(vi) ***Organic acid :***

Formic acid	-	Utrica dioca (stinking nettle)
Tartaric acid	-	Grapes and Tamarind
Citric acid	-	Lemon, orange
Oxalic acid	-	***Oxalis*** (Khatti Buti)
Mullic acid	-	Leaves of Gram, Apple fruits

(2) ***Reserve materials :***

(A) ***Starch grains*** - starch occurs as stored food in plants cells.

⇒ Starch grains are either round (wheat pea) or elliptical (potato) . But starch grain which occur in late are double shaped.

⇒ Polyphedral starch grains occur in Maize endosperm

⇒ ***Canna*** have largest starch grains (1/100mm) while smallest starch grains occur in **rice** (1/200 mm) .

✡ One the basis of position of hilum starch grains are of two types

(1) ***Concentric*** - in wheat endosperm and cotyledons of pea.

(2) ***Eccentri*** - Potato and Rice.

⇒ In rice and beet root plant starch grains have more than one hilium such starch grains called compound

(B) ***Proteins :*** In endosperm and cotyledones of several plants proteins occur as stored food in form of grains. Protein grains called ***Aleurone grains.*** Cereal endosperm have a layer of cells having aleuron grains called ***Aleurone layer.***

(C) ***Hermicellulose*** - Hemicellulose is a part of cell wall. Shining white colour of Date and coconut endosperm is due to high conc. of hemicellulose.

⇒ However hemicellulose occurs as stored food in Ivory platm (***Phytelophas***) This hemicellulose is used to **manufacture artificial ivory** and **billiard balls.**

(D) ***Inulin*** - Inulin is a soluble homopolysaccharide composed of fructose units. It occues as stored food in roots of **Dahalia** and ***Artichoke.***

⇒ (Glycerrhiza provides non saccharifer sweetners)

(3) Secretory material :

These occur in special gland and sacs. These includes encymes, colouring material, Nectar.

VACUOLE

Discovered by Spallanzani

Vuoles are generally absent in animal cells and meristematic cells in plants. Cell of permanent tissue in plant have well developmed vacuoles.

acuoles are surrounded by an unit membrane called ***tonoplast.*** Inside vacuole there is a nonloving fluid called cell ap. cell sap contains water, salts, sugars, organic acids, Vitamins and waste materials of metabolism. Sometimes cell asp have certain water soluble pigments like anthocyanin (blue or violet) , anthoclor (yellow) etc. Beta cyanin in beet root cells.

Functions of vacuole →

1. Storehouse of nutrients.
2. Removes water materials from cytoplasm
3. Solutes in cell sap regulates osmotic relations of plant ell.
4. Pigments of cell sap provide different colouration to petals.
5. Mature plant cell store water in vacuole. Immature plant cells like cells of meristem store water in cytoplasm.

TOOLS AND TECHNIQUE IN CYTOLOGY

STAINING :

Stain	Finarl colour	Suitatble for
● Schultze' solution (Chlor-zing iodine)	Yellow →	Ligning, cutting, suberin & starch
	Blue/Violet →	Cellulose
● **Sudan IV**	Scarlet red	Suberin
● Sudan black and Red	Black/Red	Lipid
● **Haematoxylene**	Red	Nuclear stain ┐ Cell division
● **Acetocarmine**	Pink	Nuclear stain ┘
● **Feulgen's stain**	Red/Purple	DNA
● **Osmium tetraoxide**	Black	Fats, Stain for electon microscopy
● Toludine blue	Blue	RNA
● **Sfafranine**	Red	Lignin
● Periodic acid Schiff (PAS)	Red	Polysaccharides
● Aniline blue (Cotton blue)	Blue	Fungal hypae
● **Eosin**	Pink, Red	Cytoplasm, Cellulose
● **Janus green**	Greenish blue	Mitochondria
● Methylene blue	Blue	Nucleolus
● Phoroglucinon	Red	Linging
● **Aurantia**	Yellow	Mitochondiria
● Brazlin	Red	DNA
● Para Rosailin	Purple	DNA
● Iodine solution	Blue, Red	Starch, Glyogen
● **Crystal Violet**	Violet	Bacteria
● **Azure B**	Red	RNA

Vital Stains → Stains the cell organelles without killing cell are called as ***vital stains.*** Normally cell becomes dead after staining but vital stains keep the cell alive. **Janus Green,**

FEULGEN REACTIOIN

Feulgen reaction was discovered by **Feulgen** and **Rosen beck** (1924) . Feulgen reaction specifically stains DNA. Feulgen reaction involves two steps -

(i) ***Acid hydrolysis of material :***
It removdes the purine at the level of **purine-deoxyribose glycosidic bond** of DNA there by unmasking the aldehyde group of deoxyribose.

(ii) The free aldehyde group react with **Schiff's reagent.** The Schiff's reagent is prepared by treating **basis fuschin** with **sulfurous acid.**

MICROSCORPY

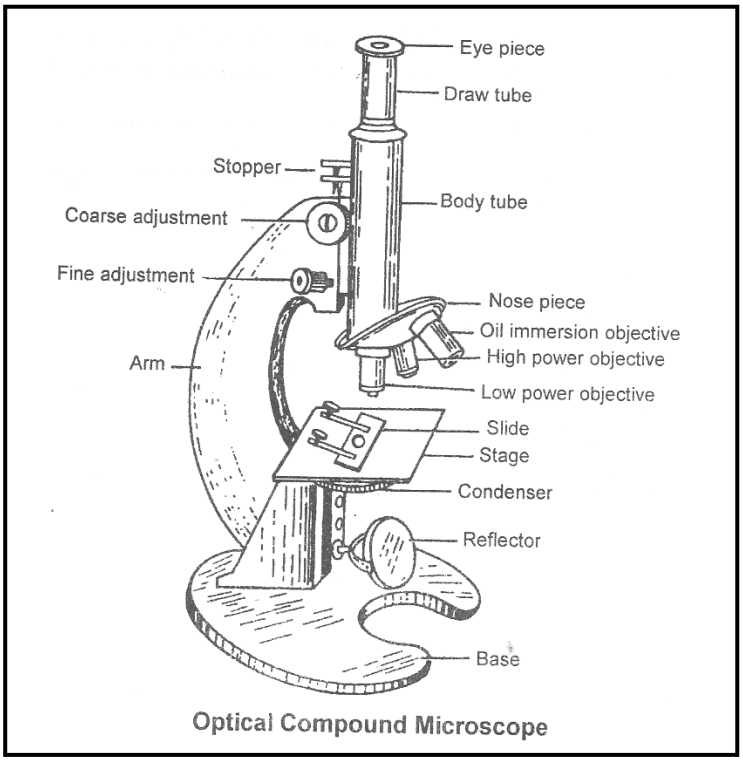
- Microscopy means the use of microscope for studying the cell.

MAGNIFICATION POWER :

- Maximum magnification power of light compound microscope is **2000.**

$$\text{Magnification} = \frac{\text{Sinze of retinal image with microscope}}{\text{Size of retinal image with unaided eves}}$$

- Magnification = power of objective lens x power of eye piece lens
- In light compound microscope maximum power of objective lens is **100 X** and maximum power of eye piece lens is **20 X.** Thus the maximum magnification achieved is 10 × 20 = **2000.**



Optical Compound Microscope

RESOLVING POWER

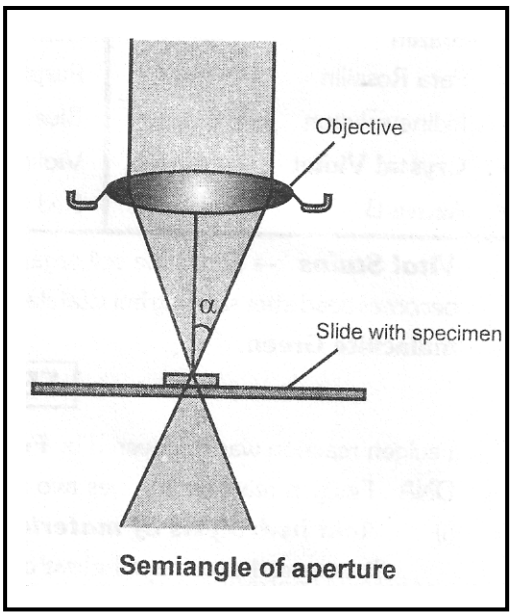
- Resolving power of microscope refers to the smallest distance between two objects which can be identified as separate image. Resolving power of human eye is **100** μm**.**
- Resolving power of light microscope is **0. 25** μm to **0. 30** μm Resolving power of microscope can be calculated with the help of the **Abbe equations.**

$$Lm = \frac{0.61 \times \lambda}{NA}$$ **[**Erns Abbe German physicist**]**

Lm is limit of resolution
NA is numerical aperture.

$$NA = n \sin \alpha$$

- n is refractive index of medium and sin α is the sine of the half angle of light entering the objective lens from the specimen
- λ is the wavelength of light used to illuminate the object.



Semiangle of aperture

- Limit of resolution is inversely proportional to resolving power. So when **Lm** becomes **smaller,** the **resolution increase.**
- Resolving power is inversely related to wavelength of light. Therefore the highest resolution is obtained. with the light of shortest wavelength i. e. (**450-500 nm**) light at the **blue end of the** visible spectrum

- The angle of light that can enter a lens depends on the refractive index of the medium as well as upon the object itself.
- Refractive index of air is **1. 00.** Since sin $\propto$ can not be higher than 1 ($\propto$ = 900 and sin 900 = 1) no lens working in air can have a numerical aperture more than 1. The semi angle of aperture for the best objective lens is 700 (sin 700 = 0. 94) , Therefore, the only way to increase the numerical aperture is to increase the refractive index by using

an oil **(ceda wood oil, olive oil)** which has a refractive index identical to that of a glass. Generally the value of NA for oil immersion lens is **1. 4**

$$Lm = \frac{0.61 \times \lambda}{1.4}$$

Therefore,resolutionofmicroscope is **roughlyonehalfof thewavelengthofilluminating light.**

LIGHT COMPOUND MICROSCOPE

- Light compound microscope was first invented by Z. Janssen and H. Jassen (1590)
- Wilson (1710) improved the microscope of Janssen and Janssen and presented it in the form of present day Laboratory Microscope.
- Normally light compound microscope is **bright filed microscope.** In a bright field microscope the image is formed when the light transmitted through the specimen
- The specimen being denser and more **opaque** than its surroundings, absorbs some of this light and the rest of the light is transmitted directly up to the eye piece. As a result, the image produced by the specimen will be darker than the surrounding brightly illuminated field.
  Use : The bright field microscope is a multipurpose instrument and can be used for life unstained materials as well as preserved and stainred materials.

DARK FIELD MICROSCOPE OR ULTRA MICROSCOPE

- Dark field microscope developed by **Zsigmondy** (1905)
- In dark field microscope, the object is illuminated by **oblique beam of light.** The resulting image is illumiated brightly against the dark background.
- The most effective advantage of Dark field microscope is tha, the **nuclei, mitochodri** and **vacuoles** are readily detected.

PHASE CONSTRAST MICROSCOPE

- It was developed by **Zernicke** (1935)
- This microscope multiples the small differences among the phases or refractive indices of different cellular constituents.
- It converts the difference of refractive index into differences in brightness with the help of a transparent **phase plate** situated at the back focal plane of objective lens.
  So different strucutres of cell appear in different brightness.
- Phase contrast microscope does not require staining of material so cell can be observed in **living** state.
- **This microscope is useful to study functional aspect of cell like cell division.**

INTERFERENCE MICROSCOPE

- It was developed by **Merten et. al.**
- It is one modification of phase contract microscope. Here, pars of cells with different thickness and refractive index appear in **contrasting colours** so as to provide clear observation.
- In this microscope, the light rays are split into **two beams** before passing through the object **by two prisms** that create contrasting colours in a image. One beam passes through the object and undergoes a phase change.
  The second beam **does not pass through** the object and remains unchanged.
- The two beams are brought together above the object.
- This microscope can determine the presence of **several light** absorbing substances like nucleic acid, proteins, lipids etc.
- By using this microscope, it is possible **to find out the dry weight of macromolecules like DNA, RNA and proteins**

FLUORESCENT MICROSCOPE

- It was developed by **Coons** (1945)
- It emloyes **invisible ultraviolet** rays instead of **oridinary light for illumination.** it is built on the principle hat certain substances absorb short wave ultra-violet radiations falling on them, get excited and emit them back but in the form of longer wave length of visible light. This penomenon is called **Fluorescence.**

- Some cellular components like chorophylls, riboflavin vitamin A collagen fibers etc. have the property of **autofluorensce.** They are therefore, visible under the fluorescent microscope.
- Other cellular componetns (e. g. cellulseo, starch glycogen lipids proteins) can be made visible under the fluorescent microscope with the help of special dyes called **fluorochromes** (e. g. **Acridine orange, coroposphine**) . The phenomenon of fluorescence with the help of fluorochromes is called **secondadry fluorescence.**
- **Fluorescent microscope is useful in detecting the microbes present inside the infected tissue. Some Fluorochrome :**
  - **(i) Fluorescein -** (emitting green light, when excited with blue light)
  - **(ii) Rhodamine -** (emitting deep red light when excited with green yellow light) .
  - **(iii) Quinie sulphate**
  - **(iv) auramine**

ELECTRON MICROSCOPE

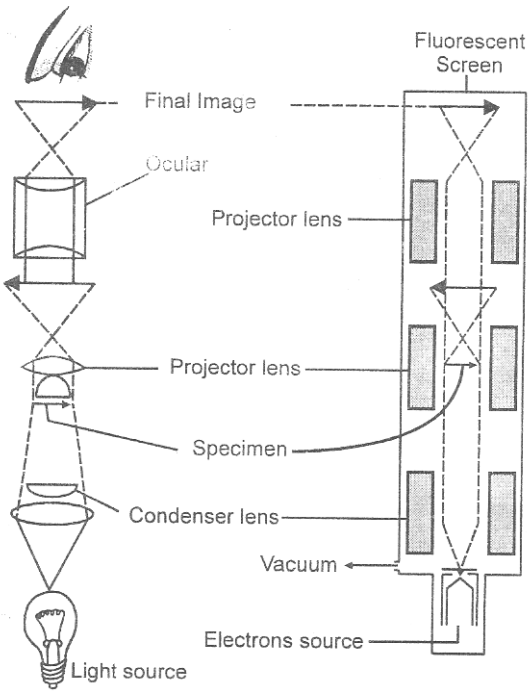
- The microscope employs a beam of electrons for illumination of object and image formation.
- A vacuum is created for the smooth passage of electrons. Electrons are produced by a cathode filament of tungsten when it receives high voltage electric current (50, 000 - 100, 000 volts)
- Three types of **electromagnetic lenses** are used similar to glass lenses of compound light microscope-**condenser, objective** and **projector.**

(i) The first acts like a **condenser,** focusing the electron stream on the object.

(ii) The second acts like **objective lens** producing a magnified intermediate image of the object.

(iii) The third act like **projector** it magnifies a portion of the intermediate image to produce a final image of the object which is inspected visually on a fluorescent screen.

- Each electromagnet has coil of wire enclosed by a soft iron casing. Image is observed on a fluorescent screen. It can also be obtained on photographic film. The same is called **electron micrograph.**



Simplified figures of the optical systems of compound microscope and electron microscope

TYPES OF ELECTRON MICROSCOPE :

1. Transmission electron microscope
2. Scanning electron microscope.

(i) Transmission Electron Microscope (TEM) :

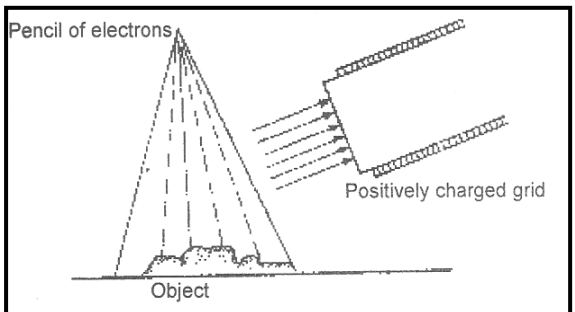
- It studies internal structure. The instrument was invented by **Knoll** and **Ruska.** (1932) . the specimen is made ultra thin (0. 1 $^{\mu m}$)) dehydrated and impregnated with heavy metal.
- It is placed in the specimen chamber. Electron beam is thrown over the specimen by electromagnet condenser.
- Electrons coming out of the specimen are spread by objective and projector electromagnets. They fall on the fluorescent screen or photographic film and give the image of internal structure of the specimen.
- Magnification is 100, 000-300, 000. Resolving power is theoretically **0. 025 Å** but is actually **2-10 Å**

1. The later depends upon wave length of the electron beam. Wave length of electron beam is connected with voltage. $\lambda \, in \, \mathring{A} = \frac{12.2}{\sqrt{V}}$ Here, λ is wavelength of electron beam while **V** is voltage.

2. Electron beam produced by an electric current of 50, 000 volts has a wavelength of 0. 05 Å. It is less than the diameter of smallest atom (hydrogen atom = 1. 06 Å) .
3. The material to be studies under electron microscope must be well preserved, fixed, completely dehydrated, ultrathina nd impregnated with chemicals that sharpen the differences among various organelles. The material ultrathin and impregnated with chemical that sharpen the differences among various organelles. The material is commonly fixed in **osmium tetra-oxide or oxmis acid.**
4. It is embedded in plastic and sectioned with the help of a diamong or glass raror attached to an **ultramicrotome.** They are then exposed to electron dense material for obtaining a proper contrast. The commonly used materials are lead acetate, uranyl acetate, palldium vapours, phosphotungstate etc.

(ii) Scanning electron Microscope (SEM)

- it gives **three-dimensional surface** view of objects. Scanning electron microscope was developed by **knoll** in 1935.
- In this microscope the beam of electrons is reflected back from the object (not transmitted through)
- The speciment is first dipped in **liquid propane** at - 1800C and then dehyddrated in **alchol** at - 700C. It surface is canend by a narrow beam (200 Å in dimater) of electrons. Secondary electrons emitted by the surface of the specimen are collected by positively charged grid for transfer to **television tube** and **fluorescent** screen.



Principle of scanning electron microscope

- Scanning electron microscope has a magnification arrange of 15-20, 000 (now upto 200, 000)

Scanning porbe microscope : Which is capable of resolbing to a very high extent the outer texture of the material and magnify it to 100 million times. This microscope has potential to image even a single atom.

Scanning tunneling microscope : Scans the surface of the electrical conductors and computer chip for detecting the defects.

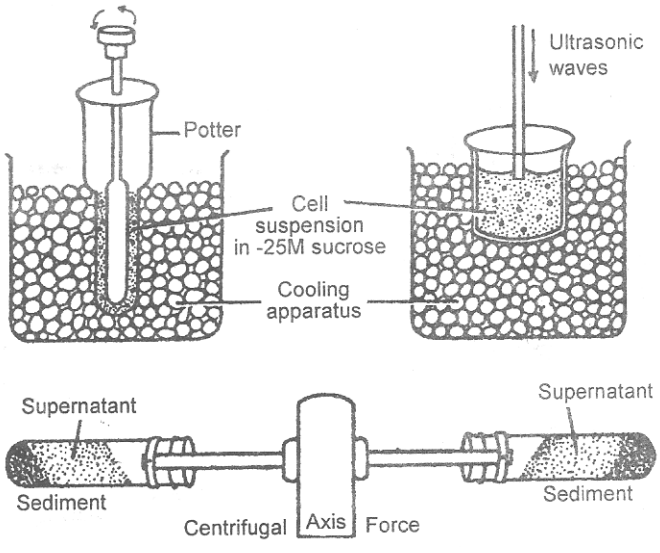
Atomic force microscope : Which is useful in viewing the detailed pattern of the biological molecules like **DNA** and **proteins.**

CELL FRACTIONATION

- It is a procedure for rupturing cells, separation and suspension of cell constituents in **isotonic medium** in order to study their structure chemical composition and function.
- Cell fractionation is done is two steps, **homogenisation** and **differential centrifugation.**

(1) Homogenisation

- Homogenisation is the release of cellular constituents in their natural state by rupturing of the cell membrances and cell wall, and preventing their clumping destruction etc. with the help of a special solution.
- The cells are placed in a 0. 25 M sugar solution at 00 - 4^0C.



Technique of cell fractionation

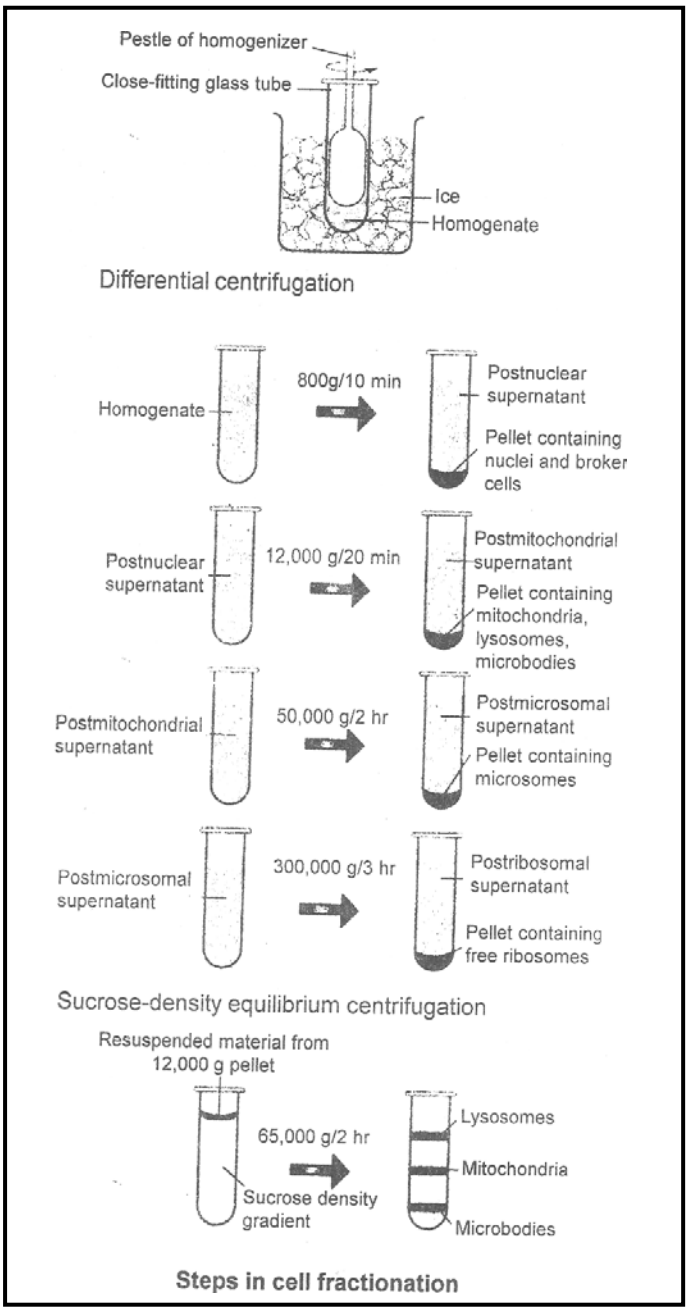
They are broken or homogenised to release their constituents either by grinding or exposing them to ultrasonic **vibrations.**

- Gridning is done is **pesle** and **mortar** or **Potter homogeniser**. The latter consists of two cylinders separated by a narrow gap. The shearing force produces by the movement of cylinders causes the rupture of cells.
- Ultrasonic waves are produced by **piezoelectric** crystal. they are transmitted to a steel rod placed in the suspension having cell. s Ultrasoniv waves produce vibrations which rupture the cells.
- The liquid containing suspension of cell organelles and other constituents is called **homogenate**. Sugar or sucrose solution preserves the cell organelles and prevents their clumping.

Differential Centrifugation

The homogenate is placed is tubes and then subjected to different centrifugal forces in machines called **centrifugues.**

- Centrifuges having more than **50, 000** rotations per minute are called **ultracentriguges.**
- Various cell fragments settle down as different centrifugal speeds depending upoen their size and weight. The denser constituents settle down at lower speeds while the lighter components do so at higerh speeds. For example, nuclei settle down when the homogenate is subjected to a centrifugal force of 800 - 1000g for ten minutes.
- The supernatant is taken to a new tube and subjected to centrifugal force of say 10000 - 12000 g for 20 minutes. **Mitochondria** settle down at this force.



Steps in cell fractionation

In it his way **nuclei, chloroplasts, mitochondria, lysosomes, peroxisomes, ribosomes,** pieces of **endoplasmic reticulum** settle down separately. The pellets (sediments) and supernatant are then subjected to microscopic and biochemical analysis.

- **Note : Normal clinical centrifuge** is motorised instru ment which can rotate upto 5000 rpm.

DENSITY GRADIENT CENTRIFUGATION

- Centrifugation through a gradient of increasing sucrose density is performed. In this, the denser solution is at the bottom and its density gradually decreases towards the top. One the gradient is formed, the homogenate is layered at the top and centrifuged. After centrifuction, components from the homogenate with different moving speeds reach equilibrium with the gradient. Hence this type of separation is also called **isophyknic (equal density) centrifugation.**

- The rate of sedimentation of the components depends on the size of the component and is described as sedimentation coefficient or **Svedberg units.**

Note 1. On the basis of the knowledge of the sedimentation coefficient, the size and composition of the cell, and types components can be determined.

2. **Peroxisomes may be separated from lysosmes** or **fragments of agranular edoplasmic reticulum from granular endoplasmic reticulum.**

BUOYANT DENSITY CENTRIFUGATION.

- **In buoyant density centrifugation, centrifugation is done using high concentration of sucrose of the caesium chloride solution.** They form a continuous density gradient in the centrifuge tube and cell components settle in density, which is equal to t heir density.
- At this point the component floats and does not move and further due to its buyoyancy. Thus, a series of distinct band are formed not due to 'S' value (Sedimentation coefficient) but according to buoant density, which is highest at the bottom and lowest at the top. This process of fractionation through centrifugation is also termed as **equilibrium sedimentation.**
- The method so sensitive that is allows even separation of molecules having heavy isotopes (with ^{13}C or ^{15}N)
- The method was developed by **Mathew Meselson** and **Franklin Stahl** to **separate heavy DNA with ^{15}N from DNA with ^{14}N** for **providing evidence for semi-conservative replication of DNA**

AUTORADIAOGRAPHY

Autoradiopgraphy is an important technique used for styding the **Biosynthesis** of molecules and to trace the metabolic events occurring inside the cell. Autoradiography is carried out with the help of radioactive isotopes called **tracer.**

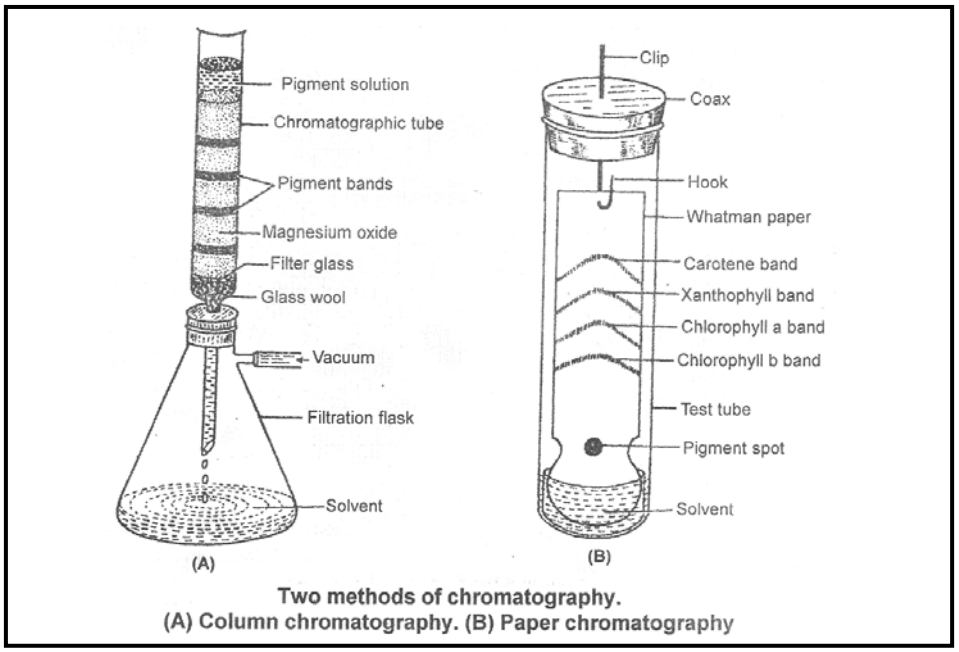
Some tracer elements :

Carbon - 14 (^{14}C) Tritium (^{3}H) Phosphorus - 32 (^{32}P) and Iodine - 131 (^{131}I)

Calvin used C^{14} isotope for the study of **photosynthesis**

CHROMATOGRAPHY

- This is one of the most common methods of separation of molecular components of the cells present in a solution of **cytosol,** lef after centrifugation. The cytosol is allowed to percolate in an **insoluble medium,** which has different affinity for molecules of different substances.



Two methods of chromatography.
(A) Column chromatography. (B) Paper chromatography

- it is a techniqie os separating various chemical in a mixture due to differential adsopriton. The sytem was discovered by Rassain botanist **Michael Tswett** in 1906.

(i) Column Chromatography : The solution is passed through a tube or column containingan adsorbent like calciu m carbonate and magnesium oxide. As the solution percolated down, various chemicals get absorbed at various levels.

* Commonly used for separation of mixture of tissue lipids.

(ii) Paper Chromtography : It used Whatman 1 or chromatographic paper. A paste of the mixture is applied over the paper 4-5 cm away from the end which dips in a solvent. As the solvent passes through the paper. it spread the different d\chemical of the mixture to different distances.

* Used for separation of amino acids, nucleotides, and low molecular weight products.

Special point

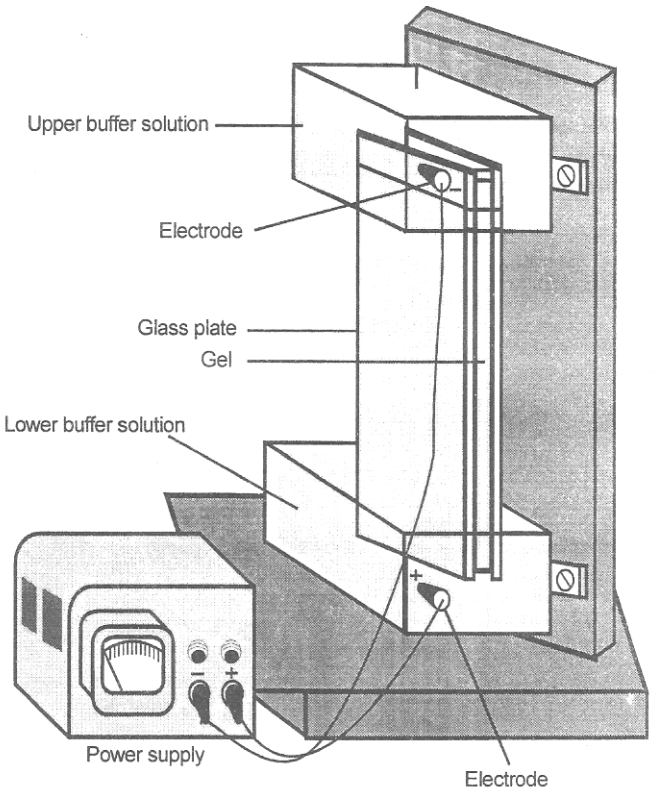
- **Ion exchagne chromatography :** Used for insulin purification and plasma frectionation.
- **Affinity chromatography :** Used for separation of immunoglobulins, cellular enxymes and mRNA.
- **Gell filtration chromatography :** Used for determination of molecular weight of proteins.
- **Thin layer Chromatography :** Used for separation of amino acids, nucleotides, and low molecualr weight products.
- **High Preferencial Liquid Chromatography**

ELECTROPHORES

●● A mixture of chemical is applkiced over a paper or film which dips at its two ends in different continers, one having a cathode and the other having the anode. Under the influence of electric current. the chemicals migrate and spread over the film or paper depending upon their electric charge.

- It is used in separation of nucleic acids, proteins amino acids, nucleotides, etc.

Immuno - eletrophoresis : It is extremely sensitive end the molecule can be separated in **picoram to nanogram quantities.** Antibodies coupled with radioisotopes, specific enzymes or fluoresnce dyes can be used for detection of specific proteins (even, proteins that differ by a single amino acid can be resolved) .



Electrophoresis apparatus