.

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.1 .	Attempt any three	12
a)	**Draw block diagram of General Elements of an analytical instrumentation & describe function of each block. (Description 2marks + Diagram 2marks)**  **Fig: General Elements of an analytical instrumentation** **characteristic module □----------processing module---------** 1) Chemical information source—It generates a set of signal containing necessary information. It may be the sample itself. 2)Processing module- Transducer: It converts the signal to a one of the different nature. It is generally used to convert nonelectrical phenomenon associated with the analysis of the sample. foreg. Photodiode. 3) Signal Conditioner: It converts the o/p of transducer in to an electrical quantity suitable for operation of the display system.2)It also increases sensitivity of instrument by amplification of original signal. 4) Display System: It provide a visible presentation of quantity as a displacement of scale or chart or record.	04
b)	**List four applications of incinerator.** 1. Dispose of Medical wastes 2. Dispose of damaged organs 3.Dispose of Burning of Placenta 4. Disposable needle syringes 5. Surgical pads 6. Hand glows which are used in hospital 7.To burn hygienic waste generated daily may be also saline bottles,dressing cottons & dangerous body parts.damage blood bags.	04

c)	**Draw a neat labelled diagram of conductive blood cell counter.**  **Fig: Electro conductive blood cell counter** Blood cell counters operating on the principle of conductivity change which occurs each time a cell passes through an orifice are generally known as coulter counters. The technique is extremely useful for determine the number and size of the particles suspended in an electrically conductive liquid. The principle of the measurement is that blood is a poor conductor of electricity whereas certain diluents are good conductors. For a cell count, blood is diluted and the suspension is drawn through a small orifice. By means of a constant current source a direct current is maintained between two electrodes located on either side of the orifice. As a blood cell is carried through the orifice, it displaces some of the conductive fluid and increases the electrical resistance between the electrodes. A voltage pulse of magnitude proportional to the particle volume is thus produced. The resulting series of pulses are electronically amplified, scaled and displayed on a suitable display	04
d)	**State types of electronic microscope also list its different parts. (Types 2 marks + Parts 2 marks)** **Types of Electronic microscope**: 1)SEM:Scanning Electron Microscope. 2) TEM:Transmission Electron microscope **Different parts**: 1) Light source **(Any four)** 2)mirror lenses. 3)condenser system 4)diaphragm 5)Eye piece. 6)photomiographic system	04

B)	**Attempt any One**	**06**
a)	**With neat diagram explain the construction and working of scanning electron microscope.** **(Diagram – 3marks, Working – 3marks)**	06

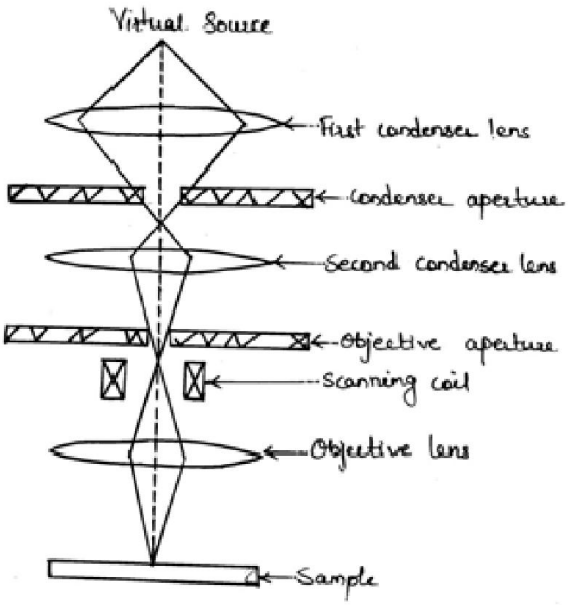


Fig : Scanning electron microscope

SEM is used to provide 3d image of cells in scanning electron microscopy. The electron beam does not pass through the specimen, instead the surface of the cell is coated with a heavy metal & a beam of electrons is used to scan across the specimen. Electrons that are scattered or emitted from the sample surface are collected to generates a 3d image as the electron beam moves across the cell because the resolution of scanning electron microscopy is only about 10nm, its use is a generally restricted to studying whole cells rather than subcellular organelles or micro molecules.

Scanning electron microscope & its optical system are shown in the figure. The virtual source at the top represents the electron gun, producing a stream of mono chromatic electrons. The stream is condensed by the first condenser lenses which are usually controlled by the course probe current knob. It works in conjunction with condenser a aperture to eliminate the high angle electrons from the beam. The beam is then constructed by the condenser aperture. Eliminating some high angle electrons. The second condenser lenses forms the electrons into a thin light coherent beam and it's usually controlled by a fine probe current knob.

A user selectable objective aperture further eliminates high angle electrons from the beam. A set of coils then scans or sweeps the beam in a grid fashion, Dwelling of points for a period of time determined by the scan. Speed casually in the microsecond range. The final lenses, the objective focuses the scanning beam onto the part of the specimen desired. When the beam strike the sample & dwells for few microseconds. Interaction occur inside the sample are detected with various instruments. Before the

	beam moves to its next Dwell point, these instruments count the number of interaction & display a pixel. A pixel is one of the many tiny dots that make up the – of a picture in computer memory. This process is repeated until the grid is finished & then repeated, the entire pattern can be scanned 30 times per seconds.	
b)	**State working principle of centrifuge. Give its classification & any four application of it.**	06

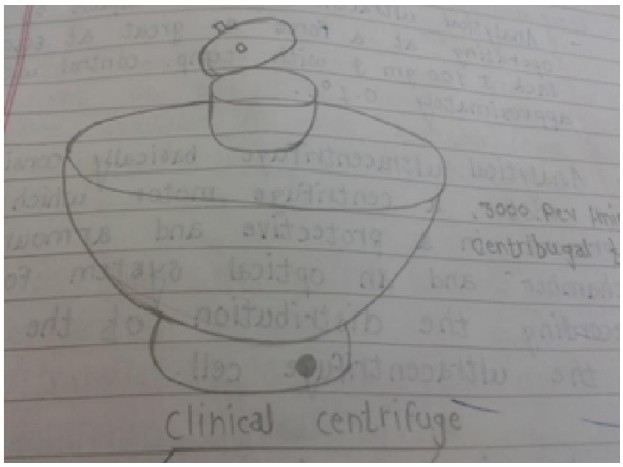


Fig: Clinical Centrifuge

Working principal of centrifuge. It is a device that spins liquid sample at a high speed &create a strong centripetal force causing the denser material to travels towards bottom of centrifuge tube more rapidly than gravitational force.

The basic idea behind centrifuge is the sedimentation process & it is depend on the applied centrifugal force.

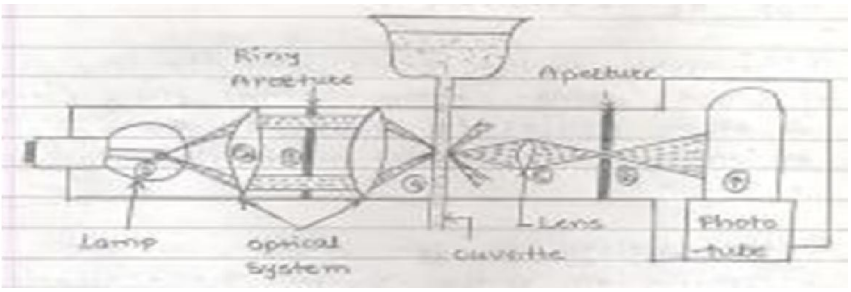
Classification of Centrifuge

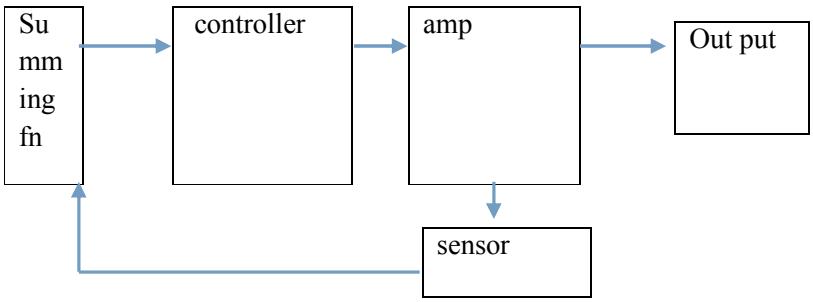
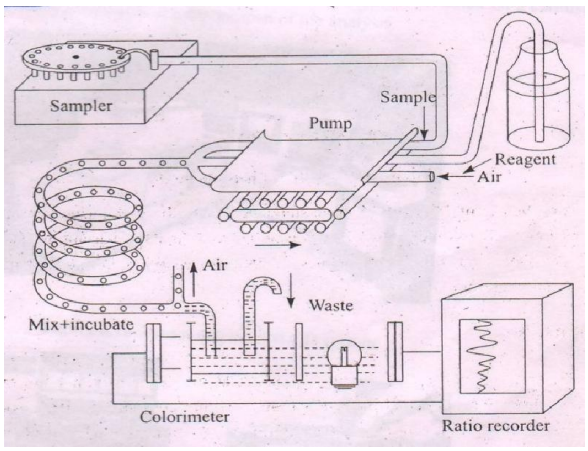
1.Clinical Centrifuge

2. Ultra Centrifuge

Applications of centrifuge:

1. It is used to separate urine components
2. It is used in labs & forensic labs to separate compounds of blood.
3. To separate RBC's, WBC's & Plasma in a blood.
4. To separate substances of different densities using centrifugal force
5. To determine relative molecular mass of macromolecules such as proteins & DNA.

Q.**2.**	**Attempt any four**	**16**
a)	**Define chromatography. Give classification of it.** Chromatography is basically a group of methods for separating a mixture of substance into component parts. One phase is fixed liquid or solid and the other phase is mobile gas or liquid. In chromatography differences in the rate of movement of components of the mixture in the mobile phase caused by interaction of these components with the stationary phase are used to separate the components. Classification of Chromatography : a. Gas chromatography b. Liquid chromatography	04
b)	**Draw neat labelled diagram of dark field blood cell counter and state its working principle.**  Fig : Dark field blood cell count **Working :** The diluted blood flows through a thin cuvette.The cuvette is illuminated by a cone shaped light beam obtained from a lamp through ring aperture.and Optical system.The cuvette is imaged on the cathode of a phototube by means of lens & an aperture.Normally no light. reaches the phototube until a blood cell passes through the cuvette and reflects a flash of light on the phototube.	04

c)	**Draw labelled diagram of hot air oven and give its two specification.** **Ans:** When electricity is passed through the heating coil electrical energy is converted to heat energy. The temperature is controlled by thermostat. It is most widely used method of dry sterilization by dry heat. The oven uses electrical as heat source. The oven is fitted with a fan to ensure adequate and even distribution of hot air in the chamber.  Fig : Hot air oven Specifications Supply voltage 230 V Ac Temperature range upto 300 degree centigrade	04
d)	**Draw neat labeled diagram auto-analyzer and describe its working.**  Block diagram autoanalyzer. The autoanalyzer sequentially measures blood chemistry and displays this on a graphic readout. **Working :** The autoanalyzer includes following elements: 1) Sampler- aspirates samples, standards, and wash solutions to the autoanalyzer system.	04

2) Proportioning pump and manifold- introduces (mixes) samples with reagents to effect the proper chemical color reaction to be read by the colorimeter. It also pumps fluids at precise flow rates to other modules, as proper color development depends on reaction time and temperature.

3) Dialyzer- separates interfacing substances from the sample material by permitting selective passage of sample components through a semipermeable membrane.

4) Heating bath- heats fluids continuously to exact temperature (typically 37^0C incubation, equivalent to body temperature). Temperature is critical to color development.

5) Colorimeter- monitors the changes in optical density of the fluid stream flowing through a tabular flow cell. Color intensities (optical densities) proportional to substance concentrations are converted to equivalent electrical voltages.

Recorder- converts optical density electrical signal from the colorimeter into a graphic display on a moving chart.

e) **Draw neat labelled diagram of PO_2electrode and state its principle.** 04

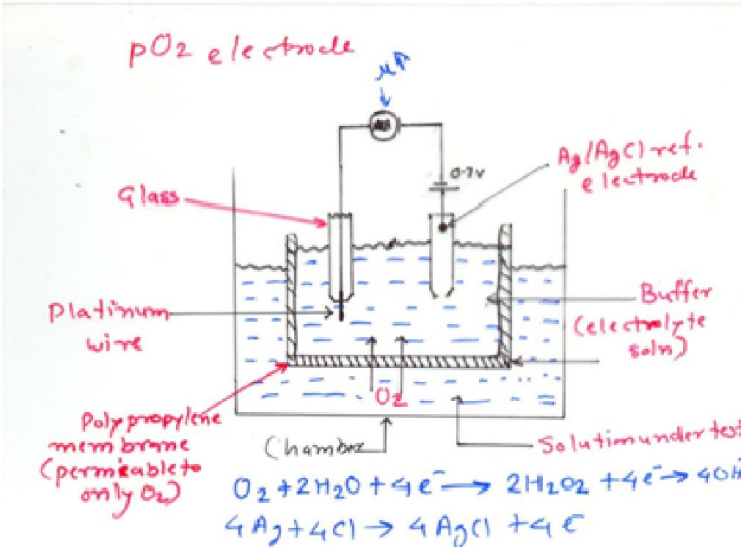


Fig : PO2 electrode

The PO_2 electrode is known as Clark electrode after its inventor and it is an O_2 sensor for blood. The electrode arrangement consists of two chambers and they are separated by polypropylene membrane i.e. permeable to O_2. The blood sample is injected into lower sample chamber as shown in the figure. The upper chamber contains the electrode. The O_2 in the blood permits the polypropylene membrane and reacts chemically with a phosphate buffer contained in the upper chamber. The buffer maintains the solution pH at a constant level. The O_2 combines with water in the buffer producing electrons proportion to the number of O_2 molecules according to the formula:

$O_2 + 2H_2O + 4e^- \rightarrow 4[OH]^+$

The electron current is measured by the ammeter. It is directly proportional to PO_2. Electrons on the left side of the equation are produced by a source voltage that polarizes the electrode and has value 0.7V. This voltage is called polorographic voltage. The electrode is called Clark's polorographic electrode. The meter scale is calibrated in units of PO_2 in the blood. This electrode current depends on current blood in the solution rather than membrane potential as it was in pH measurement.

f) **Define electrophoresis? State its working principle and give its classification.** 04

Ans.

Electrophoresis is a process of separating electrically charge particles under the influence of electric

field.

Which is givien by fallowing en
F=qE
E=Intensity of electric field
q=Charge
F=Column's farce.

Working :

It is based on the principle that the individual component of the colloidal solution migrates in a liquid of different speech when subjected to an electric field. Separation of such particles of similar geometry but different charge & particles of like charge but different geometry migrate at different towards and oppositely charge electrode. Therefore when the current is passed for a certain time through such a solution. Various component present in the solution. Would more through difference distance in their effect to migrates towards the electrode. Therefore a substance which may be a mixture is thus separated into it's component along the migration distance according to a definite low.

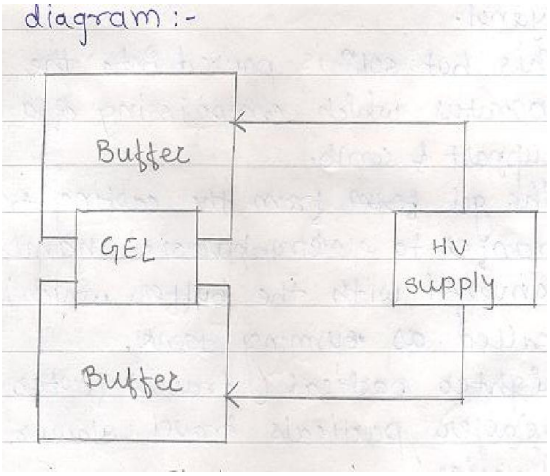
Measurement of the concentration along this migration distance would therefore provide the quantities result of the analysis. Accordingly electrophoresis is separation rate depending upon their total charge size and shape.

Thin layer Electrophoresis.

In this method of electrophoresis we can separate out only two components of a mixture.To overcome this limitation we are going to use the paper electrophoresis technique. The basic principle of paper electrophoresis method here we use paper as supporting medium under the action of electric field the charged molecule migrate through the paper just as they might thorough an unbounded solvent however the advantage of this method. Is that it is possible to obtained a complete separation into zones of different migration & not as boundary separation of overlapping zone in the liquid phase.

The separation zones are located by applying various active region.Paper electrophoresis has developed into a generally applieded& valuable routine clinical method.

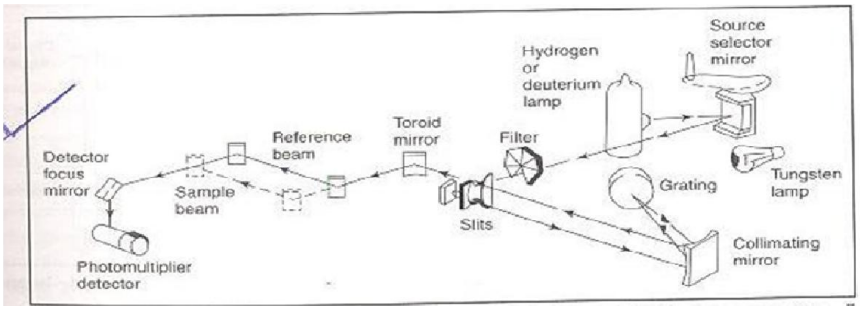
The main advantage of this technique is small substance is required for analysis.



Block diagram of Electrophoresis

Classification of electrophoresis :

a. Paper Electrophoresis
b. Gel Electrophoresis
c. Micro – Immuno
d. Thin layer
e. Cellulose acetate electrophoresis
f. Capillary electrophoresis

Q.3.	Attempt any four	16
a)	**Draw neat labelled diagram of dual beam spectrophotometer and describe its working.(Diagram 2 marks + Description 2marks)**  **Fig : Dual beam spectrophotometer** **Description** : Energy of appropriate wavelength is produced by the appropriate source lamp. This energy is converted to monochromatic light by using filter-grating optical system. The filters are necessary to eliminate unwanted orders from the gating. Six different filters cover the wavelength regions from 300 to 900nm.The filter wheel is driven by a dc motor, which is synchronized with the wavelength cam. As the wavelength cam moves, it causes the filter motor to drive till the correct filter comes into position. The wavelength cam drives the wavelength arm which causes the grating to pivot on its own axis.It causes the wavelength of light coming out of the monochromator to change.The monochromatic light is then directed to the sample and reference via a vibrating mirror bridge, which vibrates horizontally at a certain frequency.This bridge allows light to pass into sample and reference cell holders alternately, with a frequency equal to displacement frequency of the bridge.The vibrating bridge is controlled by the bridge drive circuitry.The reference and sample pulse train is then passed to the photomultiplier tube, which converts the monochromatic light pulses to current pulses.	04
b)	**State importance of sterilization. List different methods of sterilization. (Importance 02 marks + Methods 02 marks)** **Importance of sterilization:** Sterilization is a term referring to any process that removes or kills all forms of microbial life, including transmissible agents (such as fungi, bacteria, viruses, spore forms, etc.) present on a surface, contained in a fluid, in medication, or in a compound such as biological culture media. **Different methods of sterilization.** 1.Autoclave. 2.Hot air oven. 3.Ultrasonic cleaner. 4.Water bath. 5.Freezer.	04

c)	**Draw neat labeled diagram of liquid chromatography &explain it.(Diagram 02 marks + Description 02 marks)**	04

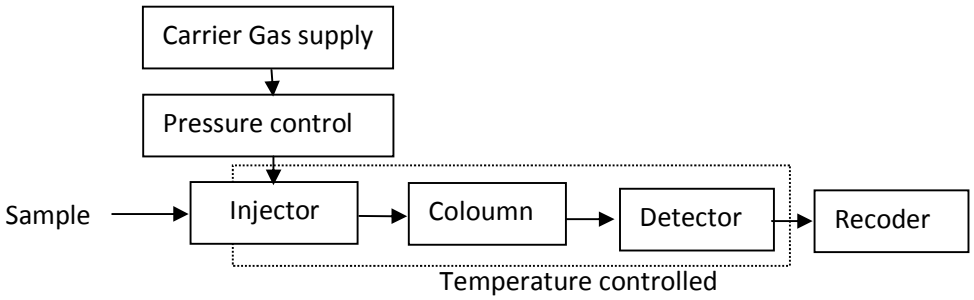


Fig: Block diagram of Gas-Liquid Chromatography

Description :

The basic parts of a liquid chromatograph are shown in figure. It consists of the following parts.

- Carrier liquid supply along with pressure regulator and flow monitor.

- Sample injection system.

- Chromatographic column

- Thermal compartment of thermostat

- The detection system

- The strip chart recorder

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 T1, 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 vapor mixes almost instantaneously with the flowing carrier gas and is swept into the chromatographic column, which is the heart of the chromatography.

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 T2. 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 potentiometer recorder and a plot of the time signal amplitude called chromatogram is obtained.

d)	**Draw neat labelled diagram of colorimeter & describe its working. List any two application of it. (Diagram 01 marks + working 02 marks + twoApplications 01 marks)**	04
e)	**State Beer's of Lamberts law. State its mathematical expression.**	04

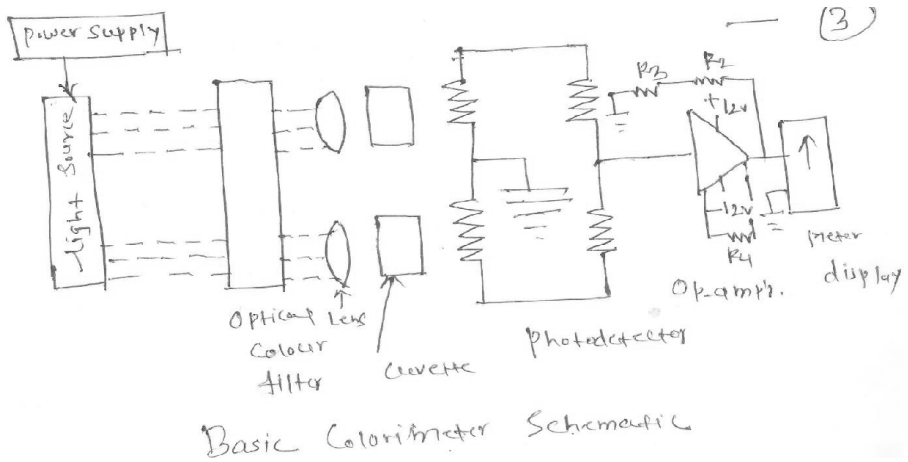


Fig: Colorimeter

Working : -

First operational amplifier error is removed by balancing potentiometer for this both input are grounded Then reference liquid like distill water is kept in both cuvettes outputs of indicator is adjusted to zero both cuvettes contents same fluid hence differential output is zero. Reference liquid is retain in cuvette to is replaced by blood or another liquid. Now the differential reading given by the meter is indicator liquid absorbance of light.

Application of colorimeter:- (Any two)

01. Chemistry section deals with the analysis of blood, urine, cerebrospinal fluid ((csf) and other fluids determine the quantity of various important substances they contain.

02. Hematology section deals with the determination of the number and characteristics of the can statements of the constituents of the blood particularly the blood cells.

03. Microbiology section is which studies are performed on various body tissues and fluids to determine the presence of pathological miro-organisms.

(State 02 marks + Expression 02 marks)

A combination of the two laws, known jointly as the Beer Lambert law, defines 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= £ cb, Where A= absorbance (no unit of measurement) £ = molar absorptivity (dm3 mol-1 cm-1) c= molar concentration (mol dm-3) b= path length (cm). It may be noted that £ is a function of wavelength. so, the Beer Lambert Law is true only for light of a single wavelength or monochromatic light.	