*International Journal of Scientific Research and Engineering Development-– Volume 8 Issue 4, July Year 2025*
*Available at www.ijsred.com*

RESEARCH ARTICLE OPEN ACCESS

# Precision in Life Science: Mastering Instrumental Approaches to Biological Research

Ankita Pradhan
BSc Final Year,Botany Honours, University Rank Holder , 2017-2018
Vikash Group of Institutions, Bargarh, 768028
Affiliated to the Sambalpur University, Jyoti Vihar, Burla, Odisha, 768019
ankitapradhan860@gmail.com

## Abstract
The phrase 'Precision in Life Science: Mastering Instrumental Approaches to Biological Research' highlights the essential role of instrumentation in advancing the biological discovery. Modern life science depends heavily on precise and accurate measurements. This enable the researchers to explore biological phenomena at molecular, cellular and organismal levels. This section provides an overview of some key instrumental approaches (like Spectrophotometer, Paper chromatography, Centrifugation and Electron Microscope techniques) which play important roles in the world of biological sciences.
This emphasizes the fundamental principles of instruments and their working mechanism along with their practical applications in biological research. It encloses experimental design, data acquisition and analysis which are essential crucial for obtaining reliable and reproducible results. By understanding and effectively utilizing these instrumental tools, researchers can gain deeper insights into complex biological processes. By exploring these techniques students can gain hands-on experience in handling these instruments under the guidance of teaching professionals. This serves as an invaluable resource for students and researchers aiming to master the instrumental techniques essential for conducting high-impact, precision-driven biological research.

**Keywords –** biological phenomenon, accurate measurements, instrumental tools

## INTRODUCTION
Instrumental techniques in biology involve using specialized instruments to analyse biological samples and processes. These techniques are crucial for characterizing biomolecules, studying biological systems, and gaining insights into various aspects of life sciences. Instrumental techniques for this particular paper enclosing the followings

- Spectrophotometer
- Paper chromatography
- Electron microscope
- Centrifugation

## [ I ] SPECTROPHOTOMETER
It is the instrument that is used to study the absorption and emission of electromagnetic radiation as a function of the wavelength.
A Spectrophotometer measures the amount of light a substance absorbs at different wavelengths, allowing for identification and quantification of the substance. The core principle is based on the Beer-Lambert Law. This law states that the absorbance of a solution is directly proportional to the concentration of the solute and the path length of the light beam through the solution.
A spectrophotometer is a sophisticated analytical instrument that measures the amount of light absorbed or transmitted by a sample. It works by passing a beam of light through a solution and then measuring how much of that light passes through (transmittance) or is absorbed by the sample (absorbance). The fundamental principle behind its operation is that every chemical compound absorbs or transmits light over a specific range of wavelengths.

The relationship between light absorption and concentration is governed by the Beer-Lambert Law, which states that the absorbance of a substance in a solution is directly proportional to its concentration and the path length of the light through the sample. This allows spectrophotometers to quantify the amount of a specific substance in a solution.

History- The first highly accurate and commercially viable spectrophotometer, the Beckman DU spectrophotometer, was invented by Arnold O. Beckman in 1940. This invention revolutionized analytical chemistry and biology.

## Principle of Spectrophotometer

1. Bouger Lambert's Law- When a beam of monochromatic light is passed through ab absorbing medium, the amount of light absorbed is directly proportional to the path length ( thickness) of the absorbing medium and is independent of the intensity of the incident light.
   Mathematically, $2.303 \log I^0/I = Kl$
   Where, $I^0$ = intensity of incident light , I = intensity of transmitted light, K = absorption coefficient.
2. Beer's Law- When a beam of monochromatic light is passed through an absorbing medium, the amount of light absorbed is directly proportional to the concentration of absorbing medium.
   Mathematically, $2.303 \log I^0/I = K'c$
   Where, $I^0$ = intensity of incident light , I = intensity of transmitted light, c= concentration of absorbing medium, K'= absorptivity constant.

By combining both law 1, 2 it can be called as Beer – Lambert's Law. It says, when a beam of monochromatic light is passed through an absorbing medium the amount of light absorbed is directly proportional to the path length and concentration of absorbing medium.

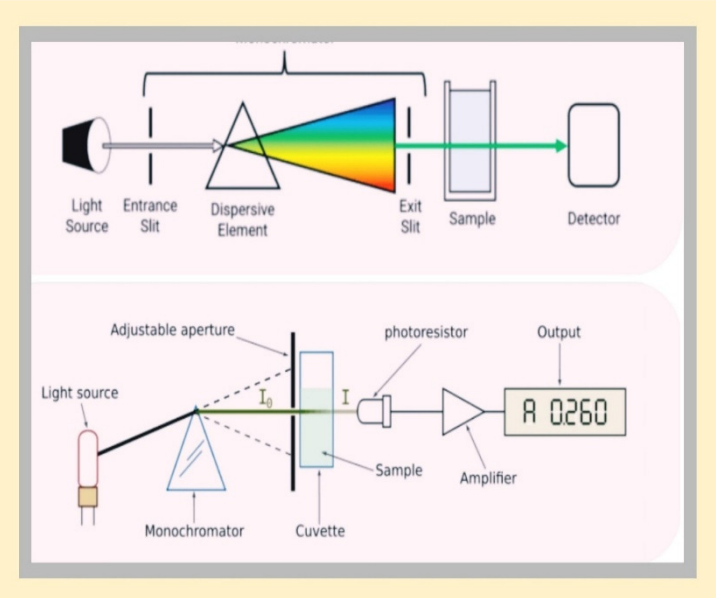
## Instrumentation of Spectrophotometer

The essential components of Spectrophotometer includes

1. Radiant energy source – A stable radiant energy source is required to spectrophotometric use. The most commonly used source are as follows. For visible radiation, Tungsten Filament Lamp is used; for UV radiation, Hydrogen and Deuterium Lamp are used; for IR radiation, Nernst Glower and Globar are used.
2. Wavelength selector – All light sources used for spectrophotometry provide continuous radiation over a wide range of wavelengths, but laws of absorption of light can be applied only for monochromatic radiation. Therefore, a narrow band width radiation is essential for spectrophotometry. Spectrophotometer is made possible by the use of wavelengths. Here 2 types of wavelengths can be used such as Filter and Monochromator. Filters are used in colorimeters and monochromators are used in spectrophotometer.
   Monochromators – they resolves the polychromatic light into its individual wavelengths and form narrow band width radiation.
   Components of monochromators includes ---
   - An entrance slit for receiving polychromatic light from the source,
   - A collaborative device ( lens or mirror) which collimates the polychromatic light onto the dispersion device,
   - A wavelength resolving device (prism) that breaks the polychromatic light into monochromatic light,
   - A focusing lens / a mirror which emits monochromatic light,
   - An exit slit that allows the monochromatic light to escape.

3. Sample holder – The sample to be studied in UV or Visible region are usually solutions which are put in special shell called as 'cuvette'. Cuvette used for visible region are made up of glass and cuvette used for UV region are made up of quartz or silica.
4. Detection Device – These works on the principle of photoelectric effect, where the incident light liberates electron from the material surface and electrons are emitted and measured as current. Current is directly proportional to the intensity of incident light and provide a measure of it. Detectors are 3 types.
    - Photo voltaic cell
    - Photo tubes
    - Photo multiplier tubes
5. Amplifier and Readout – The detectors generate electric signals which are proportional to the transmitted light. The signals need to be transmitted into such a form which is easy to interpret. This can be achieved by the use of amplifier, ammeter, potentiometer and recorder.




[Fig 1 : This is the diagrammatic representation of instrumentation of Spectrophotometer]

**Types of Spectrophotometer**

( depending on application, and working mechanism design based on electromagnetic spectrum)

- UV-Visible Spectrophotometers – These are the most common type of spectrophotometers. They uses the light in the ultraviolet (185-400 nm) and visible (400-700 nm) region of the electromagnetic spectrum.
- IR Spectrophotometers – They uses the infrared light (700-15000 nm) of the electromagnetic spectrum. They provide results on molecular vibrations and structural characteristics.
- NIR Spectrophotometers (Near-Infrared) – They are used for food analysis due to their ability to target organic bonds.
- Single-beam spectrophotometers – They use a single light beam. Here light beam passes through both the reference and sample and it require a sequential measurements.They are cost-effective.
- Double-beam spectrophotometers – These uses the light which splits into two beams. Here one beam is for the sample and another one is for a reference. It allows for simultaneous measurement. Its accuracy and stability are higher.

## Applications of Spectrophotometry

1. Quantitative Analysis – The unknown concentration of a solution can be determined with the help of a Spectrophotometer. A graph has to be plotted by taking the concentration of known sample versus absorbance and then by cross-matching the absorbance of a sample whose concentration is unknown. In the concentration vs absorbance plot of the known sample, the concentration of sample can be determined.
2. Qualitative Analysis - The visible and UV spectra may be used to identify different classes of compounds. This can be done by the absorption spectrum curve, because this is specific for a particular class of compound.
3. Enzyme Assay – The quantitative assay of enzyme activity is carried out when the substance or product is coloured and absorb light because the rate of appearance and disappearance of substance can be followed with a Spectrophotometer which gives a record of the progress of the reaction.
4. Molecular Weight Determination – Molecular weight of compound can be determined on the basis of its absorption data. Molecular Weight= £wl/ OD
5. Study of Cis- Trans Isomerism – Since the geometrical isomers differ in the arrangements of groups so the absorption spectra of isomers also differ, and here absorption spectrometry can therefore be utilised for study of cis- trans isomerism.
6. Control of Purification – The impurities present in a compound can be detected by Spectrophotometry by verifying whether the compound shows an absorption maxima which is not a character of it.
7. In chemistry this instrument can be used in determining concentrations of chemical substances, studying reaction kinetics, and identifying compounds.
8. In biology and biochemistry this instrument is used in quantifying DNA, RNA and protein concentrations and also used in monitoring microbial growth and studying enzyme activity.
9. In pharmaceuticals this instrument can be used in examining quality control of drugs, identifying impurities and dissolution testing.
10. In food and beverage industry this instrument can be used in measuring ripeness of fruits, alcohol content in beverages and colour analysis.
11. For environmental monitoring this can be used in analysing water quality and detecting pollutants.
12. In material science, spectrophotometers can be used in characterizing the optical properties of various materials.
13. The Spectrophotometer is used for non-destructive analysis where the sample remains intact after analysis which is valuable for precious or limited samples especially in quality control.
14. In the miniaturization and automation, the modern spectrophotometers have become increasingly miniaturized and integrate with advanced software and automation systems which make them user-friendly and capable of high-throughput analysis.

# [II] PAPER CHROMATOGRAPHY

Chromatography can be described as a crucial bio-physical technique which describes the separation, identification and purification of the components of a mixture for qualitative and quantitative analysis. The Russian botanist Mikhail Tswett coined the term chromatography in 1906.

The very first analytical use of chromatography was described by James and Martin in 1952. They used it for gas chromatography for the analysis of fatty acid mixtures.

Chromatography technique which is developed by Russian botanist Mikhail Tswett to study plant pigments. Mikhail Tswett's research focused on separating chlorophyll and carotenoids from leaves, leading to the development of adsorption chromatography.

Chromatography is an analytical technique which deals with the separation of closely related compounds from a mixture. This can be utilised for separation of proteins, amino acids, lipids, carbohydrates and plant pigments.
Types of Chromatography

- Partition chromatography
- Adsorption chromatography
- Ion- exchange chromatography
- Affinity chromatography
- Gel filtration chromatography
- Thin layer chromatography
- Gas – liquid chromatography
- High performance liquid chromatography

**Paper Chromatography**
Paper chromatography is a simple yet effective analytical technique used to separate and identify components of a mixture. It's a type of partition chromatography, meaning it relies on the differential distribution of substances between two phases: a stationary phase and a mobile phase. Here components that are less soluble in the mobile phase and have a stronger affinity for the stationary phase will travel shorter distances. The Rf value is characteristic of a particular compound under specific chromatographic conditions (stationary phase, mobile phase, temperature).
It is a type of partition chromatography where the molecules of a mixture get partition between the stationary phase and mobile phase.

**Principle of Paper chromatography**
Three components form the basis of the chromatography technique.

1. Stationary phase - This is always composed of a solid phase or a layer of a liquid adsorbed on the surface solid support.
2. Mobile phase - This is always composed of liquid or gaseous component. It has substances to be separated along with the solvent system.
3. Separated molecules - The type of interaction between the stationary phase, mobile phase and substances contained in the mixture is the basic component effective on the separation of molecules from each other.

**Apparatus and Paper Development**
The apparatus of paper chromatography consists of

- A support for paper
- Solvent through in which the chromatogram will develop
- An air tight container

In case of paper chromatography, firstly a few drops of solution containing the mixture of substances to be separated is applied at one end, which is usually 2cm above the paper. Then the paper is collected and dried and dipped into a solvent system.
**Types of Paper chromatography**

- Ascending type – Here paper is suspended in such a manner that the base of paper is in contact with the solvent at the base of the chamber. The sample's spot should be in a position just above the surface of the solvent so that as the solvent moves vertically upwards through the paper, then there is separation of components. Here the required set-up is very simple. It also provides the

better result because in this type the two forces ( capillary force and gravitational force) works in the opposite direction.

- Descending type – Here the end of the paper (where sample is located) is held in a through at the top of the apparatus, then the rest of the paper is allowed to hang vertically. So the separation of sample is achieved as the solvent moves downward under gravitational force. Here both forces ( capillary force and gravitational force) works in the same direction. So the resolution is quite poor as compared to the ascending chromatography.
- 2-Dimentional Chromatography - The sample component which can't be separated by using ascending or descending type ( 1 – Dimensional tech with one solvent system) they can easily be separated by using 2 – dimensional system by using two solvent systems. The paper with a sample applied, first developed normally by either ascending or descending and then continue till the solvent approaches the end of paper. The paper then removed and rotated 90º and developed for 2nd time using another solvent system having different eluting property.
- Radial (Circular) Chromatography: The sample is applied at the center, and the solvent moves outwards in a circular pattern.
- Two-Dimensional Chromatography: A powerful technique where the chromatogram is developed in one direction, dried, rotated 90 degrees, and then developed in a second solvent system. This provides much better separation for complex mixtures, especially for compounds with similar polarities.

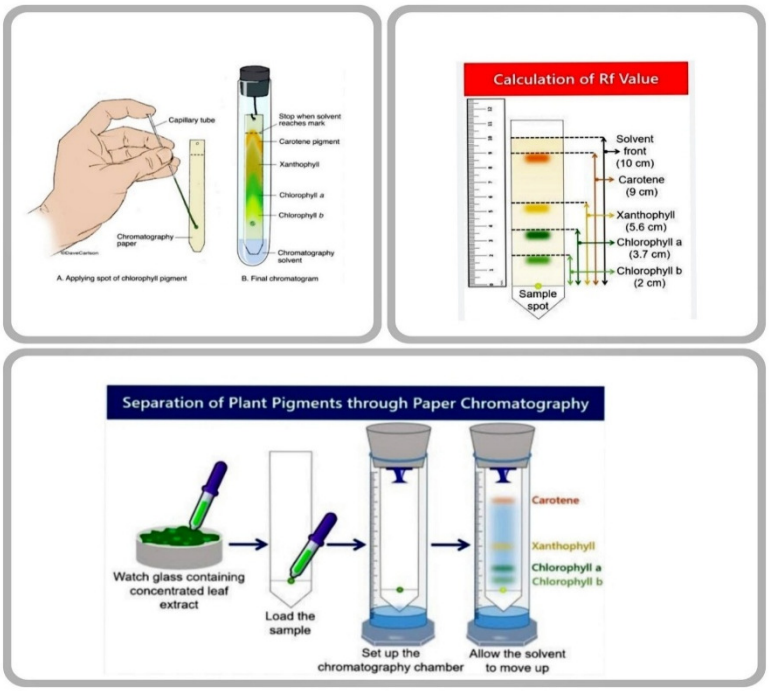
Choice of the solvent system – The mobile phase is a mixture of various solvent like alcohols, acids, phenols, ketones and hydrocarbons. The solvent system is selected in such a way that there would be better resolution of sample component.

Detection of components –

- Once the chromatogram is developed the paper is removed and then identified for different components of the sample.
- If the sample components are coloured the analysis becomes very easier because the colour components identify themselves.
- If the sample components are colourless, they have to be imparted colours by using some colour producing reagents.

- The identification of different components may also be made on the basis of Rf value ( Retardation Factor ) . It is the ratio between distance travelled by the substance and the distance travelled by the solvent front.




## Applications of Paper Chromatography

- Biochemistry and Medical Studies - Identifying substances in bodily fluids (like urine, blood). Studying fermentation and ripening processes. Analysing reaction mixtures in biochemical labs. Checking the purity of pharmaceuticals.
- Chemical Analysis - Identifying unknown compounds by comparing Rf values. Determining the rate of chemical reactions. Monitoring reaction progress.
- Food and Beverage Industry - Detecting contaminants in drinks and foods. Analyzing food colours and identifying artificial colours and preservatives.
- Forensics- Analyzing inks and dyes in documents.
- Environmental Testing- Analyzing pollutants in environmental samples.
- Plant Biology- Studying leaf pigments.
- Other Applications- Inspecting cosmetics. Detecting adulterants. Separating amino acids, plant pigments, and other compounds. Separating inorganic ions. Identifying rubber accelerators and antioxidants. Assaying pharmaceutical compounds.
- They are simply and cost-effective and due to this they can easily be used for educational purposes, basic laboratory separations and preliminary analyses where high-end equipment is not necessary or not available.
- They are used for the visualization of the ‘Invisible’. Many compounds are colourless. The ability to use specific visualizing agents to make these separated components visible is a key aspect that allows for identification and analysis of a vast array of substances.

## [III] ELECTION MICROSCOPE

An electron microscope is a microscope that uses a beam of electrons as a source of illumination. They use electron optics that are analogous to the glass lenses of an optical light microscope to control the electron beam, for instance focusing it to produce magnified images or electron diffraction patterns. As the wavelength of an electron can be up to 100,000 times smaller than that of visible light, electron microscopes have a much higher resolution of about 0.1 nm, which compares to about 200 nm for light microscopes.

The electron microscope was invented by Ernst Ruska and Max Knoll. Ernst Ruska (1906-1988), a German physicist and engineer, is credited with the initial invention of the electron microscope.

Max Knoll (1897-1953), also a German scientist, collaborated with Ruska in developing the first electron microscope in 1931.

The first practical electron microscope, capable of magnification up to 400x, was developed by Ruska and Knoll.

Broadly Election microscope are 2 types.

1) Transmission Electron Microscope
2) Scanning Electron Microscope

## General Principle of Electron Microscope

An electron microscope uses electron beam to produce image of object and magnification can be done by the use of electromagnetic lens. In contrast, the optical light microscope uses the simple optical lens for magnification.

Wavelength of light is in directly proportional to the resolving power . Wavelength of election beam is 1000 times shorter than the visible light. As a result of this, the resolving power of election microscope is very high.

- In case of the Transmission Electron Microscope, an electron beam from an electron gun is transmitted through a thin section of samples and electrons are projected onto the florescent screen which forms the image of the object.
- In case of the Scanning Electron Microscope, the electrons are scattered from the surface of the specimen and the secondary electrons generated are finally projected on the screen which forms the image of the object.

## Sample Preparation for Election Microscope

The sample to be viewed under an electron microscope requires the following processing.

1) Fixation – The chemical fixation for the biological sample aims to stable the mobile macromolecular structure by means of chemical crosslinking. Mobile macromolecules are large molecules, like proteins or RNAs, that can move between cells in a plant, often traveling through the phloem, a specialized vascular tissue. Chemical crosslinking is a process where two or more molecules are joined together by covalent bonds, forming a network or larger structure. Eg : For chemical crosslinking of proteins, gluteraldehyde, Imidoesters, N-Hydroxysuccinimide Esters etc are used.
2) Dehydration – It involves the replacement of water molecules from the organic solvent like acetone or ethanol which is then followed by a critical point rank.
3) Sectioning – As the electrons are having very less penetration power, the specimen (under study)must need to be extremely thin slices (ultra-thin). Here the sample can be cut on an ultra-microtome. It is a specialized instrument which is used to cut specimens into ultrathin sections, for examination under electron microscopes to analyse their internal anatomy. These sections, typically between 20 and 150 nanometres thick. Ultra microtomes uses specialized knives (which are typically made of glass or diamond) to cut the sections with extreme precision.

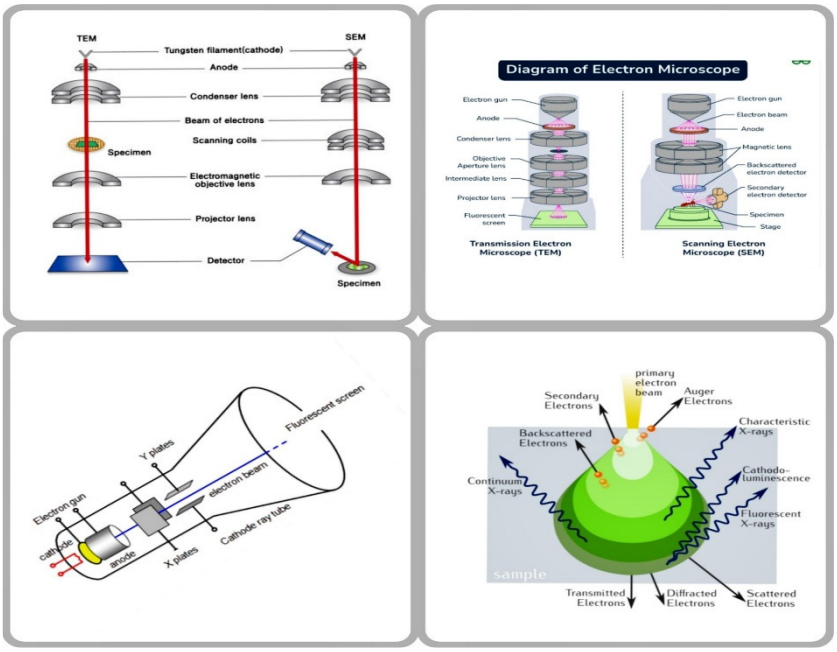
4) Staining – In the electron microscope, the contrast depends upon the atomic number of molecules present in the specimen/sample. Since most of the biological specimen have the elements like Hydrogen Carbon, Nitrogen and Oxygen having small atomic number (01,06,07,08 respectively) they have to be impregnated with heavy metals like Gold, Lead and Uranium (with atomic number 79, 82 and 92 respectively)mainly for scattering electrons and thus give better result.
5) Shadowing – In the electron microscope, sometimes the specimen after fixation and drying is sprayed with heavy metals in such a way that the coating is thicker at some portion and thinner at some other portions. This creates a shadow effect and in turn increase the contrast. This is called as shadow casting .
6) Freeze Fracture Technique – This is mainly used for examining the lipid membranes and their incorporated proteins. Here at first the sample is frozen rapidly ( called as cryo- fixation) they it is fractured using a microtome (at -4$^{o}$c ). The cold fractured surface is then shadowed with Platinum and Arum. Then the specimen is allowed to return to the room temperature and the metal replica of fractured surface is released from the underlined biological materials by treatment with acetic acid and SDS . This replica is then thoroughly washed, dried and viewed under the electron microscope.

**Instrumentation of the Electron Microscope**

1) A High Voltage Supply –It is essential for accelerating the electrons. It enable image formation and analysis. It provides the necessary voltage to create an electron beam ranging from 10-1000 kV. The stability and precision of the high-voltage supply are essential for maintaining a focused beam and achieving high-resolution images.
2) An Internal Vacuum Chamber – It allows the electrons to travel without being deflected or scattered by air molecules. This ensures a focused and accurate electron beam. This leads to the high-resolution images. The vacuum chamber protects the electron gun and other sensitive components from contamination by atmospheric gases and particles. High vacuum also increases the collection efficiency of electrons by the detectors and further enhance the image quality.
3) A Coding System – It refers to the processes used to represent and interpret the information obtained from the interaction of electrons with the sample. It refers to the entire process of converting the interaction between electrons and the sample into a meaningful representation that can be used to understand the structure, composition, and other properties of the sample.
4) Tungsten Filament – This is the primary source of electrons in the microscope. It provides the stream of electrons used to form the image. The filament is typically heated and this causes it to emit electrons. These electrons are then accelerated and focused by the lenses. Tungsten is most popularly used due to its high melting point and ability to withstand high temperatures.
5) Electromagnetic Lens ( Condenser lens and Objective lens) – These lenses use magnetic fields which are generated by coils of wire within pole pieces to control the path of the electron beam. By adjusting the current through the coils, the strength of the magnetic field and thus the focusing power of the lens can be controlled.
6) A Photographic Plate – This is used to record the image generated by the electron beam. This plate is made up of a thin glass or plastic sheet and is coated with a light-sensitive emulsion. This could be exposed to electron beam and creating a latent image.
7) A Beam Deflector / fast beam blankers (FBBs) – They are used to control the direction and intensity of electron beam. They are essential for creating images of a sample, prevent unwanted

exposure and generate pulsed electron beams. They are used for stroboscopic scanning electron microscopy (SEM).



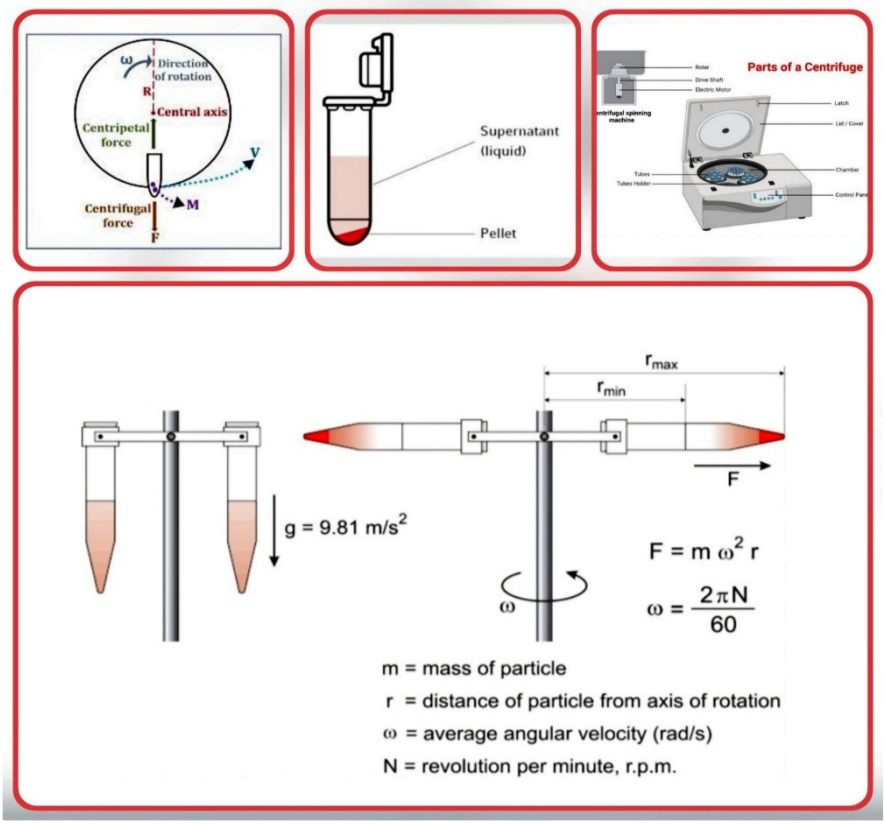

At first the specimen is fixed and dehydrated and then it is cut into very thin slices followed by staining with heavy metals. Then electron microscope uses the electron beam to produce the image of the object.
In case of TEM, the electrons from heated tungsten filament ( electron gun) passes through specimen via the electromagnetic lens and the electrons that are transmitted are finally projected onto the photographic plate and the enlarged image of the object can be seen. It is helpful in the study of cell organelles which produces 2-D images.
In case of TEM, the electrons from heated tungsten filament ( electron gun) reaches the specimen via electromagnetic lens and a pair of beam deflector. Then the electrons are scattered from the surface of the specimen and secondary electrons are collected by the detector and finally projected onto the photographic plate where the enlarged image of the object can be seen. This is used to study the surface structure of cells which produces 3-D image.

## [IV] CENTRIFUGATION

Centrifugation is a mechanical process which involves the use of the centrifugal force to separate particles from a solution according to their size, shape, density, medium viscosity and rotor speed. The denser components of the mixture migrate away from the axis of the centrifuge, while the less dense components of the mixture migrate towards the axis. Chemists and biologists may increase the effective gravitational force of the test tube so that the precipitate (pellet) will travel quickly and fully to the bottom of the tube. The remaining liquid that lies above the precipitate is called a supernatant or superannuate.
There is a correlation between the size and density of a particle and the rate that the particle separates from a heterogeneous mixture, when the only force applied is that of gravity. The larger the size and the larger the density of the particles, the faster they separate from the mixture.
The rate of centrifugation is specified by the angular velocity usually expressed as revolutions per minute (RPM), or acceleration due to gravity (expressed as g ). The conversion factor between RPM and g depends on the radius of the centrifuge rotor. The particles' settling velocity in centrifugation is a function

of their size and shape, centrifugal acceleration, the volume fraction of solids present, the density difference between the particle and the liquid, and the viscosity.




## Types of Centrifuges

**Micro centrifuges** - These are light weight having small-volume rotors that are capable of very fast acceleration (approx. 17,000 rpm ) They can be used for short-time centrifugation of samples around 0.2–2.0 mL. They are portable. They can be operated in cold room. They can be refrigerated or not. Micro centrifuges designed for high-speed operation up to 35,000 rpm are called high-speed microcentrifuges.

**Low-speed centrifuges** – They are used to collect chemical precipitates, intact cells nuclei, chloroplasts, large mitochondria and larger plasma-membrane fragments. They have rotor speeds of less than 10 000 rpm. They are floor-standing centrifuges.

**High-speed centrifuges** - They are typically used to collect microorganisms, viruses, mitochondria, lysosomes, peroxisomes and intact tubular golgi membranes. They are super-speed centrifuges and can handle larger sample volumes. They have higher angular velocities (around 30,000 rpm).

**Ultra-centrifuges** - They have exceptionally high speeds (150,000 rpm). They are used to collect all membrane vesicles derived from the plasma membrane, endoplasmic reticulum and golgi membrane, endosomes, ribosomes, ribosomal subunits, plasmids, DNA, RNA and proteins in fixed-angle rotors. They can isolate much smaller particles and also separate molecules in batch or continuous flow systems. Ultracentrifugation is employed for separation of macromolecules /ligand binding kinetic studies, separation of various lipoprotein fractions from plasma and deprotonisation of physiological fluids for amino acid analysis.

- **Analytical ultracentrifugation** – They can be used for determination of the properties of macromolecules : shape, mass, composition and conformation. It is a commonly used for analysis of bio-molecules, for determination of stoichiometry subunits , for identification and characterization of macromolecular conformational changes, and for calculating equilibrium constants and thermodynamic parameters for self-associating and hetero-associating systems.

They incorporate a scanning visible /ultraviolet light-based optical detection system for real-time monitoring of the progress of a sample during a spin.

- **Preparative ultracentrifuges** - They are often used for separating particles according to their densities, isolating and/or harvesting denser particles for collection in the pellet, and clarifying suspensions containing particles. Sometimes researchers also use preparative ultracentrifuges if they need the flexibility to change the type of rotor in the instrument. Preparative ultracentrifuges can be equipped with a wide range of different rotor types, which can spin samples of different numbers, at different angles, and at different speeds.

**Differential Centrifugation** - The simplest method of fractionation by centrifugation, commonly used to separate organelles and membranes found in cells. Organelles generally differ from each other in density and in size, making the use of differential centrifugation, and centrifugation in general, possible. The organelles can then be identified by testing for indicators that are unique to the specific organelles. The most widely used application of this technique is to produce crude subcellular fractions from a tissue homogenate such as that from rat liver. Particles of different densities or sizes in a suspension are sedimented at different rates, with the larger and denser particles sedimenting faster. These sedimentation rates can be increased by using centrifugal force.

**Density Gradient Centrifugation** - Known to be one of the most efficient methods for separating suspended particles, and is used both as a separation technique and as a method for measuring the density of particles or molecules in a mixture. It is used to separate particles on the basis of size, shape, and density by using a medium of graded densities. During a relatively short or slow centrifugation, the particles are separated by size, with larger particles sedimenting farther than smaller ones. Over a long or fast centrifugation, particles travel to locations in the gradient where the density of the medium is the same as that of the particle density; $(\rho p - \rho m) \rightarrow 0$.

**Applications of Centrifugation**

- Centrifuge can be used to isolate small quantities of solids retained in suspension from liquids, such as in the separation of chalk powder from water.
- In biological research, it can be used in the purification of mammalian cells, fractionation of subcellular organelles, fractionation of membrane vesicles, fractionation of macromolecules and macromolecular complexes, etc.
- Centrifugation is used in many different ways in the food industry. For example, in the dairy industry, it is typically used in the clarification and skimming of milk, extraction of cream, production and recovery of casein, cheese production, removing bacterial contaminants, etc.
- This processing technique is also used in the production of beverages, juices, coffee, tea, beer, wine, soy milk, oil and fat processing/recovery, cocoa butter, sugar production, etc. It is also used in the clarification and stabilization of wine.
- In forensic and research laboratories, it can be used in the separation of urine and blood components. It also aids in separation of proteins using purification techniques such as salting out, e.g. ammonium sulfate precipitation.
- Centrifugation is also an important technique in waste treatment, being one of the most common processes used for sludge dewatering.
- This process also plays a role in cyclonic separation, where particles are separated from an air-flow without the use of filters.
- In a cyclone collector, air moves in a helical path. Particles with high inertia are separated by the centrifugal force whilst smaller particles continue with the air-flow.

## REFERENCES

- Ballou, David P.; Benore, Marilee; Ninfa, Alexander J. (2008). Fundamental laboratory approaches for biochemistry and biotechnology (2nd ed.). Hoboken, N.J.: Wiley. P. 43. ISBN 9780470087664.
- Consden, R.; Gordon, A. H.; Martin, A. J. P. (1944). "Qualitative analysis of proteins: A partition chromatographic method using paper". Biochemical Journal. 38 (3): 224–232. Doi:10.1042/bj0380224. PMC 1258072. PMID 16747784.
- Edward Voigtman and Patricia A. Snyder (2008). "The Noise Behavior of Polar Solvents in Laser-Excited Photoionization, "Pages: 1-13
- Frei, Mark. "Centrifugation Basics". Sigma-Aldrich. Retrieved 10 May 2016.
- Taylor and Francis "Instrumentation Science & Technology", Volume 19, Issue 1 (1990) https://www.tandfonline.com/
- Edward Voigtman and Patricia A. Snyder (2008). "The Noise Behavior of Polar Solvents in Laser-Excited Photoionization, "Pages: 1-13
- Frei, Mark. "Centrifugation Basics". Sigma-Aldrich. Retrieved 10 May 2016.
- http://www.biologydiscussion.com/biochemistry/centrifugation/centrifuge-introduction-types-uses-and-other-details-with-diagram/12489
- Kasas S, Dumas G, Dietler G, Catsicas S, Adrian M (July 2003). "Vitrification of cryoelectron microscopy specimens revealed by high-speed photographic imaging". Journal of Microscopy. 211 (Pt 1): 48–53. Doi:10.1046/j.1365-2818.2003.01193.x. PMID 12839550
- Trumbo, Toni A.; Schultz, Emeric; Borland, Michael G.; Pugh, Michael Eugene (April 27, 2013). "Applied Spectrophotometry: Analysis of a Biochemical Mixture". Biochemistry and Molecular Biology Education. 41 (4): 242–50. Doi:10.1002/bmb.20694. PMID 23625877
- Wilson, K., Walker, J. (2018). Principles and Techniques of Biochemistry and Molecular Biology. Eighth edition. Cambridge University Press: New York.