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TOOL: 01

ANALYTICAL INSTRUMENTS:

Analytical instruments are a large class of instruments used for analytical applications in chemical, pharmaceutical, clinical, food-processing laboratories and oil refineries. The instruments help in analysing materials and establishing the composition. Among the most common types of analytical equipment are spectrophotometer, refractometer, calorimeter, electrochemical Instrument, conductivity meter, automatic density meter, automatic titrators, colony counter, demagnetizers, fiberscopes and several others.

1. BALANCE:

Balances are those instruments that are used to analyse the weights of given compounds. Balances are important instruments used to measure the weights of chemicals, solid substances, biological tissues etc. These balances can measure the weight as small as 100 micrograms to certain kilograms (3-5kg).

The analytical balances are highly sensitive and are to be maintained thoroughly to get accuracy in results. These balances should be kept in separate room which is free from dust since the accumulation of dust may alter its accuracy. Further chemical reactions can occur because of the humidity present in the atmosphere leading to corrosion. In the process of transferring certain salts, some amount may spill on the balance and lead to corrosion and damage to the balance. To avoid spillage, spatula to be used and before the measurement, butter paper or filter paper to be spread on weighing pan.

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PRINCIPLE:

The working of balance is based on the principle of parallel forces. The direction of the resultant two of the parallel forces are same as that of either of two forces, if parallel forces are represented both equal by magnitude and direction. They have no effect on the body. In the physical balances, the two suspended balances are of equal weights.

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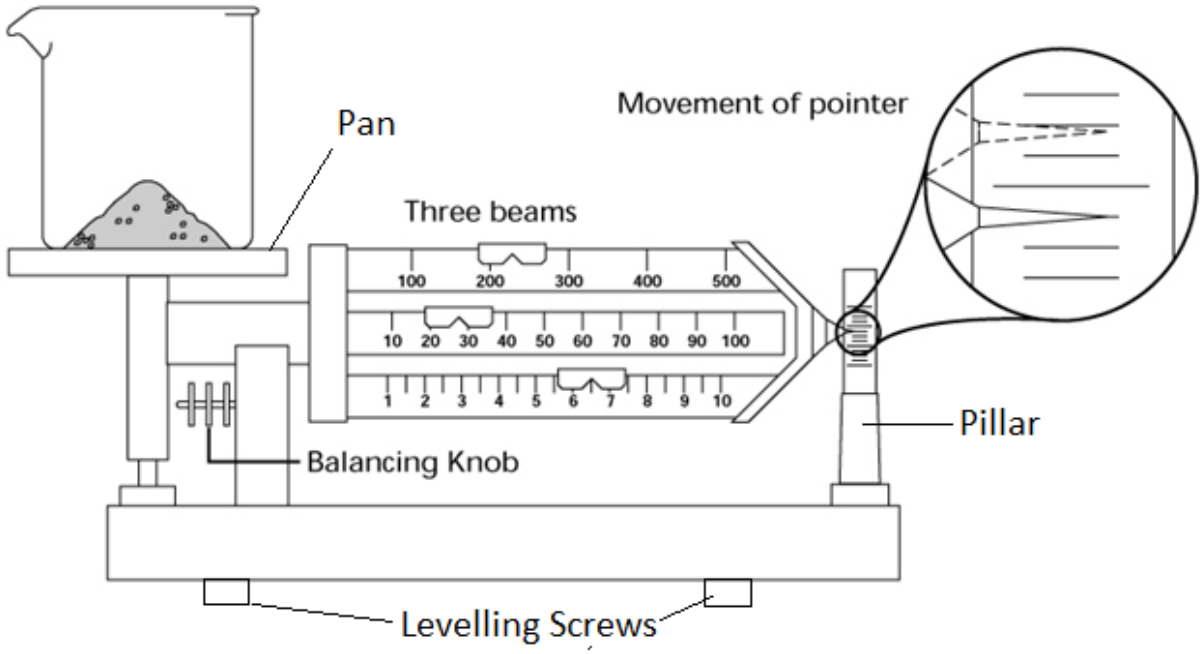
When the pans are empty, the pointer remains at rest. An addition of weight to either of pans will oscillate the pointer. The pointer will oscillate more towards the lighter side than that of heavier side. There are different types of analytical balances based on its utility and the way of measurements. They are categorised as;

1. Triple beam single pan balance,
2. Two pan single beam physical balance,
3. Single pan electrical balance,
4. Top loading digital electronic balance.

I. TRIPLE BEAM SINGLE PAN BALANCE:

This balance consists of three horizontal beams, the first two beams can measure weights ranging from 0-10gm and 0-100gm respectively. The end of third beam consists of a pointer that moves over a graduated scale.



TRIPLE BEAM SINGLE PAN BALANCE

When the pan is empty, the pointer rests at zero and the pendulum is in equilibrium. The pendulum can be controlled by adjusting the three screws at the base of the balance. The principle

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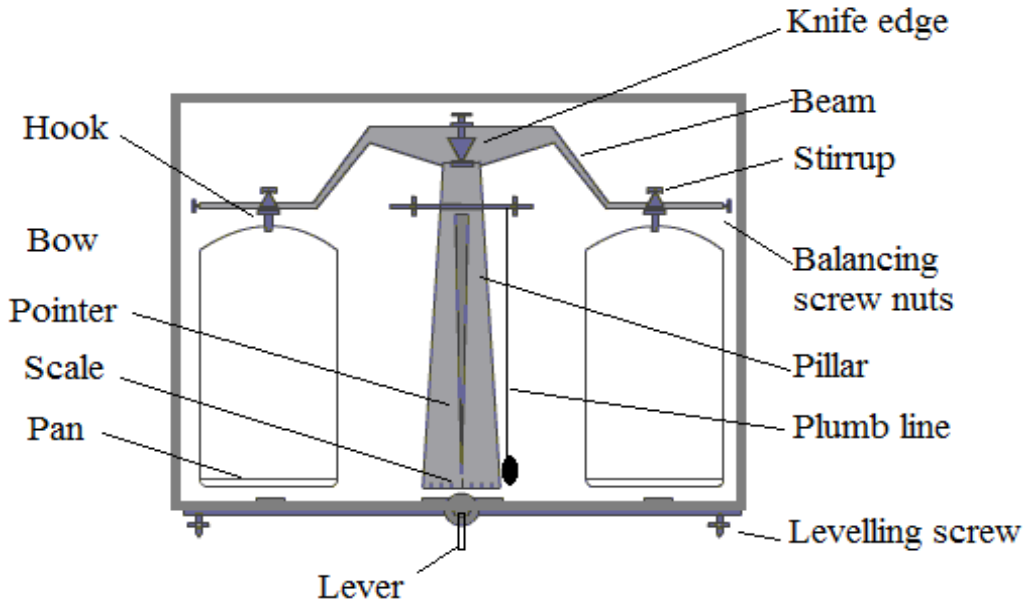
behind its working rests on the fact that the pan goes down on addition of weight and the pointer partition change to obtain the equilibrium. Again, a change in weight is brought about by moving the metal indicator over. The beams give the rough estimate of the weight. This balance is used in biological studies to weigh biological samples for estimation.

II. TWO PAN SINGLE BEAM PHYSICAL BALANCE:

This balance consists of two plates or pans suspended at equal distances from a fulcrum. One plate holds an object of unknown mass (to be measured), while known masses are added to the other plate until static equilibrium is achieved.

The balance consists of a horizontal beam on which the pans are suspended. Primarily the pans are checked for dust and proper placement of them (pans) in their respective positions. Observe if the plumb line is vertical over the indoor, this can be achieved by rotating the two front levelling screws at the base of the balance. Make sure that the balance is properly adjusted and release the beam of the balance by turning the knob clockwise and observe the movement of the pointer on the scale. If the pointer moves equal, i.e., same number of divisions on either side of the zero, the alignment is said to be correct. If this isn't achieved, it can be adjusted with the aid of adjustment screws on either side of the beam.



TWO PAN SINGLE BEAM PHYSICAL BALANCE

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Never weigh the chemicals directly on the pan but weigh in a suitable container such as weighing bottle, beaker, watch glass etc. Always place the substance which is to be weighed on the left-hand pan and weights on the right-hand pan. They should be placed exactly at the centre of the respective pans to get accurate readings. Do not weigh an object hotter or colder than the surrounding air. Before weighing (hot or cold), allow them to fall to the room temperature.

Do not handle the weights by bare hands but with the aid of forceps. Do not add or remove anything from the pan while the balance is swinging. Arrest the balance before adding or removing anything from the pan. Care is taken to avoid the spillage on pan and on the floor of the balance. Do not over load the balance. Post weighing, arrest the beam, replace the weights in their respective positions. The side doors are closed once the weighing is complete.

GENERAL DIRECTIONS FOR WEIGHING UPTO THE FOURTH DECIMAL PLACE:

- With the balance at rest, place the object to be weighed on the left pan.
- Add weights as needed on to the right pan.
- First start from the largest denomination and then in the descending order.
- Now place the ridges weighing 10mg on the right-hand side, close the balance case so as to avoid the convection currents.

NOTE:

If the rider is used on the right side of the beam scale, the weight corresponding the rider reading is taken as positive.

If the beam rider is taken on the left side of the beam scale, reading is taken as negative. The centre of balance is observed for the movements of the pointer directly for the centre of the scales.

III. SINGLE PAN ELECTRIC BALANCE:

This balance contains a single pan, it contains knobs to adjust the weights in milligrams and grams. Weights that have a range of 0.1-1g, 1-10g, 10-100g are used. There is vernier scale

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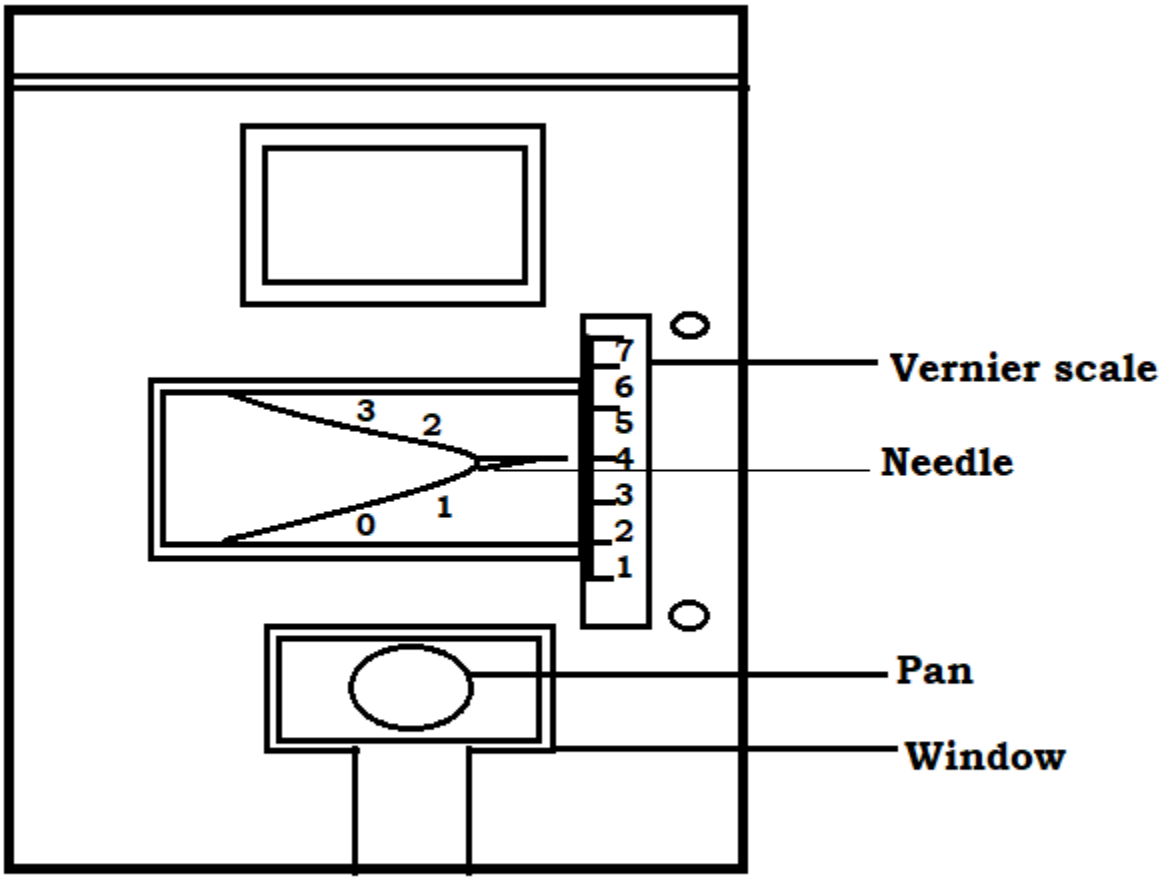
ranging from 0-10. The correct weight of the sample is determined by placing it on the pan and the zero of the vernier scale co-incised with a specific scale in a small window. This marking on the scale gives the correct measurements of the sample. Weights up to 100g can be measured in this balance. It is called electrical balance because light is provided by use of electricity.



SINGLE PAN ELECTRIC BALANCE

IV. TOP LOADING DIGITAL ELECTRONIC BALANCE:

Compared to other balances, pan can be lifted and lead to rest freely in air whereas in other electronic balances, there is high (very high) sensitivity and pans are never seen to move up and down.

The sample is directly added on the pan (care is taken to avoid the direct contact of chemical with pan), there is a sensor at the

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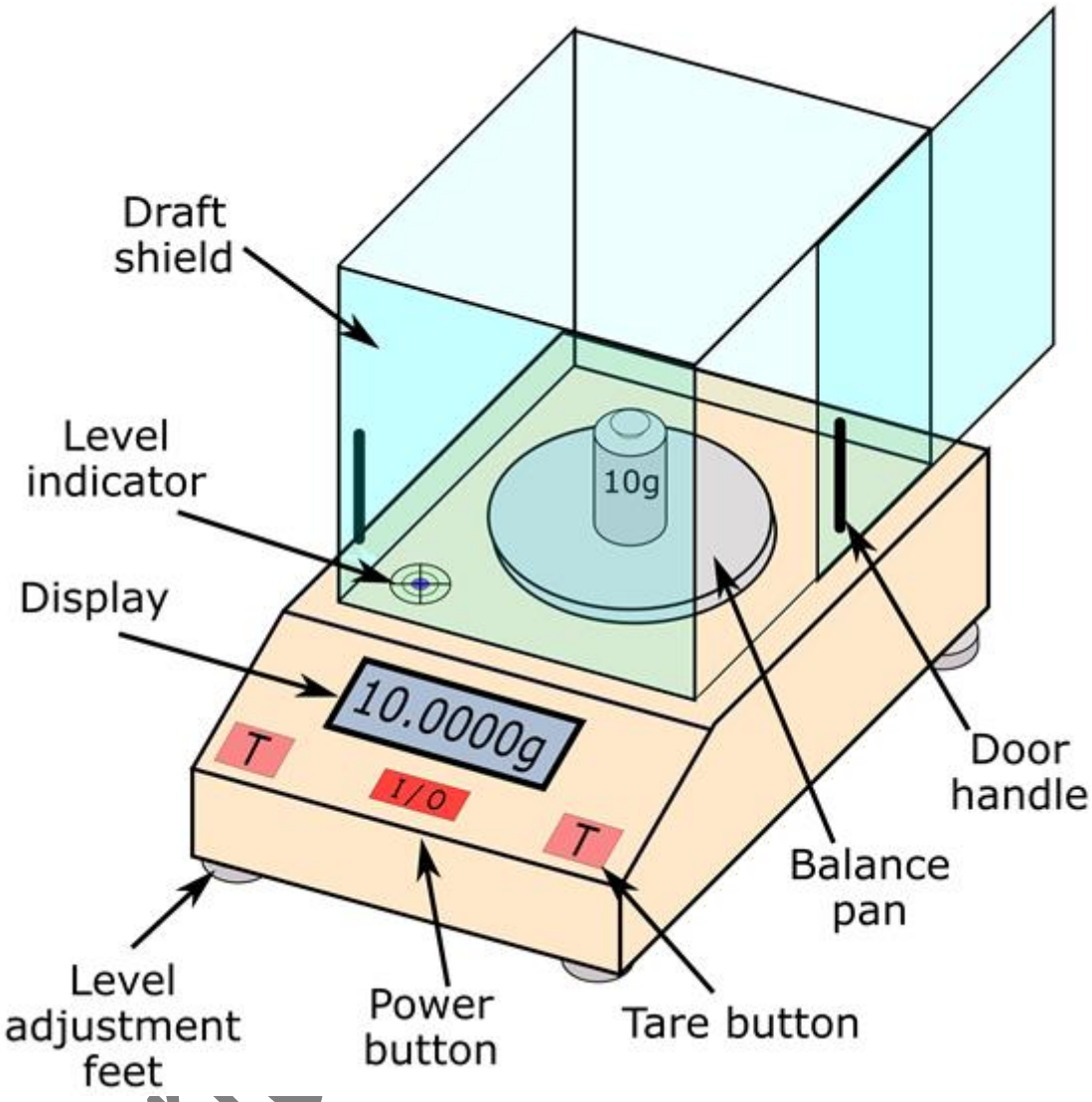
base to transit the information of the weights of the sample. If sensor is damaged, the digits are not displayed.



TOP LOADING DIGITAL ELECTRONIC BALANCE

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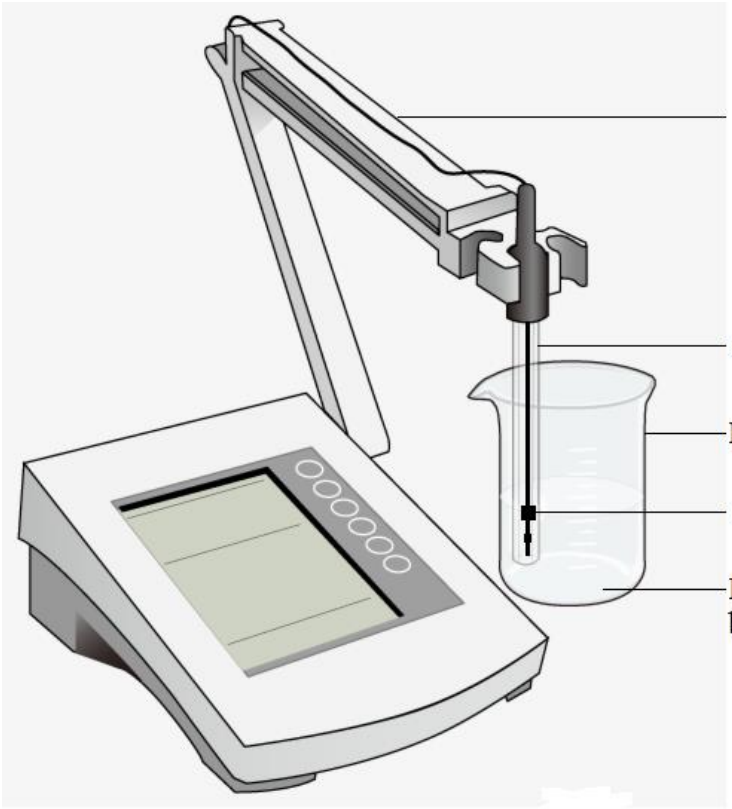
2. p^H METER:

p^H may be defined as the negative logarithm of hydrogen ion concentration that may be represented as **p^H = -log[H^+].** The p^H electrode or p^H meter is one of the most basic equipment in biological laboratory.



p^H METER

PRINCIPLE:

The p^H electrode depends on the ion exchange in the hydrated layers formed on the glass electrode surface, glass consist of silicate network among which are metal ions coordinated to oxygen atoms and it is the metal ions that exchange with H^+. the glass electrode acts like a battery where voltage depends on H^+ activity of solution in which it is immersed. The size of the potential (E) due to H^+ is given by equation;

$$E = 2.303\frac{RT}{F}\log_{10}\left[\frac{(H^+)_i}{(H^+)_o}\right]$$

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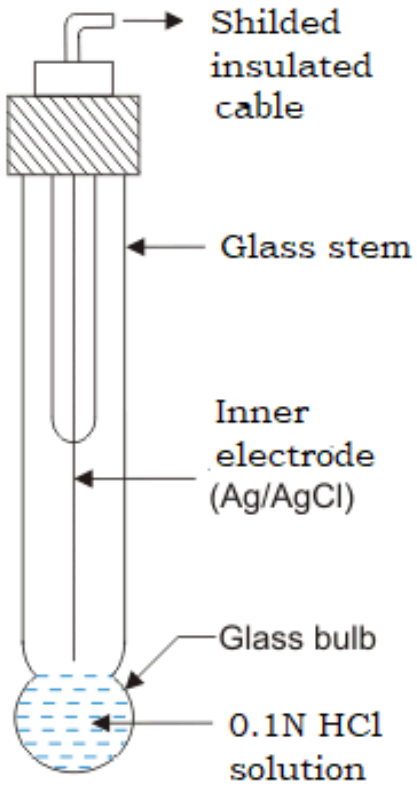
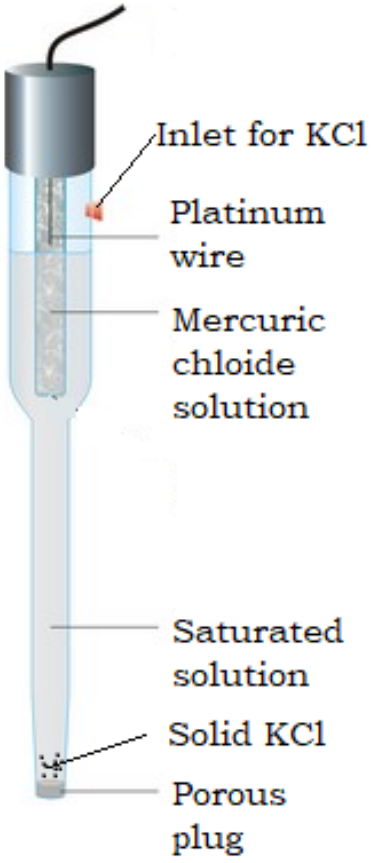
Where;

- E= Experimental potential
- $2.303\frac{RT}{F}$ (=5) is the Nernst factor or shape
- R= Molar gas constant
- T= Absolute temperature
- $\frac{(H^+)_i}{(H^+)_o}$ are the molar concentration of H^+ inside and outside the glass electrode respectively. H^+ is fixed and is generally 10^{-1} because the electrode contains 0.1M HCl, **{p^H = -log[H^+]}.** The developed potential is directly proportional to the p^H of the solution outside the electrode.

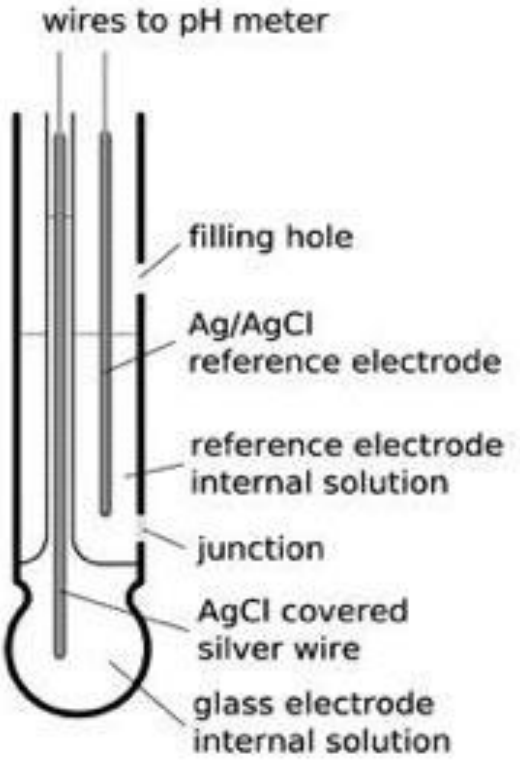


GLASS ELECTRODE



CALOMEL REFERENCE ELECTRODE

COMBINED ELECTRODE OF p^H METER:



Combination Electrode

The combined electrode is a combination of glass electrode and calomel reference electrode in a single electrode. A glass electrode consists of a thin soft glass membrane that is situated at the end of the hard glass tube in the glass electrode. An internal reference, electrode of Ag or AgCl is surrounded by an electrolyte of 0.1M HCl. This internal reference electrode gives rise to a steady potential. Thus, varying potential of glass electrode can be compared with a steady potential produced by an external reference electrode such as the standard calomel electrode. By the joining of internal and external reference electrode, p^H is obtained.

GLASS ELECTRODE	TEST SOLUTION	REFERENCE ELECTRODE
Reference H^+ glass electrode internal membrane (internal) i.e. 0.1M HCl	H^+ (external) i.e. analyte	Reference electrode (external)

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The external reference electrode can either be separate or built around the glass electrode giving a combination electrode.

INSTRUCTIONS WHILE HANDLING COMBINED ELECTRODE OR GLASS ELECTRODE:

- Stir the test solution thoroughly before measuring p^H
- When the electrode is in use or stored, the reference solution drops down, so it should be filled with freshly prepared 2N KCl solution started with a gel through filling the whole provided with an electrode cap below.
- Record the temperature of the solution.
- Set the temperature compensator on the meter to approximate value.
- Electrode should be cleaned every time by rinsing with water (do not use tap water).
- Sticky solution may be removed by whipping electrode with a moist pad of cotton.
- In prolonged storage, the outer surface of the bulb may remain dry.
- Electrode must be dipped in 4.0 p^H buffer for 24 hours.
- Electrode which fails to respond to above treatment can be activated by dipping in 0.1N HCl for 24 hours and washed with distilled water.
- Do not use electrode beyond p^H and temperature range, permanent damage may occur if used beyond range 4.9

SIGNIFICANCE OF BUFFER:

An Ideal buffer pose the following characters and is impermeable to biological membranes.

- Buffer is a mixture of weak acid a conjugate base.
- Biological stability i.e. a lack of interference with metabolism and biological processes are seen.
- Lacks in formation of insoluble components with cations.
- Minimum effect of ionic composition and salt concentration.
- Limited p^H change is seen in response to temperature increase.

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3. CENTRIFUGE:

Centrifuge is an analytical separation technique which gets separated under the applied centrifuge field. If a force is applied which is greater than the gravitational force of Earth, there is partial sedimentation more rapidly. This is the basis of centrifugation separation technique.

Particles which differ in density, size or shape, sediment at different rate of centrifugal field. The sedimentation rate of each particle is directly proportional to applied centrifugal field. The centrifuge is an instrument which is used to spin substances at high speed. Centrifugation techniques are broadly classified into two major types, they are;

A. PREPARATIVE CENTRIFUGATION:

This technique deals with separation, isolation and purification of subcellular organelles. This technique needs large amounts of biological samples to study its properties.

B. ANALYTICAL CENTRIFUGATION:

It deals with study of purified macromolecules. This technique requires only small amounts of samples.

PRINCIPLE:

If a particle is in a centrifugal field generated by a spinning rotor with angular velocity (w), the centrifugal force is given by the equation;

$$\mathbf{F=mw^2r^2}$$

Where;

m=mass of particle,

r=radial density,

w=angular velocity of rotor $\{\mathbf{w=}\frac{2\pi}{60}\ \mathbf{rpm}\}$

substituting: w=F= $\frac{4\pi^2 rpmr^2 m}{3600}$

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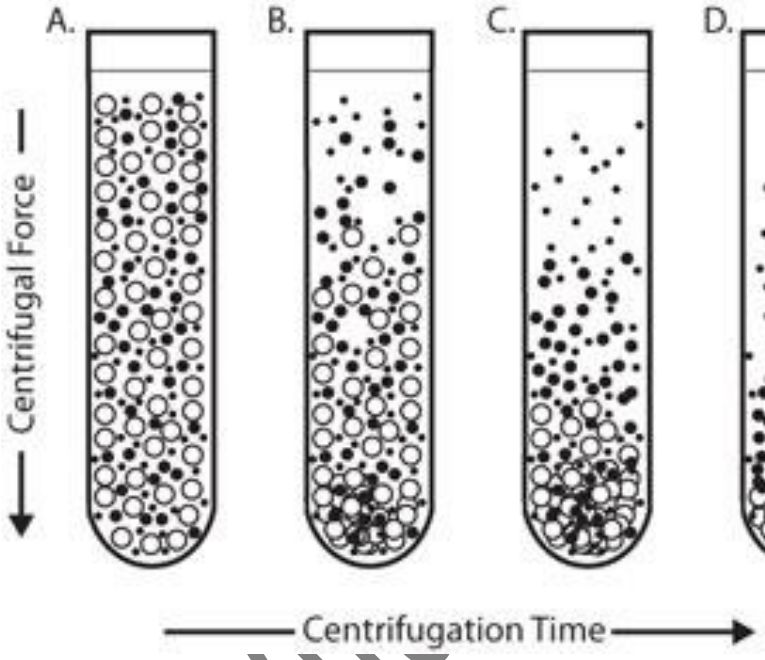
The ration of weight of the particle in centrifugal field to weight of the particle when acted by gravity alone is known as relative centrifugal field (RCF). From above relationship it is clear that RCF depends on rpm and radius of rotation 'r'. If 'r' is constant for a given rotor, thus variation in rpm alone determines the variation in RCF.



DIFFERENTIAL SEDIMENTATION OF PARTICLATE SUSPENSION IN THE FIELD

TYPES OF CENTRIFUGE:

Centrifuge can be categorized based on their rotation speed.

a. DESKTOP CENTRIFUGE:

It has a medium speed of below 3000 rpm and operate at room temperature. This is also called clinical centrifuge.

b. BENCHTOP CENTRIFUGE:

Speed ranges between 5000-16000 rpm. It's useful for centrifuging 50-100ml solution.

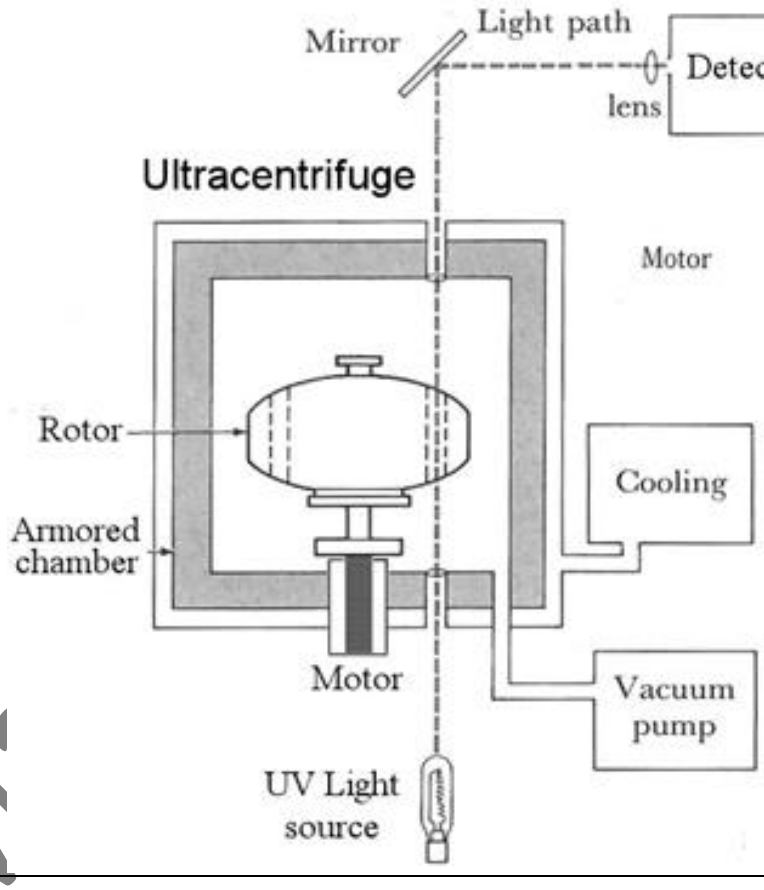
c. HIGHSPEED CENTRIFUGE:

Speed ranges between 10000-25000 rpm. They are usually equipped with refrigeration to remove heat generated due to

friction between air and spinning rotor. It is used to collect micro-organisms, cell debris, subcellular organelles etc.

d. ULTRA-CENTRIFUGE:

Speed ranges between 75000 and 1 lakh rpm. It is constructed with a vacuum rotor chamber and refrigeration. Rotors are made of aluminium alloys, which can withstand high centrifugal force. It can be used to isolate and purify the components {preparation ultra-centrifuge} or to characterize sample {analytical ultra-centrifuge}.



COMPONENTS OF CENTRIFUGE:

Every centrifuge basically has two components, they are motor and rotor. Apart from this, ultra-centrifuge also possesses optical detecting system. Hence, it not only isolates the biological samples but also helps to study structure and function of components of the sample. Rotor is kept in air cooled chamber and is suspended on a wire emerging from drive shaft of rotor. Tip of

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rotor contains an upper lens and lower lens; lower lens allows passage of light so that sample is illuminated and upper lens focuses light to camera lens which in turn directs it to photographic film.

Application of centrifugal force causes net motion of solute. Solute sediments with a finite velocity towards the bottom of the cell. Rate at which particle sediments depends on centrifugal force, density and viscosity of the medium. Sedimentation rate depends upon size and shape.

APPLICATIONS OF ULTRA-CENTRIFUGE:

- Used to determine relative molecular mas of macro-molecule like protein, DNA etc.
- Helps to determine size and shape of macro-molecules.
- It is used to detect conformational changes in macro-molecules.
- It is the only technique with which we can accurately determine molecular weight of a protein.
- Used to obtain stoichiometry of a protein complex.
- Used to isolate satellite DNA and viruses.
- Inter and intra molecular interaction like protein-protein interaction can be studied.
- It is also employed to study binding of 10 factor to an enzyme. Example: biding of NADH to lactate dehydrogenase.

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TOOL: 02

MICROPHOTOGRAPHY

INTRODUCTION:

Photographic recording of images through a microscope is called microphotography or photomicrography. Wide ranging subjects from a whole mount of a small insect to an organelle in a cell, can be photographed using an appropriate microscope, lens and lighting. The fields in which microphotography is used are cytology, histology, electron microscopy (TEM and SEM) and autoradiography etc.

AIM:

To study the working principle of microphotography.

PRINCIPLE:

Light is the basis of photography and it is an electromagnetic energy having both wave and particle properties. It is considered to be in the form of photons (the smallest unit of light energy) which are discrete particles with zero mass and no electrical charge. Photons travel in waves and are able to strike objects as if they were particles and after the physical and chemical nature of the molecules on the surface of the objects. Specifically, when they strike silver molecules, they turn them black. It is this property of light that is made use in photography.

COMPONENTS AND MAGNIFICATION:

The picture is composed with the aid of eyepiece. This special ocular contains a reticle with one or more frames indicating the region of microscopic field that will be include on the film frame with different sized film (the 35mm frame is 24mm x 36mm and polarised print frame is 8.3cm x 10.8cm). If the field you would like to photograph is larger than the reticle frames, the total magnification must be reduced by turning the eye piece, selecting the region of the field that could be included in the frame work; when good competition is achieved, the camera is turned so that it is positioned at the same angle as the reticle frame.

FOCUSSING:

The focussing eye piece enables you to focus the microscope image on the film plane of the camera, first the collar of the

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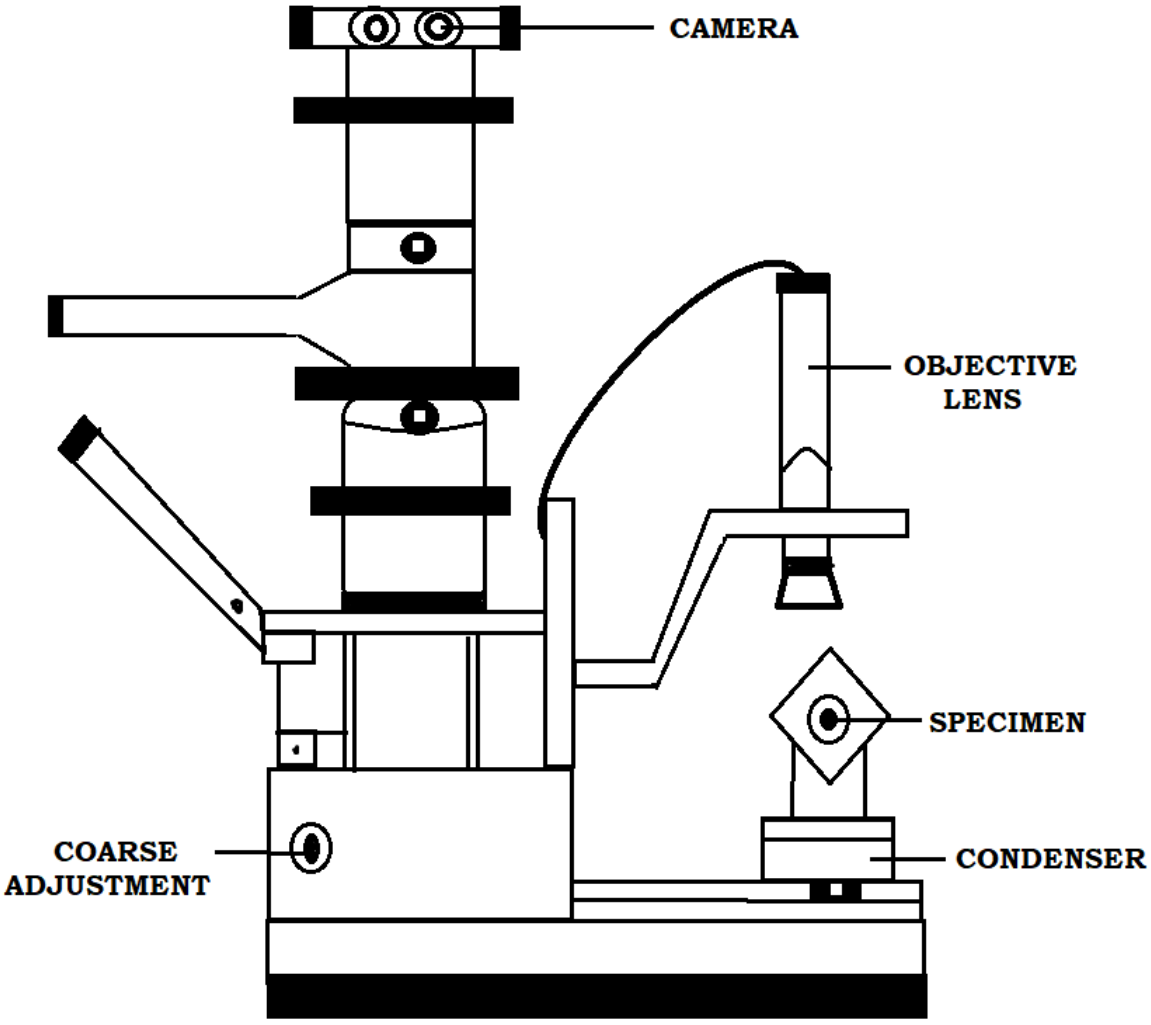
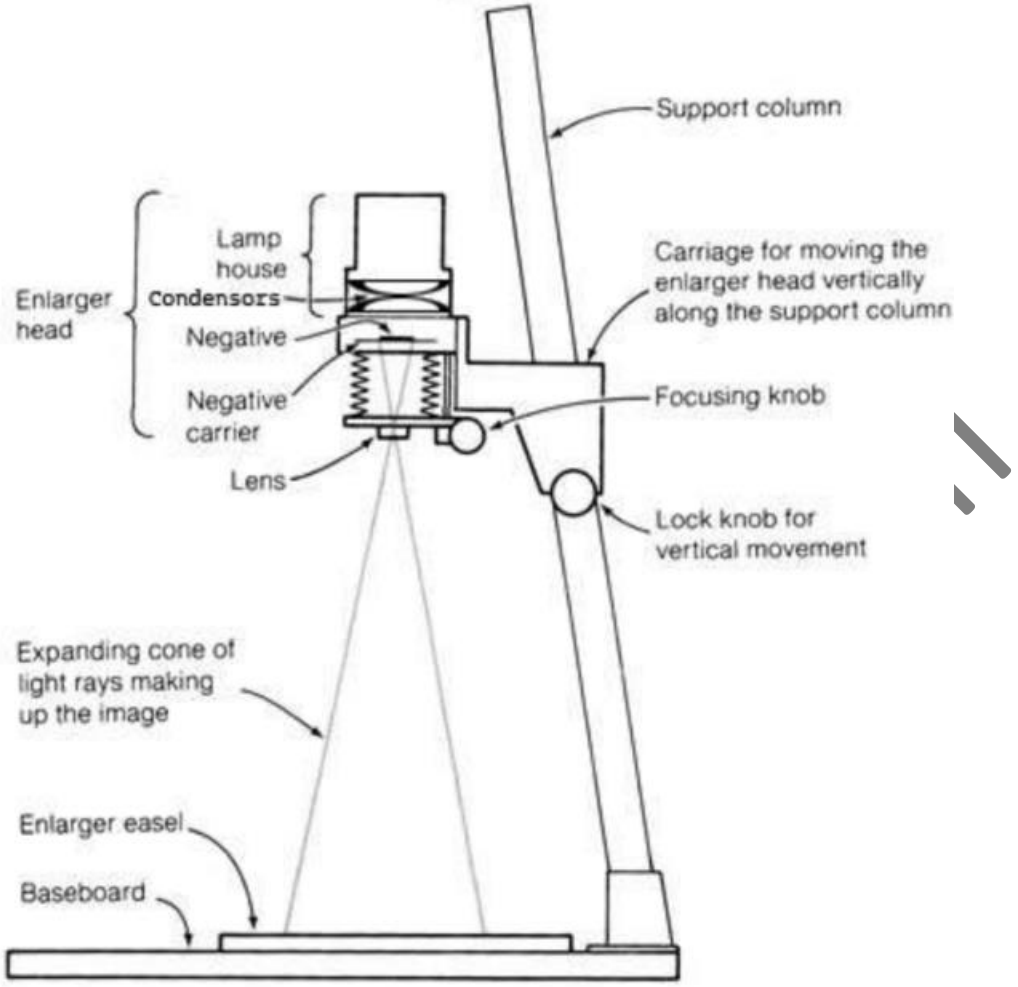
focussing eye piece is turned until the image on the reticle is in the sharp focus. Then the image is in to focus at the fine adjustment knob.

DETERMINING CORRECT EXPOSURE:

In all photography, the exposure **(E)** is determined by the light intensity (I) and exposure time **(T)** and **E=IT**, thus the exposure remains the same. Example: if the light intensity is half the exposure time and is simultaneously doubled in photomicrography, the intensity of the light that reaches the film is effected by many factors, this included transformer settings, which determines the voltage, reaching the illuminator bulb, any filter in the light path, the magnification of objective or ocular and focus, the nature of the specimen. Light intensity is controlled by changing transformer settings or filter in the light path.



MICROPHOTOGRAPHY



PHOTOGRAPHIC ENLARGER

FILTER:

To enhance the contrast in black and white photomicrography, we use the filter with colours that are complementary to that of the stained specimen, for the red and the purple stained cells and tissues a green filter is used. The green filter also minimises the effect of line aberrations since objectives are optically corrected in the spectral region.

PRINTING (Black and white wet process method):

35 mm film; Kodak technical pan film 24TS is an excellent 35mm filter for photomicrography. It has high resolving power and has extremely fine grades permitting the negative to be printed with considerable enlargement.

FILM PROCESSING:

All film contains two basic layers. There is a light sensitive emulsion, which is a layer of gelatine containing crystals of silver halides and a base which is a polyester or a cellulose acetate support surface. The silver halides are very light sensitive and even a small amount of light reduce some of the silver ions to metallic silver in small region of the crystal producing a greater visible image when the film is developed.

The developer, chemically reduce more of silver ions in the exposed crystal producing a grain of metallic silver. Following development, the film is fixed or made permanent with fixative which contains sodium hypo-sulphide and a chemical that hardens the gelatine coat. The sodium hypo-sulphide makes the image permanent by converting the exposed silver halide crystal to soluble compounds, which can be rinsed away easily.

PRINTING:

In this process, light is allowed to pass through the negative ion to the print paper to enlarge the image on the negative, a photographic enlarger is required. The main components of the enlarger are; lamp, filter, drawer, condenser or negative carrier and focusable lens system. When the negative is placed in the negative carrier and the enlarger lamp is turned on, the image is projected on to the print paper below. The print paper held flat is an enlarger placed on the base board. As light stirs the paper emulsion, a latent image is formed just as with film, a developer is required to convert the latent image to a visual image and fixed is required to remove the unexposed silver halide crystals making a permanent print.

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TOOL: 03

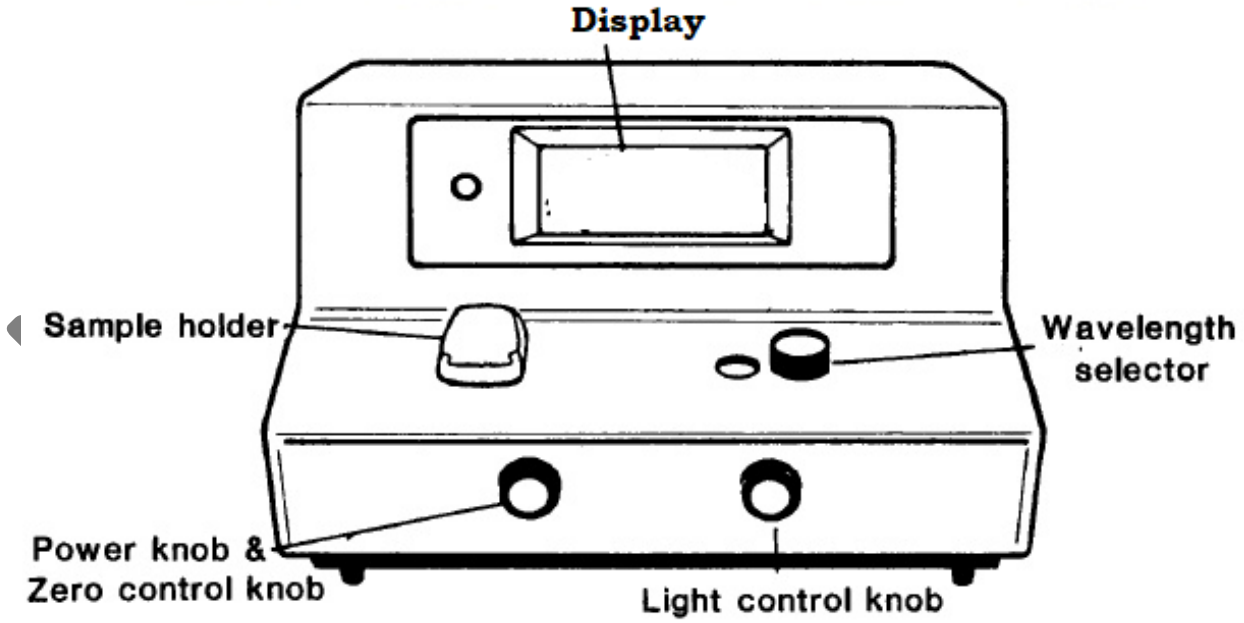
COLORIMETER AND SPECTROPHOTOMETER

COLORIMETER:

Colorimeter is a light sensitive device that measures the transmittance and absorbance of the light passing through a liquid sample. The device measures the intensity or concentration of the colour that develops upon introducing a specific reagent into a solution. Colorimetry is the study of colour in a relatively broad range of wavelength of visible light. There are two types of colorimeters;

1. Colour densitometer which measures the density,
2. Colour photometer that measures colour, reflection and transmission.

The main components of colorimeter are, a light source, a cuvette and photocell (coloured filter to generate colours). Colorimeter operates only in visible light of electromagnetic spectra. Colorimetry is the study of colour in a relatively broad range of wavelength of visible light.



COLORIMETER

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PRINCIPLE:

The basis of colorimetry in the relationship between the concentration of a substance in a solution and the colour intensity of that solution, the colour intensity being proportional to the concentration.

The intensity of absorbance of a colour depends on the chemical being used and the arrangement of the electrons and energy levels within the decimal. The colour we use in a solution is complementary to the colour absorbed by the chemical. When light pass through a solution, light of certain wavelength is absorbed by the coloured chemical substance (absorbance) and the remaining light is transmitted (transmittance).

TRANSMITTANCE AND ABSORBANCE:

When monochromatic light (light with specific wavelength) passes through a homogenous solution in a cell (cuvette), the intensity of emergent radiation depends on the path length (l) and the concentration (c) of the solution. Consider the figure where I_0 is the intensity of the incident light passing through a solution in a cuvette with a diameter (l) i.e. light path length and I_1 the intensity of the transmitted light. The ration of $\frac{I_1}{I_0}$ is the transmittance (T).

Transmittance $T = \frac{I_1}{I_0}$

Transmittance is often expressed in percentage; the percentage of transmittance (%T) is the difference between the intensity of incident light and that of the transmitted light and it is due to absorbance of some light by the solution in the cuvette.

Cuvette absorbance(A) is given by the $\log \frac{I_0}{I_1}$

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Absorbance $A = \log \frac{I_1}{I_0} = \log \frac{1}{T} = \log(100\%T)$

The relationship between absorbance of a solution, the concentration of the solute being measured and the path length is given by the Beer-Lambert's law which states that for a given path length, the concentration of a substance in solution provided that no other solute is absorbing at the specific wavelength. The Beer-Lambert's law is valid only for monochromatic radiation (radiation of single wavelength) and only where there is no change in the physical or chemical state of the solute. With changes in concentration, the Beer-Lambert law is written as;

A=KCl

Where, A is the absorbance (no units, since A= $\log \frac{I_0}{I_1}$)

K is the molar absorptivity with units of $mol^{-1}Lcm^{-1}$

C is the concentration of the compound in solution ($molL^{-1}$)

L is the path length of the sample, i.e. the diameter of the cuvette in which the sample is contained (in cm).

The Beer-Lambert's law lets us know that the absorbance depends on the total quality of the absorbing compound (concentration) and light path through the cuvette.

CONSTRUCTION OF COLORIMETER:

A colorimeter has the following basic components;

a) Illumination source,
b) Filter,
c) Sample handler (cuvette),
d) Detector,
e) Display.

LIGHT SOURCE:

Light in the visible range, a tungsten filament lamp is used as the source radiation. Tungsten lamps emit continuous radiation in the range of 350nm to 2500nm.

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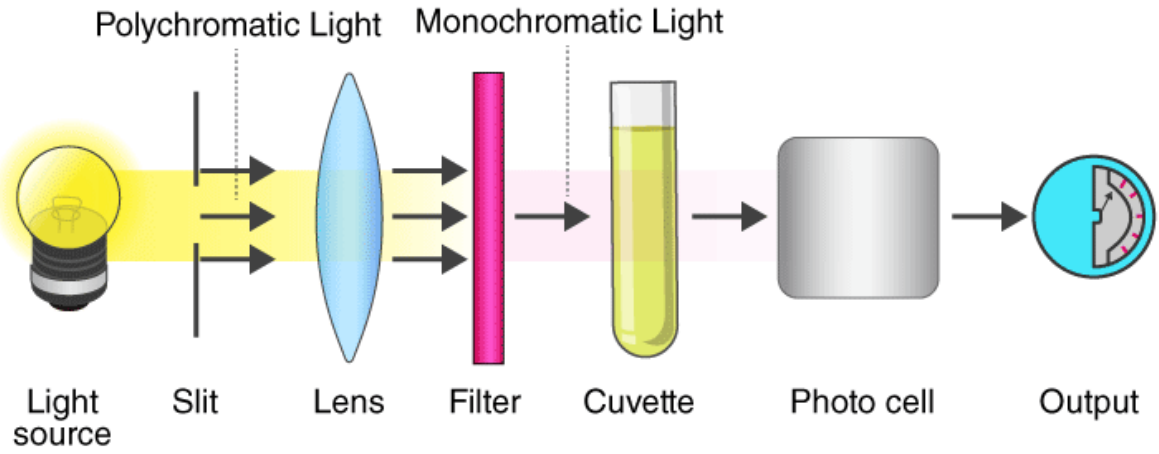


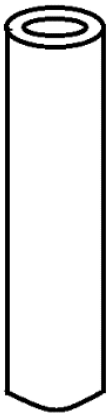
THE MAIN COMPONENTS OF COLORIMETER

FILTER:

A monochromator is required to select a specific wavelength of light. Filters are used for this purpose in colorimeters. A filter absorbs light of all the wavelengths except that of a specific band of wavelength. A filter may be thin gelatine layer stained with an organic dye held between two thin layers of glass or simply a tinted plane glass. The band width transmitted by filter is generally about 100nm to 150nm. Increasing the concentration of the dye in the gelatine or the tint of the glass may reduce the transmittance with band width.

CUVETTE:

The sample solution which is to be analysed for the absorbance (optical density), is contained in a tube i.e. cuvette of specific diameter (1cm) and made up of high-quality glass.



CUVETTE

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DETECTOR:

A detector of a colorimeter is a device that converts electromagnetic energy (light energy) into electrical energy, which can be measured using an appropriate ammeter. Hence the colorimeter is often referred to as photo-electric cell. A photo cell consists of thin film of silver coated on to the surface of semi-conductor material (ex: Cadmium sulphide, silicon and selenium) fitted onto a steel base. Electrodes are connected to the silver layer (anode) and the steel bas (cathode).

THE ABSORBANCE CURVE OF TWO DYES:

AIM:

To determine the absorbance curve of two dyes.

PRINCIPLE:

Coloured compounds have their own characteristic absorption spectra and careful selection of the wave lengths, where maximum absorption is found enables a mixture of two-coloured substances to be analysed.

MATERIALS REQUIRED:

a) Colorimeter with series of filter
b) Solution A (50mg/50ml distilled water)
c) Solution B (50mg/50ml distilled water)
d) An unknown mixture of 2 dyes.

METHOD:

i. Switch on the colorimeter for 20 minutes to stabilize and for the lamp to warm up to use.
ii. Select the first filter (minimum wavelength).
iii. Take distilled water in the cuvette and set the instrument to read zero absorption.
iv. Determine the extinct of each dye in turn against the range of filter supplied the instrument must be set to zero with distilled water in the cuvette for each filter.
v. Tabulate your results.

OBSERVATION:

Table showing the optical density of solution A and B at different wavelengths (of filter):

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	Filter wavelength (nm)	Optical Density (O.D)	
		Solution A	Solution B
01	400		
02	420		
03	480		
04	500		
05	520		
06	540		
07	620		
08	680		

A graph of optical density v/s wavelength is plotted (absorption curve).

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DEMONSTRATION OF BEER'S LAW

PRINCIPLE:

Beer's law states that, when a ray of monochromatic light passes through an absorbing medium, its intensity decreases exponentially as the concentration of the medium increases.

MATERIALS REQUIRED:

i. Colorimeter with a series of filter,
ii. Solution A (50mg/50ml distilled water)

METHOD:

i. Prepare a range of concentrations of two dyes by setting up a series of tubes as shown in the table.
ii. Select the filter that gives the maximum extinction of light path and set the colorimeter to zero with distilled water (Solution A had highest OD at _____nm and solution B has highest OD at _____nm respectively).
iii. Record the absorbance of each solution A and B respectively and plot against the concentration of dye in each tube (mg/ml).

RESULT:

i. The highest OD for solution A was observed at_____nm
ii. The highest OD for solution B was observed at_____nm

	Solution A (ml)	Distilled water (ml)	Solution A O.D at ____nm	Solution b O.D at ____nm
01	00	05		
02	01	04		
03	02	03		
04	03	02		
05	04	01		

A bar-graph is plotted based on the results tabulated.

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SPECTROPHOTOMETER:

A Spectrophotometer is an instrument that measures the number of photons (the intensity of light) absorbed after it passes through a sample solution. With the spectrophotometer, the amount of a known chemical substance (concentrations) can be determined by measuring the intensity of the light detected.

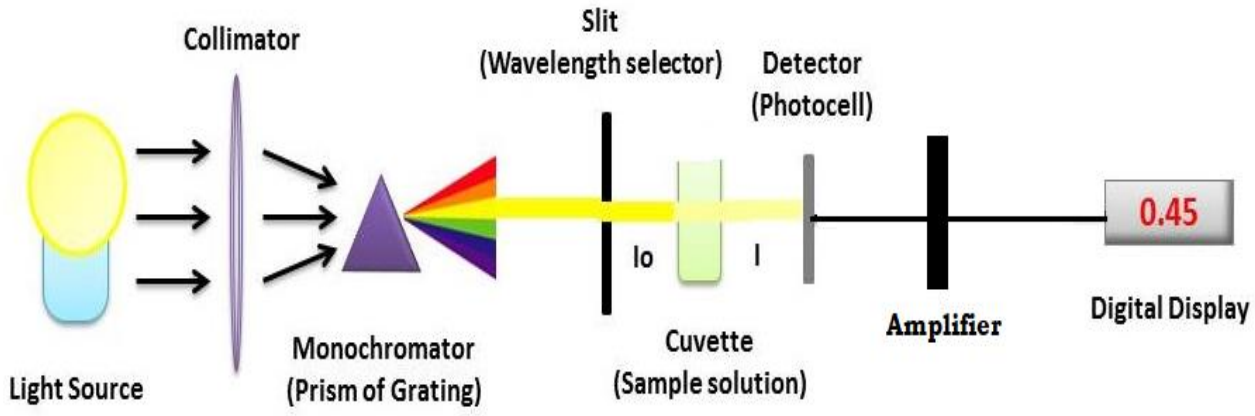
Spectrophotometry is the study of colour of a very narrow range of a specific wavelength in the UV, visible and IR regions of the spectra. Depending up on the range of wavelength of light source, spectrophotometers can be classified into;

a. UV-Visible spectrophotometer,
b. IR spectrophotometer.

CONSTRUCTION OF SPECTROPHOTOMETER:

A spectrophotometer consists of the following main components;

i. Radiation source,
ii. Monochromator,
iii. Sample handler (cuvette),
iv. Detector,
v. Amplifier,
vi. Display and recorder.



COMPONENTS OF SPECTROPHOTOMETER

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RADIATION SOURCE:

Since spectrophotometer are meant to measure absorbance of radiations in the visible, UV, IR regions of electromagnetic spectrum, different sources are provided for each. An incandescent lamp with tungsten filament which produces white light is used as the source of visible radiation in the wavelength range of 350-2500nm. A gas discharge tube which emits one or more wavelengths such as hydrogen lamp and deuterium lamp are the common source of UV radiation in the wavelength range of 180-350nm. Glow-bars of carborundum and Nernst glowers of rare earth oxides are commonly used sources of IR radiation. Glow-bars provides IR radiation in the band width range of 1-40mm and Nernst glower in the range 0.4-20mm.

MONOCHROMATOR:

In a spectrophotometer, a monochromator is used as the result of polychromatic radiation into its component wavelength to isolate them into narrow bands and focus a specific band into sample solution contained in the cuvette. A monochromator consists of:

a. An entrance slit through which a narrow beam of polychromatic light from the radiation source is admitted.
b. A collimating lens or mirror that directs the radiations as parallel beams on to the scattering device.
c. A prism for scattering the radiation into its component wavelength.
d. A focusing lens to focus the required specific narrow band of wavelength.
e. An exit slit through which the focused band is directed to pass through the sample solution.

A prism is made up of simple glass used for dispersion of light in the visible range. Fused silica or quartz or fluorite prism is used for dispersion of radiation in the UV region. Ionic crystalline materials such as NaCl, KCl and C_3Br are used for dispersion of radiation in the UV region. Ionic crystalline materials such as NaCl, KCl and C_3Br are used for dispersion of radiation in the IR region. The advantage of prism is that it is simple and inexpensive to make. Disadvantage is that the dispersion of light is angular, non-linear and that is temperature sensitive.

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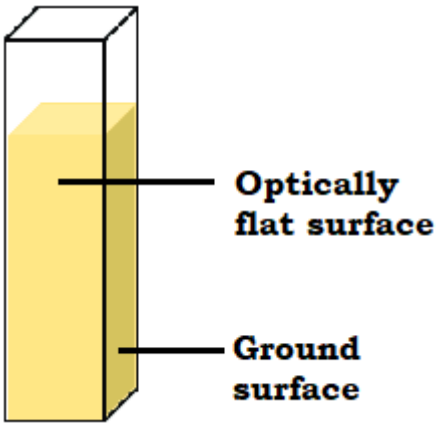
CUVETTE:



Containers referred to as cuvettes are made up of glass, quartz or fused silica and are used to hold the sample and reference solution in a spectrophotometer. Glass cuvette can be used in visible region but silica cuvettes are appropriate o measurements in the UV region. The cuvettes used in spectrophotometer are four sided containers out of which two opposite surfaces are precision grounded and polished to be optically flat for passage of radiation. The other two opposite surfaces are rough rounded and meant for handling. The radiation path length of the cuvette is 4cm.

DETECTOR:

The intensity of the radiation transmitted by the sample solution is measured with the help of a detector. The detectors used in spectrophotometer generally function on the principle of photoelectric effect. The photoelectric effect is the emission of electrons where light strikes the surface. The number of electrons emitted is proportional to the intensity of the radiation.

A common detector found in many spectrophotometers is a phototube. Two conducting electrodes, anode and cathode are enclosed in a evacuated glass tube, a source of potential difference creates an electric field in the direction from anode to cathode. Light falling on the surface of the cathode causes a current flow (flow of emitted photoelectrons as a result of photoelectric effect) in the external circuit. The current is being measured by a galvanometer.

DISPLAY AND RECORDING:

Manual spectrophotometers apply an analogue voltage output from the detector to a comparator circuit such as Wheatstone bridge or potentiometric recorder. In modern spectrophotometer the analogue voltage output is converted to digital data and fed into a computer and printer for display, recording and printout. The instrument itself may have an in-built computer for the operation and for giving printout of the results.

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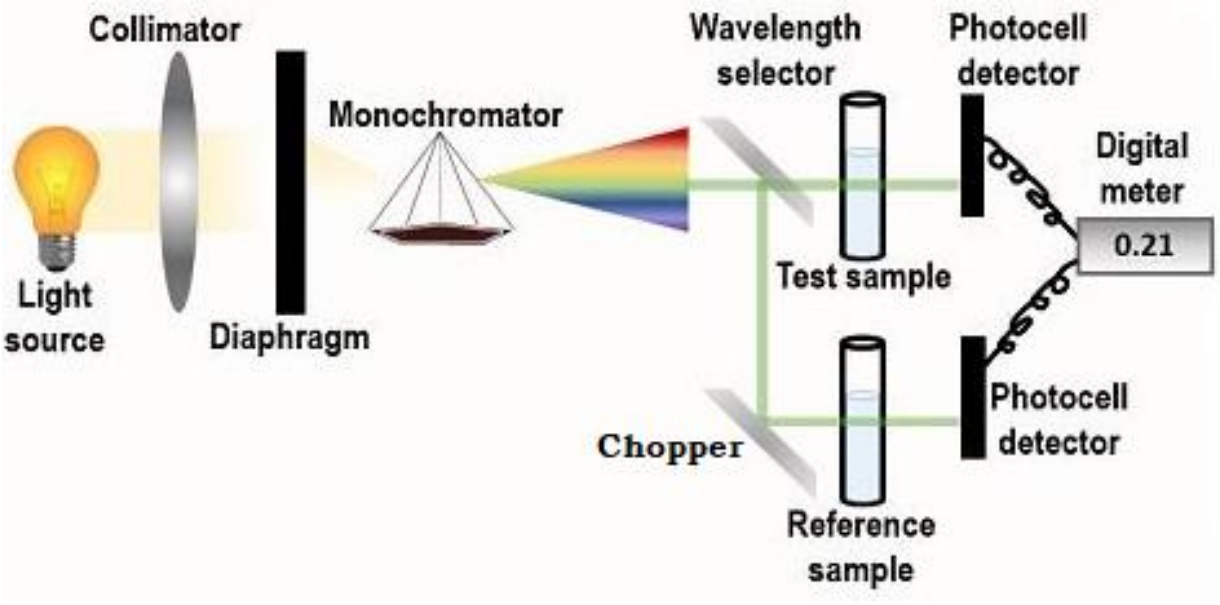
SINGLE BEAM SPECTROPHOTOMETER:

The single beam spectrophotometer is the simplest design in which we have to first measure the absorbance of the blank and then the absorbance of the sample using the same cuvette. In practice, wet set absorbance of the blank at zero and then read the absorbance of the sample. Modern single beam spectrophotometer has in-built electronic facilities to store the absorbance of the blank and to calculate the absorbance of the blank.

The main advantage of single beam spectrophotometer is that significant amount of time for the first reading (blank and reference) and the second reading (sample) is reduced. There can be problems with variation in the intensity of radiation (drift). However, modern days single beam spectrophotometers have better electronics and more stable lamps to overcome the problems of the earlier version.

DOUBLE BEAM SPECTROPHOTOMETER:

A design of the double beam spectrophotometer consists of a chopper alternately transmitting and reflecting the radiation so that it travels down the blank and the sample. The chopper causes the radiation to switch paths at about 50Hz enabling the detector to receive signals from the blank and the sample light paths alternately in short successions. These signals are processed electronically to calculate the ratio of the reference and the sample signals and to give either transmittance or absorbance as output.



DOUBLE BEAM SPECTROPHOTOMETER

SPLIT BEAM SPECTROPHOTOMETER:

The design of the split beam spectrophotometer is almost similar to that of the double beam spectrophotometer except that a beam of splitter is used in place of the chopper to direct the radiations along the reference (blank) and the sample paths. Thus, in the design, the measurement of both the blank and the samples take place at the same moment of time. The intensities of the transmitted radiation through the reference solution and the sample solution are measured by two different phototubes.

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MICROSCOPY:

AIM:

To know the principle and working of microscope, the following microscopes are to be studied:

1) Light Microscope (bright field microscope)
2) Phase Contrast Microscope
3) Fluorescence Microscope
4) Electron Microscope

LIGHT MICROSCOPE (BRIGHT FIELD MICROSCOPE):

PRINCIPLE:

The objective lens forms an enlarged seal image within the microscope and the eye piece lens further magnifies this primary image when one looks inside a microscope. The enlarged specimen image called the virtual image appears to lie just behind the stage about 25cm away. The total magnification is calculated by multiplying the objective and eye piece magnification together. E.g. 45x objective is used with 10x eye piece, the overall magnification of the specimen will be 450x.

CONSTRUCTION AND WORKING:

The microscope consists of a steady metal body or a stand composed of a base: an aim to which the remaining parts are attached. A light source either a mirror or an elective illuminator is located in the base.

Two focusing knobs, the fine adjustment and coarse adjustment are located at the arm and can move either the stage or the microscope nose piece to focus the image. The stage is positioned about half way up the arms and hold microscope slides by either simple slide clips or a mechanical stage clip.

A mechanical stage allows the operator to move a slide around smoothly during viewing by use of stage control knobs. The sub stage condenser is mounted within or beneath the stage and focuses a cone of light on to the slide. Its position often is fixed in simple microscope but can be adjusted vertically in more advanced models.

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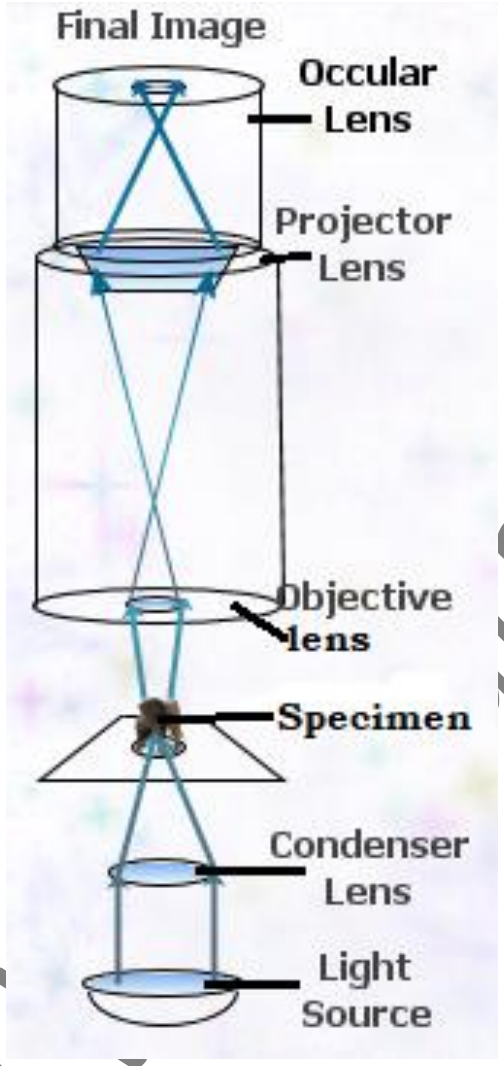


OPTICAL PATHWAY IN LIGHT MICROSCOPE

The curved part of the arm holds the body assembly to which a nose piece and one or more eye pieces or oculars are attached. Advanced microscopes have eye piece for both eyes and are called binocular microscopes. The body assemble itself contains a series of mirrors and prisms so that the barrel holding the eye piece may be tilted for the ease in viewing.

The eye piece holds three-five objectives with lenses of different magnifying power and can be rotated to position any object beneath the body assembly.

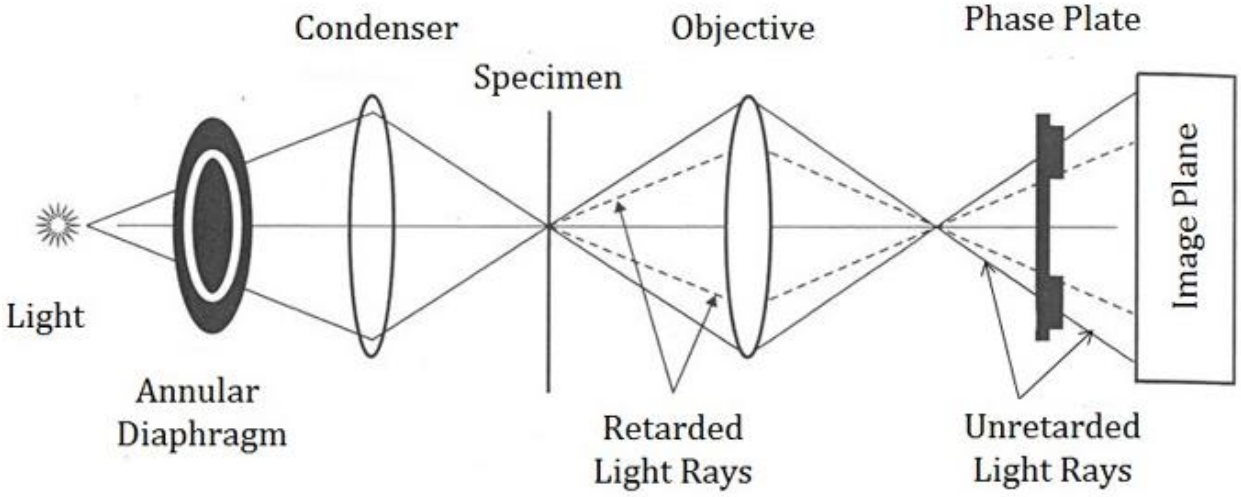
PHASE CONTRAST MICROSCOPE:

PRINCIPLE:

Phase contrast microscope converts slight differences in the refractive index and cell density into easily detected variations in light intensity and is excellent way to observe living cells.

The condenser of a Phase contrast microscope has an annular stop, an opaque disc with a thin transparent ring which produces a hollow cone of light. As this cone of light passes through a cell, some light rays are bent due to variations in the density and refractive index within the specimen and are retarded by refractive index within the specimen and are retarded by about 1/4th wavelength.

The deviated light is focussed to form an image of the object. The un-deviated light rays strike a phase ring in the phase plate or special optical disc located in the objective while the deviated rays miss the ring in the objective and pass through the rest of the plate.



OPTICAL PATHWAY IN PHASE CONTRAST MICROSCOPE

If the phase ring is constructed in such a way that the un deviated light passing through it is advanced by 1/4th wavelength,

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the deviated and un deviated waves will be about half (1/2) wavelength, out of phase and will cancel each other when they come together to form an image. The background formed by un deviated light is bright while the un stained object appears dark and well defined. Colour filters are often used to improve the image.

USES OF PHASE CONTRAST MICROSCOPE:

i. Study the microbial motility.
ii. Cytology and dynamics of live prokaryotes and eukaryotes.
iii. Detection of bacterial components such as endospores and inclusion bodies containing poly-β-hydrobutarate and poly-m-phosphate etc.
iv. For observation of materials such as lithographic patterns, fibres, latex depression, glass fragments etc.

FLUORESCENCE MICROSCOPE:

PRINCIPLE:

When some molecules absorb radiant energy, they become excited and later release much of their trapped energy as light. Any light emitted by an excited molecule will have a longer wavelength than the radiation originally absorbed. Fluorescent light is emitted very quickly by the emitted molecule as it gives up its trapped energy and return to more stable state.

The fluorescent microscope exposes a specimen to UV or blue light or violet light to form an image of the object with the resulting fluorescent light. A mercury vapour lamp produces an intense beam and heat transfer is limited by a special infused filter. The light passes through and exits filter that transmits only the desired wavelength. A dark field condenser provides a black background against which the fluorescent object glows.

Usually the specimens are stained with the die molecules called fluorochrome that show fluorescence upon exposure to light of specific wavelength. The stained specimen will appear to be fluorescent orange or green and can be detected even in the midst of the other particulate material.

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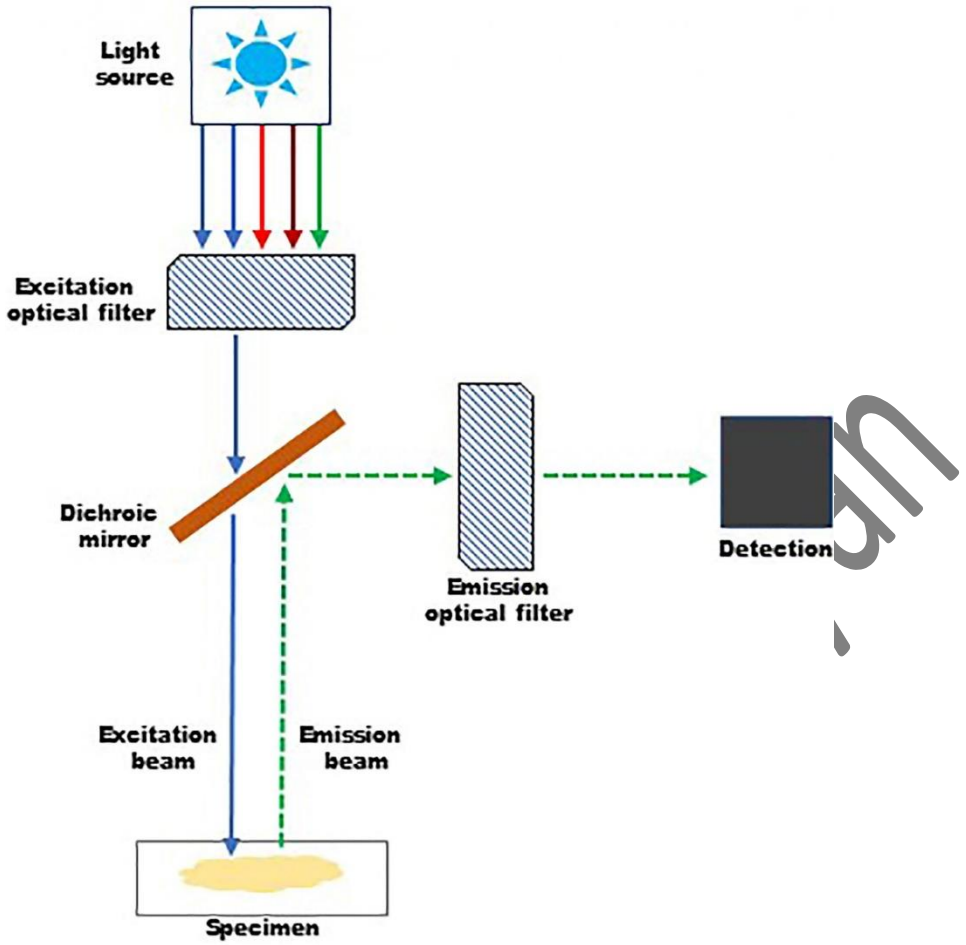


OPTICAL PATHWAY IN FLUORESCENCE MICROSCOPE

ELECTRON MICROSCOPE:

There are two types:

1. The transmission electron microscope
2. The scanning electron microscope

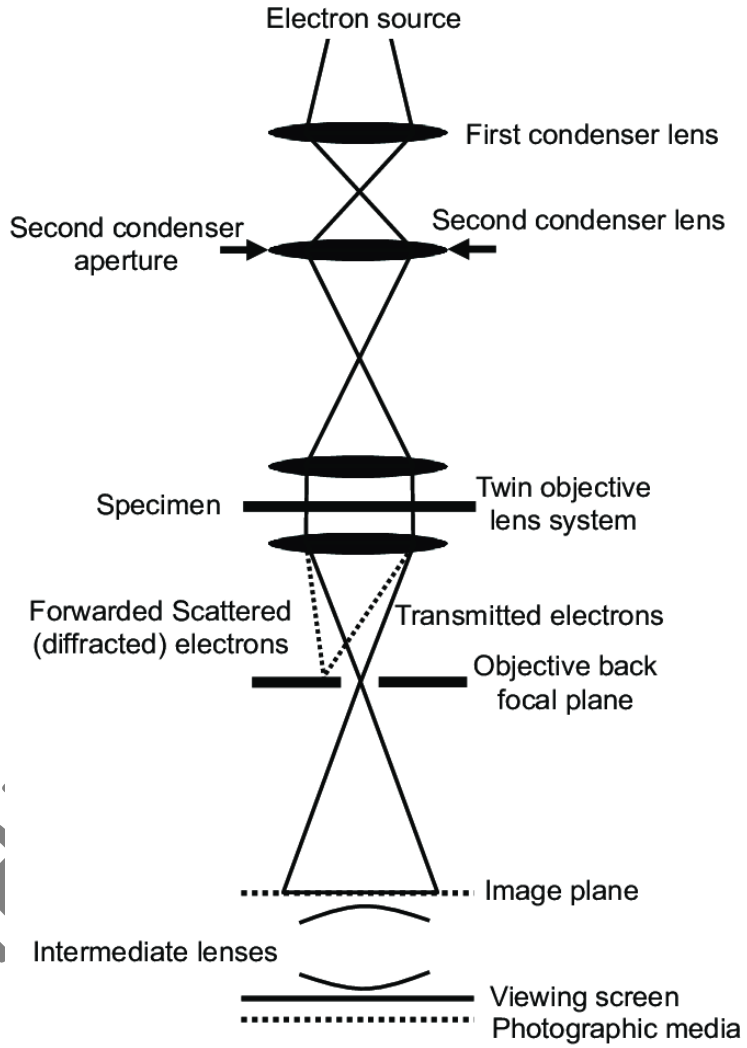
➢ TRANSMISSION ELECTRON MICROSCOPE:

PRINCIPLE:

A heated tungsten filament in the electron gun generates a beam of electrons which is then focused on the specimen by the condenser since electrons cannot pass through a glass lens,

doughnut shaped electro magnets called magnetic lenses are used to focus the beam.

The column containing the lenses and specimen must be under high vacuum to obtain a clear image, electrons are deflexed by collision within molecules. The specimen scatters electrons passing through it and the beam is focused by magnetic lenses to form an enlarged visible image of the specimen on a fluorescent screen.



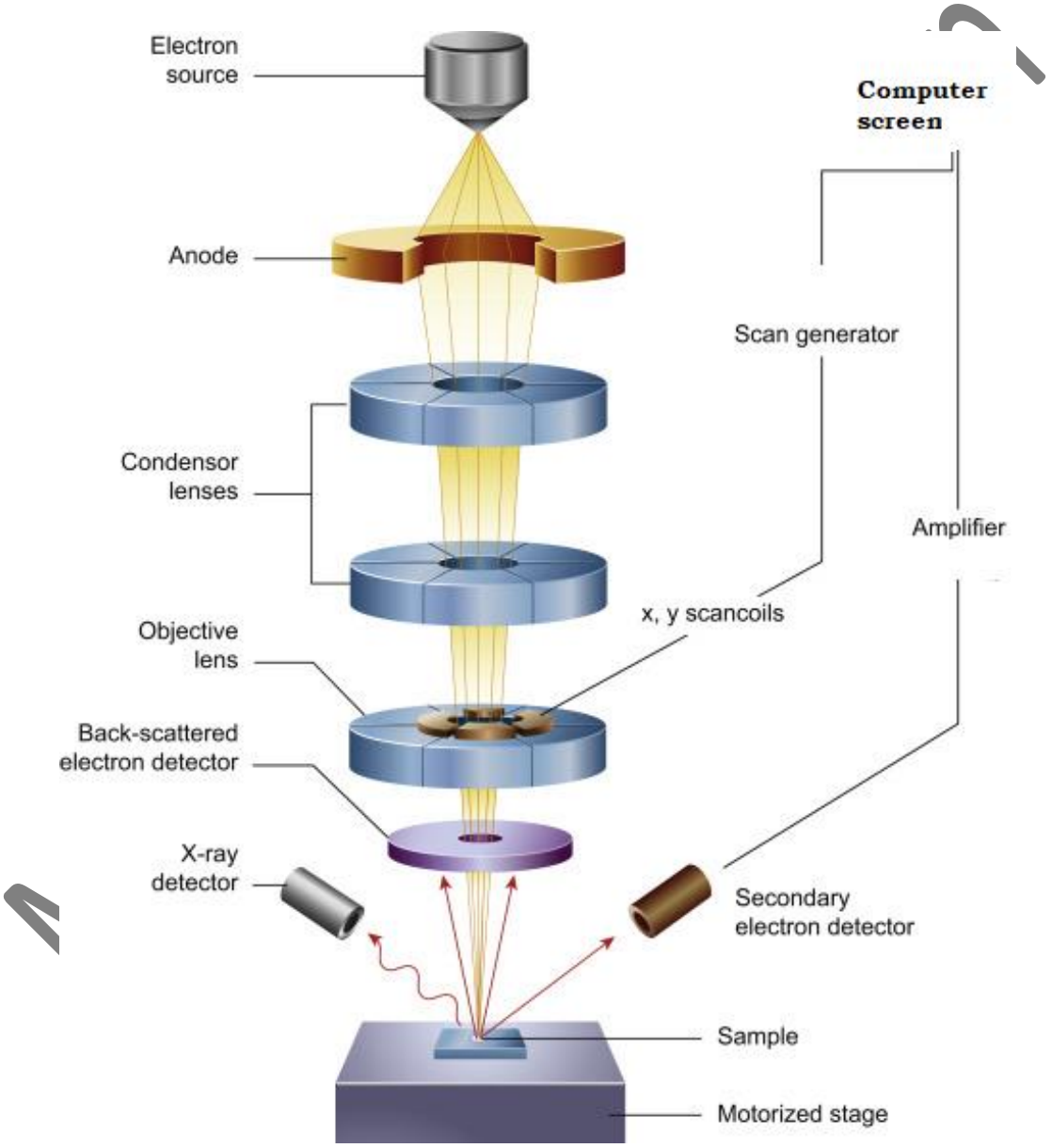
COMPONENTS AND OPTICAL PATH OF TRANSMISSION ELECTRON MICROSCOPE.

A denser region on the specimen scatter more electrons and therefore appear darker on the image. Since fewer electrons strike the screen, the contrast electron transparent regions are bright. The screen can also be moved aside and the image captured on the photographic film as a permanent record.

➢ SCANNING ELECTRON MICROSCOPE:

PRINCIPLE:

It is used to examine the surface of the specimen on great detail with a resolution of 7nm or less. The SEM produces an image from electron emitted by an object surface rather than from transmitted electrons.



COMPONENTS AND OPTICAL PATH OF SCANNING ELECTRON MICROSCOPE.

The SEM scans a narrow, tapered electron beam back and forth over the specimen. When the beam strikes aa particular area, surface atoms discharge a tiny shower of electrons called secondary electrons and these are trapped by a special detector. Secondary electrons emitted from the detector strike a scintillator causing it to emit light flashes a photo multiplayer that converts to an electrical current and amplifies. The signal is sent to a cathode ray tube and produces an image like a television picture which can be viewed or photographed. The number of secondary electrons reaching the detector depends on the nature of specimen surface. When the beam strikes a raised area, a large number of secondary electrons enter the detector, on contrast fewer electrons escape a depression in the surface and reach the detector. Thus, raised area appears lighter on the screen and depressions are darker. Thereby a realistic 3D view of the specimen can be viewed.

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SEPARATION TECHNIQUES:

- **CHROMATOGRAPHY (PAPER CHROMATOGRAPHY):**

Paper chromatography is an analytical technique for the separation of dissolved chemical substances by taking the advantage of their different rates of migration across sheets of paper. It is an inexpensive but powerful analytical technique that requires very small quantities of material.

INTRODUCTION:

It is a liquid-liquid chromatography which is based on the partition order between the two liquid phases. This technique employs a specially designed filter paper which absorbs water and holds between the cellulose fibre of the paper. This technique is primarily used for the separation of small molecular weight components like amino acids, monosaccharides, pigments etc.

PRINCIPLE:

It is partition chromatography in which the solvent molecules distribute between two liquid layers. The water layer which is held in the fibres of the paper serve as a stationary phase, often the liquid which is chosen for separation serves as mobile phase. Generally, the mobile phase is an organic solvent or non-aqueous mixture. When the paper comes in contact with the mobile phase, the solvent starts rising up by capillary action. When the solvent flows over the sample spots, the various components of this sample get separated between the stationary, move with less speed as compared with the constituents of mobile phase. The components to be separated move at different rates and appear as separate bands or spots or cones. Then he paper is dried and the spots are visualized by brushing with the visualisation reagents. The relative rates of the movement of the solvent and solute is expressed in terms of Rf value.

$$Rf = \frac{\text{Distance travelled by the pigment}}{\text{Distance travelled by the solvent}}$$

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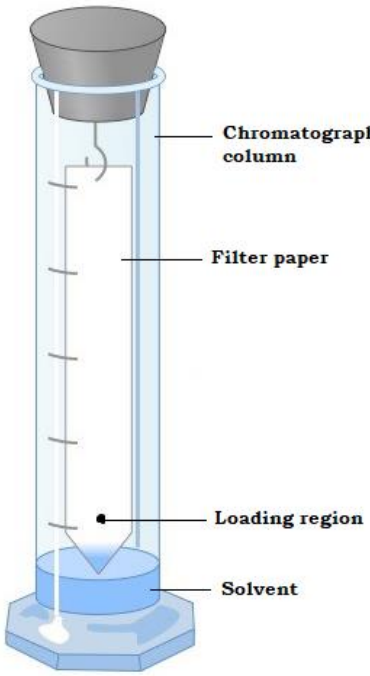
REAGENTS REQUIRED:

Acetone for the extraction of plant pigments and Propenol-petroleum ether mixture as solvent (4:96).

PROCEDURE:

Prepare the plant extract by grinding the leaves with the aid of mortar and pestle with the addition of acetone during the grinding process. Filter the extract by squeezing it through cotton cloth or filter paper.



ASCENDING PAPER CHROMATOGRAPHY COLUMN

Take a strip of Whatman's filter paper and make a small spot. On the spot, put a drop of filtrate of the plant extract and allow it to dry. Load 3-4 times by drying each time. Take 30ml of solvent in the chromatography column (tube), dip the spotted end of the paper in the solvent and cover the beaker with a lid or cork and allow the solvent to reach the top of the paper. Take out the chromatography and allow it to dry. Identify the different types of pigments and measure the distance travelled by the solvents and pigments on the chromatograph. Calculate the relative frequency (Rf) on the rate of flow value.

$$Rf = \frac{\text{Distance travelled by the pigment}}{\text{Distance travelled by the solvent}}$$

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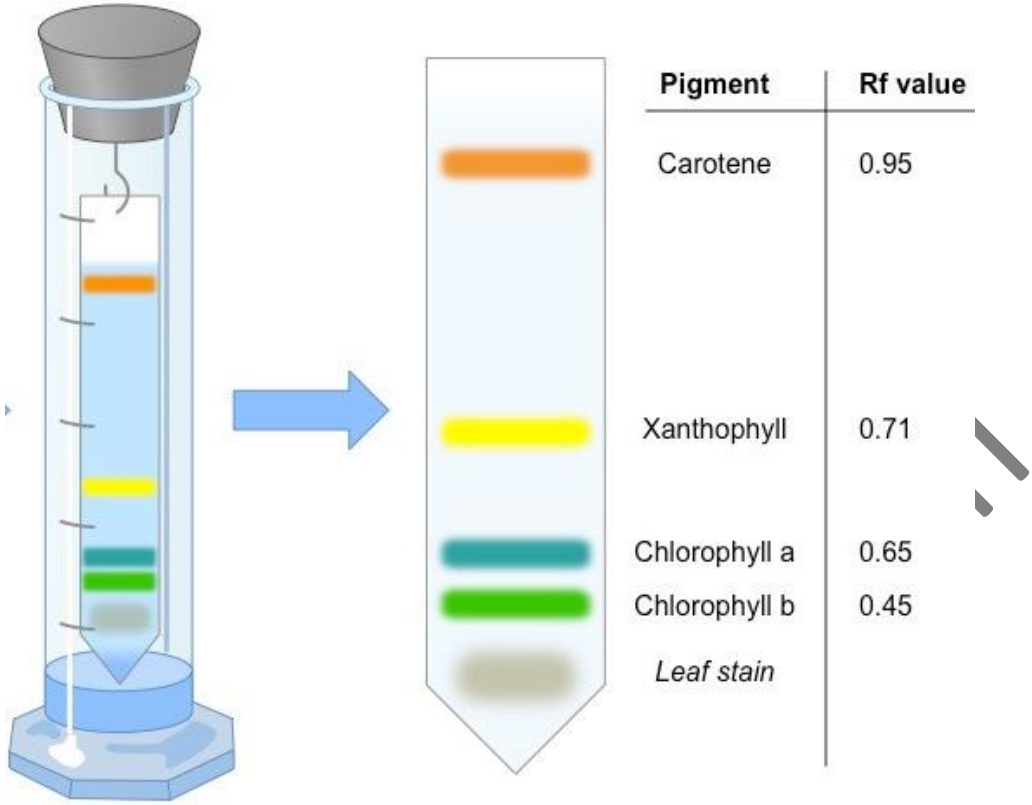

APPROXIMATE VALUES (Reference purpose)

APPLICATIONS OF PAPER CHROMATOGRAPHY:

- Paper chromatography is used to identify the inorganic compound containing ions like Ni^{2+}, Al^{2+}, CO^{2+}, Cu^{2+}, Fe^{3+} etc.
- It helps to separate, identify and isolate organic mixtures like acids, ketones, aldehydes.
- It is one of the versatile techniques to find the constituents of urine.
- It is used to study some bio chemical molecules like hormones, antibodies and enzymes etc.
- It is used to detect the adulterant and contaminants in drinks.
- It can also be used to separate drugs and to check the purity of pharmaceutical products and to analyse the constituents of cosmetics.

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• ELECTROPHORESIS:

AIM:

To study the principles and applications of electrophoresis technique.

INTRODUCTION:

Electrophoresis is the process of moving charged molecules in the media (solution or gel or paper) by application of electric field across the mixture because the molecules in an electric field move with a speed dependant on their charge, shape and size. Electrophoresis is simple and relatively rapid and is used chiefly for the analysis and purification of very large molecules such as charged sugar, amino acids, peptides, nucleotides etc. Nowadays, highly sensitive detection methods have been developed to monitor and analyse electrophoretic separation.

PRINCIPLE:

Electrophoresis is a Greek word meaning 'born by electricity'. The phenomenon of electrophoresis is mainly based on the principle of velocity (V) of a molecule. The electric field is directly proportional to its electrophoretic mobility (M) and electric field (E).

V=ME

Where,

V= Velocity of the molecules (cm/sec)

M= Electrophoretic mobility (cm2/volt/sec)

E= Electrical field strength (volt/cm)

Electrophoretic mobility of a particular molecule depends on its shape, size, surface charge, electrical field strength, and is influenced by several parameters like buffer, ionic strength, conductivity, temperature and velocity of electrophoretic medium.

The different times of electrophoretic mediums include cellulose-acetate, starch-gel, rocket immune electrophoresis, agar-gel electrophoresis, poly acrylamide gel electrophoresis (PAGE) etc. PAGE has been used as a potential medium for the separation of proteins and was first used by Davin. Acrylamide is a white

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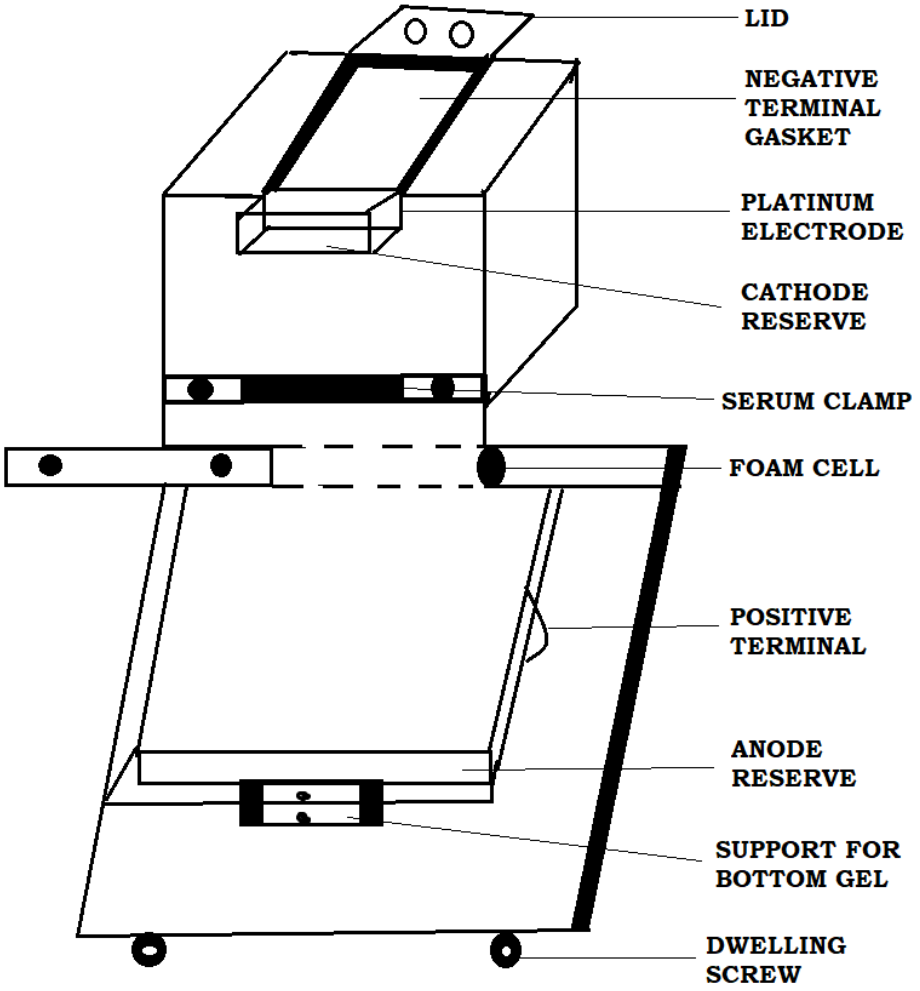
crystalline powder that is found as a major ingredient in the gel. It is a slow spontaneous process in which long chain of polymer is formed, which is most frequently used as cross-linking agent for PAGE.



POLYACRYL AMIDE GEL ELECTROPHORETIC UNIT

Polyacrylamide is a synthetic gel and is more stable, transparent, strong and relatively a good separator. It can be prepared to a wide range of sizes. It is possible to stain the gel after separation.

The charged particles migrate in the media towards the electrodes possessing opposite charge. The movement of charged particles are opposite under the influence of external electric field. This is the principle of electrophoresis.

The velocity of the molecule depends on charge, density, strength, electric field, temperature, nature of media or stored field. When a particle starts moving in an electric field and the functional forces oppose each other, the velocity of a given electric field (€) is given by

$$V = \frac{\triangle \in q}{6\pi rnd}$$

Where, q= charge, r= radius, d= distance between electrodes, n= viscosity.

FACTORS AFFECTING MOVEMENT OF MOLECULES:

The electrophoretic mobility directly depends on the charge of the sample, higher the charge greater is the electrophoretic mobility. The rate of migration depends on the size of molecules. The buffer can affect the electrophoretic mobility if it is able to bind with the components of sample being separated. The ionic strength of the buffer action alters the electrophoretic mobility of the components.

MATERIALS REQUIRED:

EQUIPMENTS: Power pack, electrophoretic unit, micropipette.

REAGENTS REQUIRED:

1. 30% acrylamide
2. 0.8% bisacrylamide solution
3. 4% resolving buffer (1.5M) (tris-chloride ^{P}H 8.8)
4. 4% stacking gel buffer (0.5M) (tris-chloride ^{P}H 6.8)
5. 10% SDS (Sodium Dodecyl Sulphate)
6. 10% ammonium sulphate (ammonium persulphate)
7. 2% treatment buffer (0.125M Tris-chloride)
8. 4% SDS
9. 20% glycerol
10. 0.2M DTT (Dithiothreitol)
11. 0.2% bromophenol blue ^{P}H 6.8
12. Tank buffer (0.025M tris, 0.192 glycine, 0.1% SDS ^{P}H 8.3)

STAINING SOLUTION:

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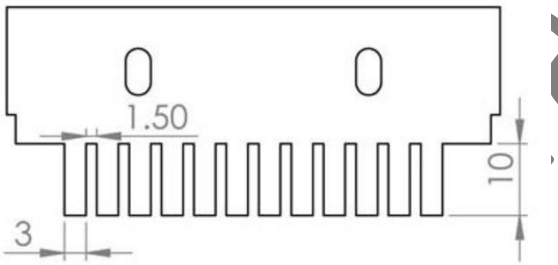
1. Coomassie brilliant blue (CBB) 0.025%
2. 40% methanol
3. 7% acetic acid
4. Stain solution (40% methanol, 7% acetic acid)
5. Preserving solution (7% acetic acid, 5% methanol).

PROCEDURE:

Assemble the two glass plates with the spaces to form a gel holder, pour the naming gel into a glass plate to a marked area. Add a layer of butanol over this and allow to polymerise. It takes approximately 40-45 minutes.



ELECTROPHORESIS COMB

Remove the butanol layer and wash the gel surface with distilled water. Place the comb carefully on the top of the gel and slowly pour the staining gel. Add a drop of butanol at the margin and allow to polymerise which takes about 40 minutes.

Remove the comb carefully and rinse the well with distilled water. Place the gel plate assembly inside the electrophoretic unit by using large dips, carefully add electrode buffer into the lower chamber without any air bubbles.

Introduce a small volume concentration of protein till the remaining volume in each well is equalised. Make sure that all wells have equal concentration of protein and rest of the upper chamber with power pack and run the gel in electric field for approximately 30-40 minutes at 300V and 30M ampere.

When marks (BPB) have reached less of the bottom margin, cut off the current supply. Carefully separate the gel from the plate

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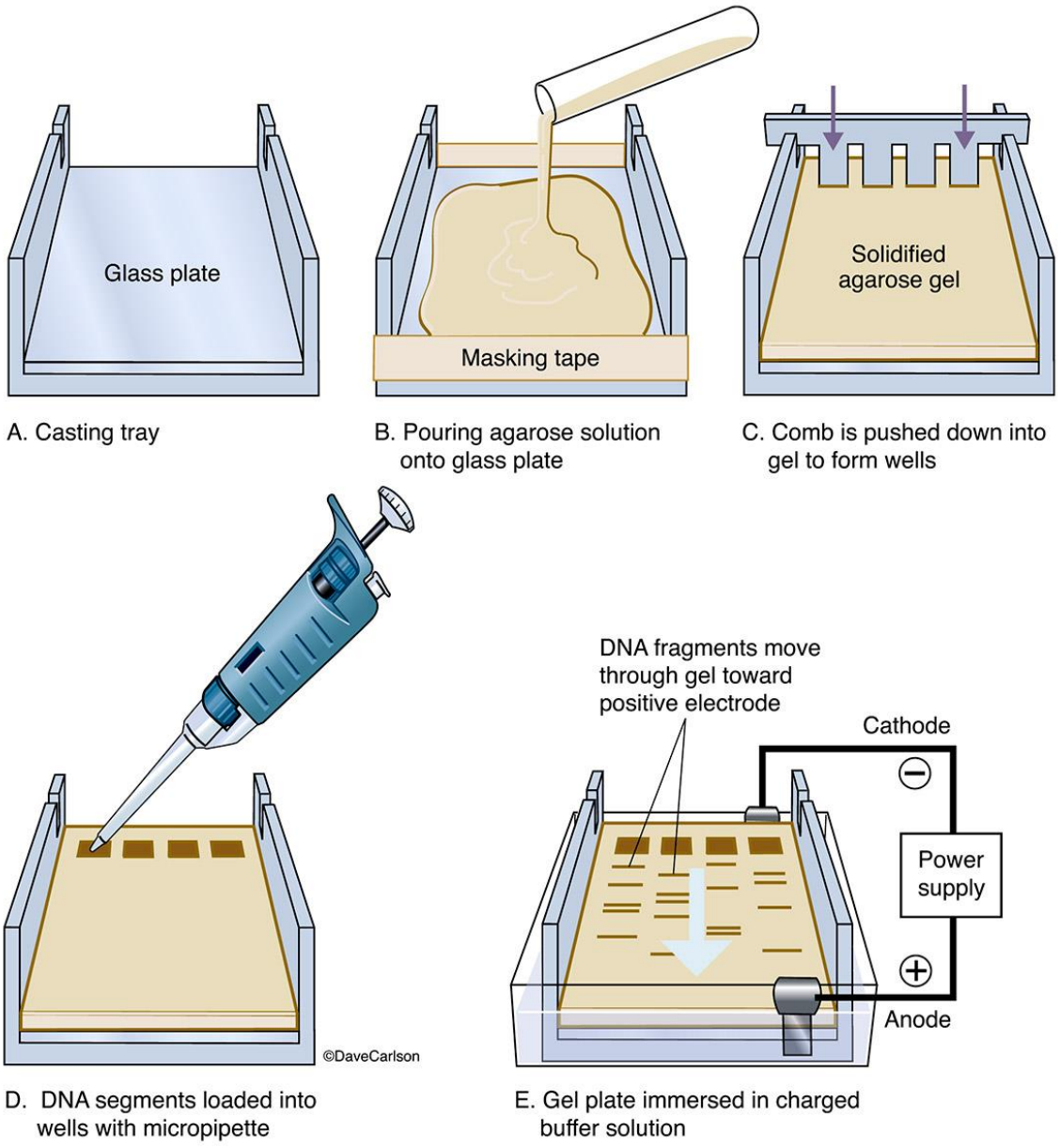
and transfer it to gel fixature for 15 minutes. Place the gel in the stain for 3 minutes. De-stain the gel overnight or even longer.



APPLICATIONS OF ELECTROPHORESIS:

a. The paper electrophoresis is used in clinical laboratory for preparation and quantification of serum protein, lipo-protein and haemoglobin.
b. Th separation and quantification of enzymes like creatinine kinase, lactate dehydrogenase etc are done using paper electrophoresis.

c. It is also used to separate components in immunological assay which is called immune electrophoresis.
d. SDS-PAGE technique is used to find the number of sub units present in the protein.
e. SDS-PAGE method is a versatile method for separation and analysis of protein, small RNA molecules and very small DNA molecules or fragments.
f. Gel electrophoresis is used for the separation and determination of relative molecular weight of macro molecules like protein, DNA etc.
g. Agarose electrophoresis can be utilised to determine the sequence of DNA.
h. Agarose electrophoresis can also be used to find out the presence of mutation in DNA or RNA (southern and northern blotting).
i. Western blotting technique is used to identify the presence of antibodies.
j. Iso electric focusing technique is used in food, agricultural industries, forensic and human genetic laboratories.

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BLOOD TYPING/BLOOD GROUPING

AIM:

Determination of ABO blood group and Rh factor.

INTRODUCTION:

Karl Landsteiner in 1900 discovered blood groups in man. In human beings with respect to blood cells there are two antigens, A and B and two serum antibodies that agglutinate them. They are grouped into four groups A, B, AB, and O based on the presence or absence of antigen on the plasma membrane of Red blood cells (RBC). Those with antigen A belong to blood group A and they have anti-B, antibody in blood serum. Those with antigen B belong to blood group B and have anti -A. Those with both A and B antigen belong to blood group AB where both antibodies are absent. Those with neither antigen belong to blood group O, have both types of antibody.

PRINCIPLE:

It is based on the principle of agglutination. The antigen-antibody reaction that occurs between a particular antigen {agglutinogen} and specific antibody {agglutinin} leads to the clumping or agglutination of cells. When the cells involved are erythrocytes the reaction is termed as HAEMATAGLUTINATION.

Normally human erythrocytes will clump or agglutinate when mixed with anti- A or anti-B[antisera]. If the cell membranes of erythrocytes possess antigen-A, agglutination is seen with anti-A and for antigen -B, agglutination is seen with anti -B.

Rh factor is identified using anti-D serum. On adding anti-D, if agglutination is seen it indicates a +ve result for Rh and is designated Rh^{+ve} and otherwise Rh^{-ve}.

MATERIALS REQUIRED:

a. Blood samples,
b. Antisera A, B and D,
c. 70% alcohol,
d. Micro slides,
e. Sterilised lancet,
f. Cotton,

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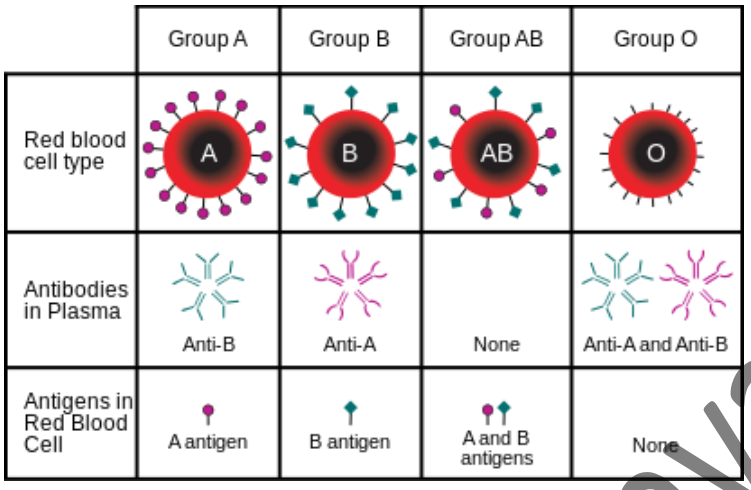
g. Applicator sticks and
h. Surgical spirit.



PROCEDURE:

1. Take a clean dry 3 cavity microslide and label the cavities as A, B, and D.
2. Sterilize the fingertip with cotton dipped in 70% alcohol or surgical spirit and allow it to dry [surface sterilization].
3. Prick the disinfected area of the finger with a sterile lancet.
4. Squeeze the fingertip and allow a drop of blood to fall into each cavity area of the slide labelled as A, B and D.
5. Add one drop of each antisera A, B and D into the respective cavities without touching the dropper tip to the blood sample.
6. Using separate applicator sticks mix the antisera and blood droplet in a circular motion
7. Swirl the slide for about two minutes.
8. Observe for the presence of agglutination reaction and record the blood group.

If the antigen-antibody reaction is not clear, the slide can be observed under the microscope for confirmation.

INTERPRETATION:

a. Agglutination of erythrocytes indicates a positive result for haemagglutination.
b. No agglutination of RBCs indicates a negative result.
c. Agglutination in first cavity, with anti – A confirms blood group A.

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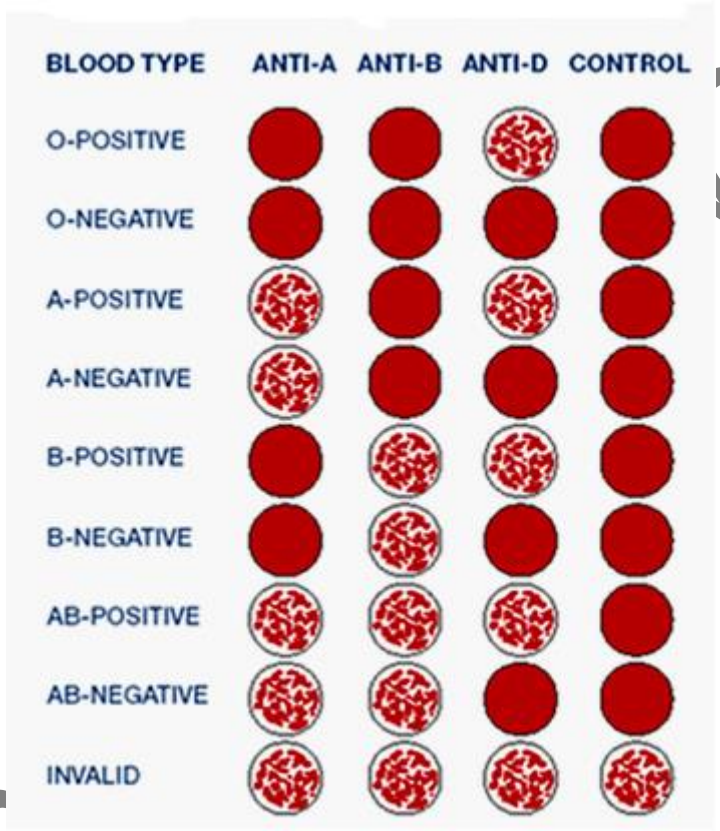
d. Agglutination in second cavity, with anti – B confirms blood group B.
e. Agglutination in both the cavities (with anti A and anti B) confirms blood group AB.
f. Lack of agglutination in both the cavities confirms blood group O.
g. Agglutination in third cavity, with anti D indicates Rh^{+ve} result and lack of agglutination confirms Rh^{-ve}.



RESULT INTERPRETATION

OBSERVATION:

i. Agglutination observed in cavity /s -----.
ii. No agglutination observed in the cavity/s -----.

RESULT:

The given blood sample was assigned to the group ------.

MICROTOMY

PART A

The study of histology requires the basic knowledge of histochemical. There are two ways of investigating the minute structure of tissue namely, the direct absorption of cell and making of permanent dead cells. To examine microscopically it is necessary that the processing of material producing little changes and preparation of material is extreme and staining is required.

TISSUE FIXATION:

The objectives of fixation are:

1. To prevent the post mortem changes in tissue,
2. Prevent cellular components as they are without distortion and shrinkage.
3. To prevent biochemical changes
4. To alter the refractive index of tissue for better observations.

Fixation ensures the various tissue constituents remain as in living state. There is no universal fixative and HCHO is more frequently used fixative. Various molecules of cell use different fixatives like,

1. Bovine's fluid (for general histological fixation)
2. HCHO-calcium fixative (for liquids)
3. Carnoy's fixative (for nucleic acid, carbohydrates and proteins).

PREPARATION OF BOVINE'S FLUID:

Bovine's fluid can be prepared by using 75ml of saturated solution of picric acid + 25ml of HCHO + 15ml of glacial acetic acid.

DEHYDRATION:

Tissue must be dehydrated which would otherwise hinder further processing such as embedding the tissue in the series of alcohol. This reduces the water content of the tissue. Th time required for each step depends on the size of the object (30 minutes-1 hour).

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CLEANING:

It is an intermediate step between dehydration and embedding. The cleaning agent (xylene) removes the opacities and makes the tissue transparent because the alcohol used for dehydration will not dissolve or mix subsequent embedding medium (paraffin wax). Also helps to remove the traces of alcohol and acid which might have entered through cell system by diffusion. The tissue must be immersed in fluid with both xylene and paraffin (1:1).

INFILTRATION:

During fixation and washing, the tissue lacks consistency for the sectioning of material. Tissue becomes soft. To prevent this, the tissue cells are filled inter cellularly (infiltration and impregnation) and enclosed extracellularly (embedding) with medium paraffin. Universally used media are paraffin and nitrocellulose.

EMBEDDING OR BLOCK MAKING:

The tissue is placed in a small container (L-block) which is already filled with paraffin and the same is cooled rapidly in water. Before transferring the tissue, warm the instrument used for transfer to prevent paraffin spillage on metal surface and to avoid air bubbles. Handle the tissue and paraffin as rapidly as possible to prevent solidification of the paraffin before tissue is oriented. The tissue is placed in a known position and carefully worked with a strip of paper in hardening the paraffin. Hardened paraffin blocks are stored in cool place to avoid melting.

SECTIONING:

The hardened paraffin blocks with material are mounted on the reader of the microtome for the sectioning. Sections are taken 5-6µ thickness after making necessary adjustments in the microtome. The sections are taken and are placed on a slide pre-smeared with egg albumin. The prepared slides are ready for staining and for histological details.

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PART B:

AIM:

To fix the given tissue for histological and histochemical studies.

MATERIALS REQUIRED:

Wax, L-blocks, spirit lamps, hot air oven, alcohol etc.

PROCEDURE:

Dissect out the required tissue from the given animal and transfer the tissue to bovine's fluid for fixation for 24 hours. Wash the tissue in 50% alcohol or distilled water with two changes of 5-10 minutes to remove excessive fixative. Transfer the tissue for dehydration in 70%, 80%, 90% and absolute alcohol for half an hour to one hour in each grade depending upon the hardness of the tissue. Later move the tissue to xylene- paraffin mixture (1:1) for half an hour for removal of excess alcohol. The material is transferred to fresh paraffin wax (58-60°C) for two-three changes of five-ten minutes in each.

The embedding of tissue material in molten paraffin wax is done by proper circulation of tissue material into the box smeared with glycine, is allowed for hardening. Now the material is ready for sectioning for which the embedded material is cut into required size and is fixed on the rider of microtome, sections of 5-6µ thickness are obtained.

The sections were transferred onto the slide previously smeared with egg albumin. Add 1-2 drops of distilled water to the slide with section and excess of water is removed and the slides are dried. These dried slides are used for different histological staining.

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IMMUNOLOGICAL TECHNIQUES:

➢ ELISA: ENZYME LINKED IMMUNO-SORBENT ASSAY.

ELISA which is abbreviated as Enzyme Linked Immuno-Sorbent Assay is an immune-technique which was invented by Peter Pearlman and Eva Engvall (Swedish scientist) in 1971. The purpose of ELISA is to determine a particular protein present in the given sample.

Different types of ELISA are;

a) Indirect ELISA,
b) Sandwich ELISA,
c) Competitive ELISA.

In the above types, sandwich ELISA is most commonly used for the protein detection.

PROCEDURE:

ELISA is performed in 96 well ELISA plates which permits high performance throughout the process. The bottom of each well is coated with a protein to which the antibody will bind.

Whole blood is allowed to clot and these cells are centrifuged to obtain the clear serum with antibodies called primary antibodies. The serum is incubated in the well and each well contains a different serum.

A positive control serum and a negative control serum would be induced among 96 samples being tested. After a while, the serum is removed and weakly adhered antibodies are washed off with a series of buffer rinse to detect the bound antibodies. The secondary antibody is added to each well. The secondary antibody may bind to all human antibodies and is typically produced in the rodents. Attached to the secondary antibodies are enzymes like peroxidase or alkaline phosphatase. The enzyme can metabolize colourless substrate into coloured product after an incubation period and the secondary antibody solution is removed and loosely adhered ones are washed off as before.

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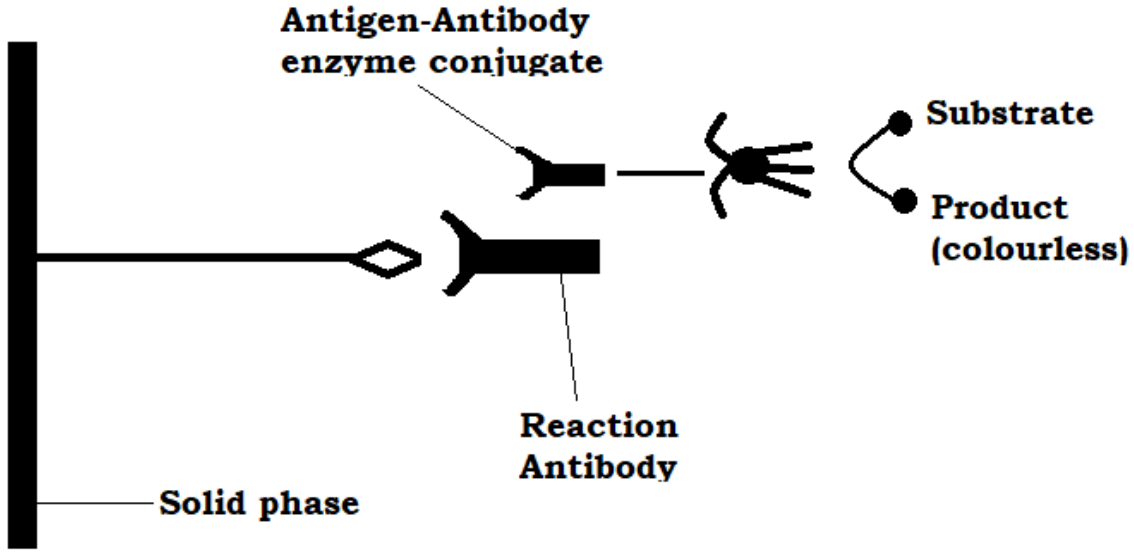
The final step is the addition of the enzyme substrate and the production of coloured products in wells where secondary antibodies are bound when the enzyme reaction is complete. The entire plate is placed into a plate reader and the optical density (O.D) is determined for each well.



ELISA

The amount of colour produced is proportional to the amount of primary antibodies bound to the proteins on the bottom of the wells.

ADVANTAGES:

- ELISA is a bio-chemical technique used mainly in immunology to detect the presence of an antibody or antigen in the sample.
- ELISA is being used as a diagnostic tool in medicine and plant pathology.

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➢ RIA: (RADIO IMMUNO-ASSAY)

RIA is abbreviated as Radio Immuno-Assay. The RIA technique was introduced in 1960 by Berson and Yalow and for the first time the hormone levels in the blood could be detected by invitro-assay.

PROCEDURE:

1. A mixture of radioactive antigen (known value) is prepared and mixed with unknown amounts of unlabelled (cold) antigen. These compete for the binding site of the antibodies.
2. At increasing concentrations of unlabelled antigens, an increasing amount of radioactive antigen is displaced from the antibody molecules.
3. The antibody bound antigen is separated from the free antigen in the supernatant fluid from which the radioactivity of each is measured.
4. For the obtained data, a standard binding curve can be drawn.
5. The sample to be assayed runs parallel.
6. After determining the ration of bound to free antigens in the unknown, the antigen concentration can be read directly from the standard curve.

ADVANTAGES:

RIA has become a major tool in the clinical laboratory where its used in the analysis of

i. Plasma level of most of our human hormones
ii. Digoxin in patients receiving drugs
iii. Certain abused drugs
iv. For the presence of Hepatitis-B surface antigen (HBS-Ag) in donated blood
v. Anti-DNA antibodies in systemic lupus erythematosus
vi. RIA is widely used because of its great sensitivity
vii. RIA is used in clinical practices in many areas like blood bank, diagnosis of allergies and endocrinology.

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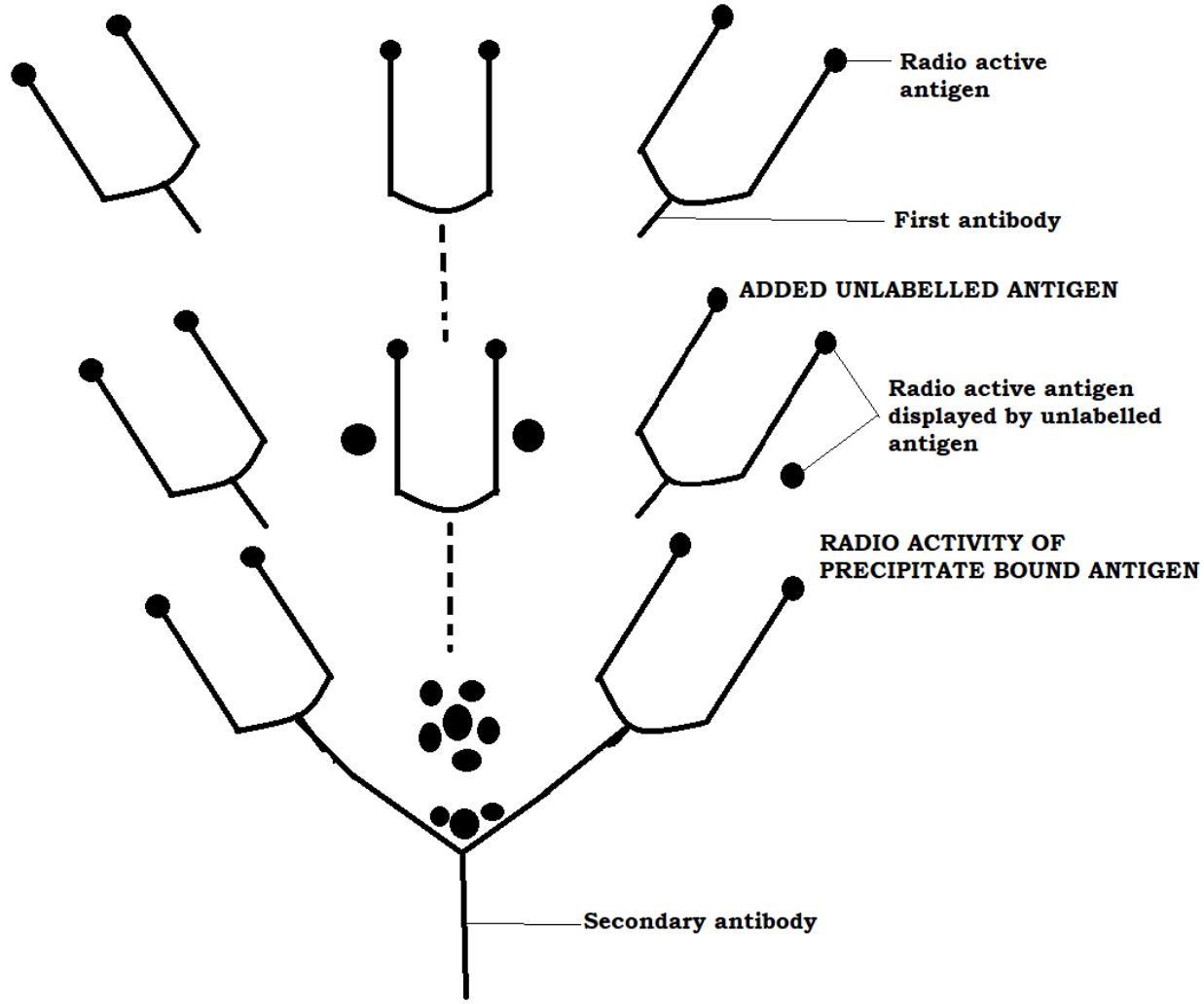
DISADVANTAGES:

The main disadvantages or drawbacks of RIA are the expenses and hazards while preparing and handling the radioactive antigens.



RADIO IMMUNO ASSAY

COMPUTER APPLICATIONS:

I. MICROSOFT EXCEL:

Excel is a software which falls under the general category known as spreadsheet. A spreadsheet is highly interactive computer program that consists of a collection of rows and columns that are displayed on screen in scrollable window. The intersection of each row and column is called a cell. A cell can hold a number, text or formulas that perform a calculation using one or more other cells. It is easy to copy cells, move cells and modify any formulas you create.

In addition, groups of numeric cells can be used to generate charts and maps. Excel files are normally called as workbooks, which consists of many spread sheets. The sheets can be worksheet, chart sheet, macro-sheet or custom dialog boxes.

FEATURES OF MS-EXCEL ARE;

1. It includes very powerful calculating features with the ability to work with both numbers and text. Data can also be presented graphically which helps in variety of data analysis.
2. It includes a number of database functions, which allows us to work with data in a tabular form.
3. The user interface provided is user friendly. Common operations such as copy, cut and paste can be easily performed.
4. The facility of dynamic data exchange (DDE) and object linking and embedding (OLE) allow excel to interact with a number of other windows applications for the purpose of exchanging data between them.
5. Excel maintains consistency in file concept. All data can be gathered in workbooks. This concept allow the user to function easily with a number of different types of worksheer stored in a single place.

SPREAD SHEET BASICS:

It is the main document that is used in excel. A worksheet is made up of rows and columns. Rows are numbered whereas columns are named with alphabets.

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There are 65,536 rows and 256 columns. Columns are named from A to 70N in a worksheet. The intersection of a row and a column is called a cell. Every cell is identified by unique cell address which includes the column alphabet with row number (Ex: A_{11}or G_{36}). The cell which is currently in use is shown with a thick rectangular box, this is called as the active cell. A workbook is the file in which data is stored, every workbook is saved with the extension of X^{15} (10^{15}).

ENTERING DATA IN A WORKSHEET:

Since a worksheet consists of cells, the data in a worksheet has to be enclosed with one cell at a time. The arrow keys are used to move from one cell to another, the tab key or the mouse can also be used for the same purpose.

All text data are called labels, if a label is too long to fit in the cell, the text will be spilled over to the next cell. If empty in the other cases, excel displays as much of the label as the column width will allow but stores the complete information into the cell. In case of long numbers, excel displays the information in scientific form or the sign in the cell.

Move the pointer over to the cell, in which the data needs to be entered and click the mouse. The cell becomes active, type the data, mistakes can be corrected by using the backspace key and to store the data into the cell and move the next cell, press enter or use ➡ ⬇ ⬆ ⬅ arrow keys.

TYPES OF DATA IN EXCEL:

A cell can hold any of the types of data listed below;

a. Values or numeric data,
b. Text or character data (labels),
c. Formulas.

i. **Values or numeric data:** Numbers can include numeric character from 0-9 and certain other characters such as +, -, (), /, $, % etc.

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ii. **Text or character data:** Non-numeric text in some of the cells, one can insert text to serve as label for values, headings of column or to provide instructions about worksheets. Text that begins with number is still considered text. Ex: if you enter a cell, excel considers this to be text rather than a value.

iii. **Formulas:** These are what makes a spreadsheet powerful. Excel lets you enter powerful formulas that use the values of cell to calculate a result. When you enter a formula into a cell, the formula is evaluated and the result appears in the cell. If you change any of the values used by the formula, the formula re-calculates and shows the new result.

Formulas are entered in the worksheet cell and must begin with an equal sign (=). Calling the cells by just their column and rows lead labels (such as A) is called relative referencing. When a formula contains relative reference and it is copied from one cell to another, excel does not create an exact copy of the formula. It will change cell address relative to the row and column they are moved to i.e. if a simple addition formula in cell **C**" **=(A_1+B_1)**, is copied to cell C_2, the formula would change to "**=(A_2+B_2)** to reflect the new row. To prevent this change, cells must be called by absolute referencing and this is accomplished by placing dollar sign **($)** within the cell addressing the formula.

Containing the previous example, the formula in the cell C_2 should be the sum of cell A_1 and B_1. Both the column and row of both cells are absolute and will not be changed. When copied, mixed, referencing can also be used where only the row or column is fixed. Example; in the formula "**=[A$$_1$+B$$_2$],** the row of cell A_1 is fixed and the column of cell B_2 is fixed.

BASIC FUNCTIONS:

For example, if you want to add the values of cell **D_1** through **D_{10},** you would type the formula **=D_1+D_2+D_3+D_4+D_5+D_6+D_7+D_8+D_9+D_{10}.** A shorter way would be to use the sum function and simply type **=sum (D_1:D_{10}).** Several other functions are listed in the adjacent table.

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Sl. NO:	FUNCTION	EXAMPLE	DESCRIPTION
01	**SUM**	Sum (A1:A100)	Find the sum of all the cells of A1 through A100
02	**AVERAGE**	Average (B1: B10)	Find the average of cells of B1 through B10
03	**MAX**	Max (C1:C100)	Retains the highest number of cells through C1-C100
04	**SQRT**	Sqrt (D10)	Find the square root of value in cell D10
05	**TODAY**	Today []	Retains the current data {leave the parentheses [] empty}

DRAWING CHARTS:

Charts allow you to present data entered into the worksheet in a visual format using a variety of graph types such as column, bar, line, pie, xy (scattered), area, doughnut, bubble, surface, stock etc.

DATABASE:

Database is collection of organized information. A database might contain employees, customers or inventory seconds. A second correspond to a worksheet row. Each record contains a collection of data elements or fields that corresponds to columns. Excel allows a user to hold up to, 65,586 rows. Excel cells can hold up to 32,767 characters. Excel limits the user to sort levels at any point of time.

FILTERS:

Excel offers the powerful database functions which is a big improvement i.e. one search criteria using data forms. Excel also offers 2 options called auto filter and advance filter.

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II. SPSS: STATISTICAL PACKAGE FOR THE SOCIAL SCIENCE:

SPSS is a software package used for statistical analysis produced by SPSS INC which was acquired by IBM in 2009. The current versions (2015) are official named as IBM SPSS statistics. The software name originally stood for Statistical Package For The Social Science.

SPSS is widely used program for statistical analysis in social science. It is also used by market researchers, health researches, survey companies, government education researchers, marketing organisations, data management sections and data documentation branches.

SPSS statistical package is one of the most popular statistical packages which can perform highly complex data manipulation and analysis with simple instructions. It is frequently used in social science. SPSS has four windows namely data editor, output viewer, syntax editor and script window.

STATISTICS INCLUDED IN THE BASE SOFTWARE:

1. Descriptive Statistics- Cross tabulation, frequencies, descriptive, explore, descriptive ratio statistics.
2. Bivariate Statistics which means t-test, ANOVA, co-relation (bivariate, partial distances) and non-parametric tests.
3. Prediction for Identifying Groups- factor analysis, cluster analysis (two steps hierarchal) and discriminant.
4. Prediction for Numerical Outcomes- linear regression.

SPSS statistics places analyses internal file structure, data types, data processing and matching files which together considerably simplify programming. SPSS data sets have two-dimensional table structure where the rows typically represent cases (such as individuals) and the columns represent measurements (such as age, sex, income etc). Only two data types are defined i.e. numeric and string or test. All data processing occurs sequentially through the files. Files can be matched one to one and one to many, but not many to many.

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The graphical user interface has two views which can be tagged by clicking on one of the two lists in the bottom left of the SPSS statistics window. The data view shows a spreadsheet view of cases (rows) and variables (columns). Unlike the spreadsheet the data cells can only contain numbers or texts and formulas cannot be found or stored in these cells. The variable view displays the meta data dictionary where each row represents a variable and shows the variable, name, variable labels, print, width, measurement types and a variety of other characteristics. Cells in both views may be edited manually defining the file structures and allowing the data entry without using command syntax. This may be sufficient for small data, larger data sets such as statistical surveys are more often created in the data entry software and extorted during complete assisted personal interviewing by scanning and using optical character recognition and optical marks recognition software or by direct from another online questionnaire. These data sets are then read into SPSS.

SPSS statistics can read and write data from an ASC 2 text files including hierarchal files and other statistical package spreadsheets and data bases. SPSS statistics can read and write to external relation data base tables via ODBC and SQL.

Statistical output is to a proprietary file format (SPV file, supporting pivot tables) for which in addition to the in-package viewer a stand alone reads can be downloaded. The proprietary output can be exported to text or Microsoft word, pdf, excel and other formats. Alternatively, output can be captured as data, text, tab-delimited text, XLS, HTML, XML, SPSS data set or a variety of graphic image formats like JPEG, PMG, BMP, EMF etc.

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III. HISTOGRAM:

A histogram is a graphical representation of the distribution of numerical data. It is a estimate of the probability distribution of a continuous variable and was first introduced by Karl Pearson.

To construct a histogram, the first step is to "bin" the range of values i.e. divide the entire range of values into a series of intervals and then count how many values fall into each interval. The lines are usually specified as consecutive, non-overlapping intervals of a variable and the lines (intervals) must be adjacent and usually equal in size.

If the bins are of equal size, a rectangle is erected over the axis with height proportional to the frequency. The number of cases in each bin in general, however bins need not be equal in width, in that case the erected rectangle has area proportional to the frequency. But density, the number of cases per unit of the variable on the horizontal axis, a histogram may also be normalized displaying relative frequencies.

Histogram then shows the proportion of cases that fall into each of several categories with the sum of the heights equalling. Examples of variables in bin width are displayed on census or bureau data below. The adjacent bins leave no gaps, the rectangular form of a histogram touch each other to indicate that original variable is continuous.

Histograms are often confused with bar charts. A histogram is used for continuous data where the bins represent ranges of data and the area of the rectangle are meaningful while a bar chart is a plot of categorial variables and the discontinuity should be indicated by having gaps between the rectangles from which only the length is meaningful. Often this is neglected which may lead to a bar chart being confused for a histogram.

CREATING THE HISTOGRAM USING EXCEL:

i. Open the Microsoft excel.
ii. Enter the data you want to be in a histogram in a row and in a different row enter appropriate bins.
iii. Click on the tab menu and select the data analysis.

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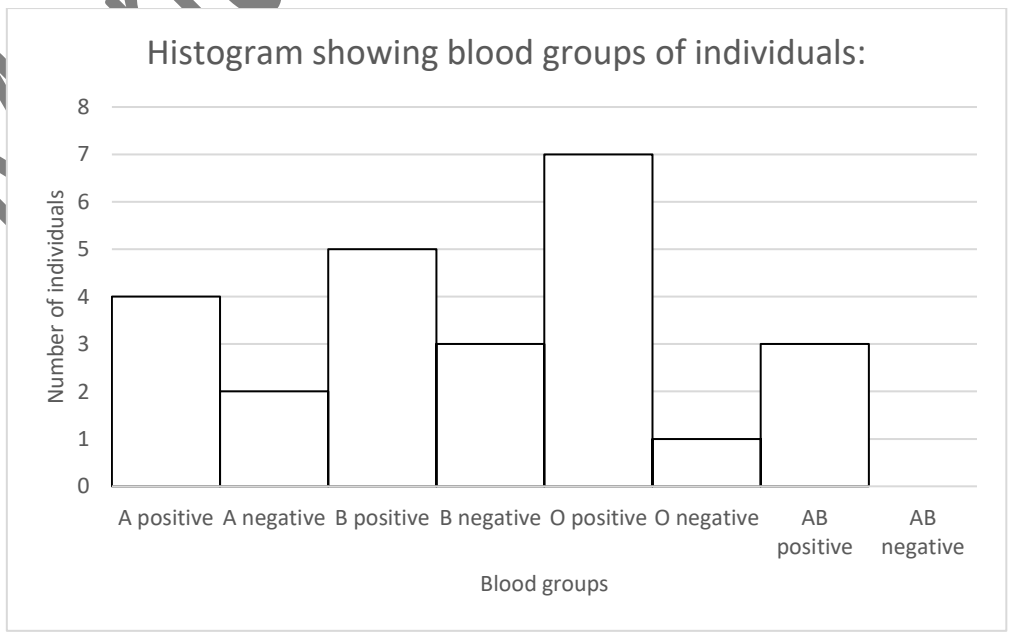
iv. Click on histogram to select the histogram option and click OK.
v. Select the input range (your data), then reset bin range.
vi. To be sure, your x-axis is labelled as bin, click on series tab- Category (x)- Labels (change the label if needed).
vii. Check the chart output box.
viii. Click ok.
ix. Finished.
x. Take print out if needed.

DATA:

Sl. NO:	BLOOD GROUP:	NUMBER OF INDIVIDUALS:
01.	A +ve	4
02.	A -ve	2
03.	B +ve	5
04.	B -ve	3
05.	O +ve	7
06.	O -ve	1
07.	AB +ve	3
08.	AB-ve	0

[Note: A Histogram is plotted by using the above data]



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IV. MINITAB

Minitab is a statistical package developed at Pennsylvania State University by researcher Barbara. F. Rayan, Thomas A Rayan, JR and Brian L Joiner in 1972. Minitab began as light version of omni tab, a statistical analysis package by NIST. The documentation for omni tab was published in 1986 and there has been no significant development since then.

Minitab is distributed by Minitab INC, a privately owned company headquarter in State College, Pennsylvania with subsidises in conveniently i.e. in UK (Minitab Tel) Paris, France (Minitab Sau) and Sydney, Australia (Minitab STY).

Today Minitab is often used in conjugation with the implementation of six sigma (MMI) and other statistics-based process improvement methods.

Minitab-17, the latest version of the software is available in eight languages i.e. French, English, German, Japanese, Korean, Portuguese, Simplified Chinese and Spanish. Minitab INC produces two other products that compliment Minitab-17. They are:

- Quality trainer cleaning package that teaches statistical tools and concepts in the context of quality improvement that integrates with Minitab-17 to simultaneously develop the user statistical knowledge and ability to use the Minitab software.
- Quality Companion 3, an integrated tool for managing in-sigma and learn manufacturing projects that allow Minitab data to be combined with project management and governance tools and documents.

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V. GPS (GLOBGAL POSITIONING SYSTEM)

The Global Positioning System (GPS) is a space-based navigation system that provides location and time, information in all weather conditions anywhere on or near the earth where there is an unobstructed sight to four or more GPS satellites. The system provides critical capabilities to military, aviation and commercial uses around the world.

The US government created this system, maintains and makes it freely accessible to anyone with a GPS receiver. The US began GPS project in 1973 which overcame the limitations to previous migration system, integrating ideas from several predecessors including a number of classical engineering designs studies from 1960s.

The US department of defence developed the system which originally used 24 satellites, which became fully operational in 1995. Roger L Easton of Naval Research Laboratory, Ivan A Getting of Aerospace Corporation and Bradford Parkinson of the Applied Physics Laboratory are credited with inventing it.

Advancement in technology and new demands on existing systems have now led to efforts to modernize GPS and implement the next generation operational control system.

In addition to GPS, other systems in use are under development. The Russian Global Navigation Satellite System (GLONASS- Global Navigation Satellite System) was developed complimenting with GPS but suffered from incomplete coverage of the globe until the mid-2000s. There are also plans from European Union Galileo positioning system for developing a positioning system. India's Indian Regional Navigation Satellite System and Japanese Quasi Zenith Satellite Systems are other positioning systems developed.

APPLICATIONS:

The free global availability and accuracy of GPS signals for positioning and timing, combined with the low cost of receiver chip sets has made the GPS preferred solution for a variety and wide range of civilian applications which include-

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- Road transport
- Aviation
- Shipping and rail transport
- Science and security
- Heavy vehicle guidance
- Surveying, mapping and geo physics
- Telecommunication
- Financial services
- Social activities.

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