BTY 104 ANALYTICAL TECHNIQUES

UNIT - 1

MICROSCOPY:

1. Definition: Microscopy is the technique of using microscopes to view objects and details too small to be seen with the naked eye.

2. Types of Microscopes:

   - Light Microscope: Uses visible light and lenses to magnify specimens.
   - Electron Microscope: Uses a beam of electrons for higher magnification and resolution.
   - Scanning Probe Microscope: Uses a physical probe to scan the specimen's surface.

3. Objective Lens: Primary lens closest to the specimen, responsible for magnification.

4. Eyepiece (Ocular Lens): Lens closest to the observer's eye, further magnifies the image.

5. Stage: Platform where the specimen is placed for viewing.

6. Light Source: Illuminates the specimen for visibility.

Types of Microscopy Techniques:

1. Bright-field Microscopy: Basic technique where the specimen is illuminated against a bright background.

2. Phase Contrast Microscopy: Enhances contrast of transparent specimens.

3. Fluorescence Microscopy: Uses fluorescence to label and visualize specific structures within cells.

4. Confocal Microscopy: Uses laser light to create sharp images at different depths in the specimen.

5. Electron Microscopy (EM):

Transmission Electron Microscopy (TEM): Passes electrons through the specimen for high-resolution imaging.

Scanning Electron Microscopy (SEM): Scans the specimen surface with electrons to create a 3D image.

Microscopy Applications:

1. Biological Research: Studying cells, tissues, and organelles.

2. Medical Diagnostics: Identifying pathogens and abnormalities.
3. Material Science: Examining material structures at microscopic levels.
4. Forensics: Analyzing trace evidence and identifying substances.
5. Quality Control: Ensuring product quality in manufacturing processes.

Microscopy Techniques in Research:

1. Immunofluorescence: Visualizing protein distribution in cells.
2. Cryogenic Electron Microscopy (Cryo-EM): Studying biological molecules in their native state.
3. Super-Resolution Microscopy: Achieving resolutions beyond the diffraction limit of light.

Challenges in Microscopy:

1. Resolution: Limitations in distinguishing fine details.
2. Sample Preparation: Ensuring specimens are properly fixed, stained, or treated.
3. Artifact Formation: Distortions or anomalies introduced during sample preparation or imaging.

These notes cover the basics of microscopy, its types, techniques, and applications across various fields of science and technology.

PRINCIPLES OF MICROSCOPY:

The principles of microscopy encompass fundamental concepts and techniques that enable the observation and analysis of microscopic objects. Here are the key principles:

1. Magnification:

   Definition: Magnification refers to the process of enlarging an object in order to view it more clearly.

   Principle: Microscopes use lenses or mirrors to magnify the image of small objects, making them visible to the human eye.

2. Resolution:

   Definition: Resolution refers to the ability of a microscope to distinguish between two separate points or details in an image.

Principle: Higher resolution microscopes can distinguish finer details and provide clearer images. Resolution is limited by the wavelength of light (in light microscopy) or the properties of electrons (in electron microscopy).

3. Contrast:

Definition: Contrast refers to the difference in brightness and color between parts of an image.

Principle: Microscopes enhance contrast using techniques such as staining (in light microscopy) or phase contrast (for transparent specimens), which make the specimen stand out from its background.

4. Illumination:

Definition: Illumination refers to the light source used to illuminate the specimen being observed.

Principle: Proper illumination is crucial for producing a clear and detailed image. Different techniques of illumination (e.g., bright-field, dark-field, fluorescence) are used depending on the specimen and desired observation.

5. Depth of Field:

Definition: Depth of field refers to the range of distances within which objects appear sharp and in focus.

Principle: Microscopes have a limited depth of field, meaning only a small portion of the specimen is in focus at a given time. Techniques such as adjusting the aperture or using specific microscopy techniques (e.g., confocal microscopy) can control depth of field.

6. Sample Preparation:

Definition: Sample preparation refers to the techniques used to prepare specimens for microscopic observation.

Principle: Proper sample preparation, including fixation, staining, sectioning (for electron microscopy), and mounting, ensures that specimens are preserved, visible, and suitable for the chosen microscopy technique.

7. Types of Microscopy:

Principle: Different types of microscopes (e.g., light microscopy, electron microscopy, scanning probe microscopy) employ distinct principles and technologies to achieve various levels of magnification, resolution, and observation capabilities.

8. Applications:

Principle: Microscopy is applied across scientific disciplines including biology, medicine, materials science, forensics, and more. Each application leverages microscopy principles to study and analyze microscopic structures, organisms, and materials.

Understanding these principles is essential for using microscopes effectively and interpreting the data obtained from microscopic observations. Each principle contributes to the overall quality and usefulness of microscopic imaging in scientific research and practical applications

UNIT - 2

PRINCIPALS OF CENTRIFUGATION:

1. Definition:

Centrifugation is a technique used to separate particles from a suspension based on their size, shape, density, and viscosity differences under the influence of centrifugal force.

2. Centrifugal Force:

Definition: Centrifugal force is the outward force that acts on an object moving around a center, arising from its inertia.

Principle: In a centrifuge, samples are spun at high speeds, causing denser particles or substances to move outward and settle at the bottom of the tube or to be separated from lighter components.

3. Components of a Centrifuge:

- Rotor: The rotor holds the sample tubes and spins them at high speeds.
- Speed Control: Centrifuges allow control of rotational speed (rpm) to achieve desired separation forces.

Temperature Control: Some centrifuges include temperature control to maintain sample integrity during separation.

4. Types of Centrifugation:

  - Differential Centrifugation: Separates particles based on their size and density differences.
  - Density Gradient Centrifugation: Separates particles based on their density differences using a gradient of a dense substance (e.g., sucrose, cesium chloride).
  - Isopycnic Centrifugation: Separates particles solely based on their density, where particles migrate to regions of the gradient where their density matches that of the surrounding medium.

5. Applications of Centrifugation:

  - Biochemistry and Cell Biology: Isolating organelles, separating proteins, and studying molecular interactions.
  - Clinical Diagnostics: Separating blood components (e.g., plasma, serum, cells) for diagnostic tests.

6. Research: Purifying viruses, separating nanoparticles, and studying physical properties of particles

7. Factors Affecting Centrifugation:

   - Rotational Speed: Higher speeds lead to greater centrifugal forces, influencing separation efficiency.
   - Time: Longer centrifugation times allow more thorough separation of components.
   - Sample Volume and Tube Size: Larger volumes and smaller tube diameters affect separation efficiency.
   - Centrifuge Rotor Type: Fixed-angle rotors are suitable for pelleting, while swinging-bucket rotors are ideal for fractionation.

GENERAL PRINCIPLES AND APPLICATIONS OF CHROMATOGRAPHY

1. Definition:

Chromatography is a technique used to separate and analyze complex mixtures based on the differential interaction between the components of the mixture and a stationary phase as it flows over or through the stationary phase.

2. Components of Chromatography:

Stationary Phase: The immobile phase through which the sample passes. It can be solid (adsorption chromatography) or liquid (partition chromatography).

Mobile Phase: The solvent or mixture of solvents that carries the sample through the stationary phase.

Column or Plate: The medium through which the separation occurs (e.g., column in column chromatography, thin layer in thin-layer chromatography).

3. Types of Chromatography:

   - Gas Chromatography (GC): Separates volatile compounds based on their distribution between a stationary liquid phase and a mobile gas phase.
   - Liquid Chromatography (LC): Uses a liquid mobile phase to separate compounds based on their affinity for the stationary phase.
   - High-Performance Liquid Chromatography (HPLC): A type of liquid chromatography that uses high-pressure pumps to achieve faster and more efficient separations.

Thin-Layer Chromatography (TLC): Separates compounds on a thin layer of adsorbent material (e.g., silica gel) coated on a glass plate or plastic sheet.

4. Principle of Separation:

    - Components of the mixture interact differently with the stationary and mobile phases, leading to differential migration rates and separation based on factors like polarity, size, charge, and affinity.

5. Detection and Analysis: After separation, components are detected based on their physical or chemical properties (e.g., UV absorption, fluorescence, mass spectrometry) to quantify and identify them.

Applications of Chromatography:

1. Analytical Chemistry:

    - Quantitative and qualitative analysis of chemical compounds in various samples (e.g., pharmaceuticals, food, environmental samples).
    - Identification of unknown compounds and determination of purity.

2. Biochemical and Biomedical Research:

    - Separation and purification of proteins, peptides, nucleic acids, and other biomolecules.
    - Drug discovery and development, including pharmacokinetic studies.

3. Environmental Monitoring:

    - Analysis of pollutants, pesticides, and contaminants in air, water, and soil samples.

4. Forensic Science:

    - Identification and analysis of trace evidence such as drugs, toxins, and biological fluids.

5. Quality Control in Industry:

Monitoring and ensuring the quality of products in pharmaceutical, food, and manufacturing industries.

6. Clinical Diagnostics:

    - Analysis of blood, urine, and other bodily fluids for diagnostic purposes (e.g., detecting metabolites, drugs, hormones).

7. Research and Development:

    - Studying chemical reactions, kinetics, and interaction mechanisms.

Chromatography is a versatile and widely used technique in analytical and preparative sciences due to its ability to separate complex mixtures efficiently and its wide range of applications across various fields of science and industry

UNIT – 3

ELECTROPHOROSIS:

Paper Electrophorosis:

1. Definition:

Paper electrophoresis is a technique used to separate and analyze charged molecules (such as proteins, amino acids, nucleic acids) based on their mobility in an electric field through a porous paper matrix.

2. Principle:

   - The separation is based on the differential migration of charged molecules in an applied electric field. Molecules with different charge-to-mass ratios move at different speeds through the paper matrix.

3. Components of Paper Electrophoresis:

   - Paper Matrix: Typically filter paper or cellulose acetate paper, which provides a medium for the separation of molecules.
   - Buffer System: A conducting solution (electrolyte) that establishes the electric field and maintains the pH for optimal separation.

Electrodes: Anode (positive) and cathode (negative) electrodes to apply the electric field across the paper matrix.

4. Procedure:

   - Sample Application: The sample is applied near one end of the paper strip or spot on a paper disc.
   - Electrophoresis: When an electric current is applied, charged molecules migrate through the paper matrix according to their charge and size.
   - Visualization: After electrophoresis, molecules are visualized using specific stains or detection methods (e.g., ninhydrin for amino acids).

5. Types of Paper Electrophoresis:

   - Horizontal Paper Electrophoresis: The paper strip is placed horizontally and immersed in the buffer solution. It is commonly used for separation of amino acids.

- Vertical Paper Electrophoresis: The paper strip is placed vertically, and the buffer solution is drawn up by capillary action through the paper. This method is suitable for separating larger molecules like proteins.

6. Applications of Paper Electrophoresis:

- Amino Acid Analysis: Identification and quantification of amino acids in biological samples.
- Protein Analysis: Separation of proteins based on their charge and size.
- Nucleic Acid Analysis: Separation of nucleic acids (DNA, RNA) fragments for size determination and purity assessment.

Clinical Diagnostics: Detection of abnormal protein patterns in diseases (e.g., hemoglobinopathies).

7. Advantages:

- Simple and inexpensive technique.
- Suitable for qualitative and quantitative analysis of charged molecules.
- Can be used for small-scale separations in research and educational settings.

8. Limitations:

- Low resolution compared to modern gel electrophoresis techniques.
- Limited applicability for large and complex molecules due to the porous nature of paper.

Paper electrophoresis remains a valuable tool in biochemical and clinical laboratories, particularly for basic separations and educational purposes, offering insights into the principles of electrophoretic separation

Gel Electrophorosis:

1. Definition:

Gel electrophoresis is a technique used to separate and analyze macromolecules (such as DNA, RNA, and proteins) based on their size, charge, and shape under the influence of an electric field.

2. Principle:

Charged molecules migrate through a gel matrix (agarose or polyacrylamide) when an electric current is applied. The separation is based on the mobility of molecules through the gel, with smaller molecules moving faster and larger molecules moving slower.

3. Components of Gel Electrophoresis:

Gel Matrix:

- Agarose Gel: Used primarily for nucleic acid separation (DNA and RNA). The pore size of the agarose gel determines the resolution of separation. Polyacrylamide Gel: Used for protein separation due to its higher resolving power. The percentage of acrylamide determines the pore size and resolution.
- Buffer System: Provides ions for conductivity and maintains pH stability during electrophoresis.

Electrodes: Anode (positive) and cathode (negative) electrodes to apply the electric field across the gel.

4. Procedure:

   - Sample Preparation: DNA, RNA, or protein samples are treated with loading dye (containing tracking dyes and sometimes reducing agents).
   - Gel Preparation: Agarose or polyacrylamide gel is prepared and cast in a gel tray or gel cassette. Combs are used to create wells for sample loading.
   - Electrophoresis: The gel tray is submerged in a buffer-filled electrophoresis chamber. When an electric current is applied, molecules migrate through the gel matrix according to their charge and size.
   - Visualization: After electrophoresis, molecules are visualized using stains or dyes specific to the type of molecule (e.g., ethidium bromide for DNA, Coomassie blue for proteins).

5. Types of Gel Electrophoresis:

   - Agarose Gel Electrophoresis: Used for separating DNA fragments and RNA molecules based on size. Suitable for larger molecules.
   - Polyacrylamide Gel Electrophoresis (PAGE):
   - Native PAGE: Separates proteins based on charge and size under native (non-denaturing) conditions.
   - SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis): Denatures proteins by breaking non-covalent bonds and separates them primarily based on size.

6. Applications of Gel Electrophoresis:

   - DNA Analysis: DNA fingerprinting, restriction fragment length polymorphism (RFLP) analysis, polymerase chain reaction (PCR) product analysis.
   - RNA Analysis: Analysis of mRNA expression levels, RNA sequencing.
   - Protein Analysis: Protein profiling, detection of post-translational modifications, analysis of protein complexes.

7. Advantages:

- High resolution and sensitivity, capable of separating complex mixtures.
- Versatile and widely applicable across biological and biochemical research.
- Quantitative analysis possible with densitometry or imaging systems.

8. Limitations:

- Gel preparation can be time-consuming.
- Resolution and sensitivity may vary depending on gel type and conditions.
- Risk of sample diffusion or distortion during staining and visualization.

Gel electrophoresis is a fundamental technique in molecular biology and biochemistry, providing critical insights into the structure, function, and interactions of biological molecules. Its applications span from basic research to clinical diagnostics and NMR:

1. Definition:

- Nuclear Magnetic Resonance (NMR) spectroscopy is a powerful analytical technique used to study the magnetic properties of atomic nuclei. It provides detailed information about the structure, dynamics, and chemical environment of molecules.

2. Principle:

- NMR exploits the magnetic properties of atomic nuclei, particularly nuclei with an odd number of protons or neutrons (e.g., 1H, 13C, 31P). When placed in a strong magnetic field and subjected to radiofrequency pulses, these nuclei absorb and emit electromagnetic radiation at characteristic frequencies.
- The chemical environment around each nucleus affects its resonance frequency, allowing NMR to provide information about molecular structure and composition.

3. Components of NMR Spectroscopy:

- Magnet: Provides a strong, stable magnetic field (typically superconducting) for aligning atomic nuclei.
- Radiofrequency (RF) Transmitter and Receiver: Generates RF pulses for exciting nuclei and detects emitted signals during relaxation.
- Sample Holder: Contains the sample (liquid or solid) placed inside the NMR probe.
- Computer System: Controls instrument operation, processes data, and analyzes spectra.

4. Types of NMR Experiments:

- 1D NMR: Standard one-dimensional NMR spectrum that provides chemical shift information along one axis.

- 2D NMR: Two-dimensional NMR techniques (e.g., COSY, NOESY, HSQC) provide additional information about proton-proton or proton-carbon connectivity and spatial arrangements in molecules.
- Solid-State NMR: Used for studying solid materials where molecules are immobilized, providing information on structure and dynamics in solids.

5. Applications of NMR:

Structure Elucidation: Determines molecular structure and stereochemistry of organic and inorganic compounds.

- Quantitative Analysis: Measures concentrations and purity of substances.
- Protein Structure Determination: Studies protein folding, dynamics, and interactions in solution.
- Metabolomics: Analyzes metabolic pathways and biomarker identification in biological samples.
- Material Science: Studies properties of polymers, ceramics, and catalysts at atomic levels.

6. Advantages of NMR:

- Non-destructive technique that requires minimal sample preparation.
- Provides detailed structural information and molecular dynamics.o High sensitivity and specificity for different nuclei.

7. Limitations of NMR:

- Expensive instrumentation and maintenance.
- Requires specialized training for operation and interpretation.
- Sensitivity decreases with larger molecular size and lower natural abundance of NMR-active nuclei.

8. Recent Developments:

- Advances in high-field NMR spectrometers for improved resolution and sensitivity.
- Development of new pulse sequences and multi-dimensional NMR techniques for complex analyses.
- Integration with other analytical techniques (e.g., mass spectrometry, X-ray crystallography) for comprehensive molecular studies.

NMR spectroscopy remains indispensable in chemistry, biochemistry, materials science, and medicine for its ability to provide atomic-level insights into molecular structure, dynamics, and interactions

UNIT-4

RADIO ISOTOPIC TRACERS:

1. Definition:

   - Radioisotopic tracers are radioactive isotopes of elements that are used to track the movement, transformation, or location of a substance in a system. They emit radiation that can be detected and measured, providing valuable information about biological, chemical, and physical processes.

2. Principle:

   - Radioactive isotopes have unstable nuclei that decay into stable isotopes, emitting radiation in the form of alpha particles, beta particles, or gamma rays.
   - By incorporating a radioactive tracer into a molecule or substance of interest, researchers can monitor its behavior within a biological system, chemical reaction, or environmental process.
   - Detection and quantification of the emitted radiation provide insights into the kinetics, pathways, and interactions involving the tracer.

3. Types of Radioisotopic Tracers:

   - Carbon-14 (^14C): Used in carbon dating and tracking the metabolism of carbon-containing molecules in biological systems.
   - Hydrogen-3 (Tritium, ^3H): Used to label organic compounds and study metabolic pathways in biological research.
   - Phosphorus-32 (^32P): Used to label nucleotides and phospholipids in biochemical studies.
   - Sulfur-35 (^35S): Used for labeling proteins and studying protein synthesis in molecular biology.
   - Iodine-125 (^125I) and Iodine-131 (^131I): Used in medical imaging (e.g., thyroid scans) and biological assays.

4. Applications of Radioisotopic Tracers:

   - Biological Studies: Tracking the uptake, distribution, and metabolism of nutrients, drugs, and biomolecules in living organisms.
   - Chemical Kinetics: Studying reaction mechanisms, rates, and pathways in chemical processes.

- Environmental Monitoring: Tracing the movement and fate of pollutants, nutrients, and contaminants in ecosystems.
- Medical Diagnostics: Imaging and diagnosing diseases (e.g., thyroid disorders, cancer) using radiopharmaceuticals.

5. Advantages of Radioisotopic Tracers:

    - High sensitivity and specificity in detecting low concentrations of labeled compounds.
    - Non-invasive and minimal disruption to biological systems.
    - Wide applicability across disciplines including biology, chemistry, medicine, and environmental science.

6. Safety Considerations:

    - Radioactive materials must be handled with care to minimize exposure and ensure proper disposal.
    - Regulatory guidelines and safety protocols govern the use, storage, and disposal of radioisotopes to protect researchers and the environment

7. Limitations:

    - Short half-lives of some radioisotopes require rapid detection and analysis.
    - Potential hazards associated with radiation exposure necessitate strict adherence

    to safety protocols.

8. Future Directions:

    - Development of new radioisotopes with longer half-lives and specific emission characteristics for advanced applications.
    - Integration of radioisotopic tracers with imaging techniques and computational models for more detailed and dynamic analysis.
    - Radioisotopic tracers continue to play a crucial role in advancing scientific knowledge and technological innovation, offering unique insights into complex biological, chemical, and environmental processes.

LYOPHILIZATION

1. Definition: Freeze drying, also known as lyophilization, is a dehydration process used to preserve perishable materials, particularly biological substances, by removing water content under low temperature and pressure conditions.

2. Principle:

   - Freeze drying utilizes the principle of sublimation, where water transitions directly from a solid (ice) to a gas (vapor) phase without passing through a liquid phase.
   - The process involves freezing the material to form ice crystals, followed by placing it under vacuum to allow the ice to sublime, leaving behind a dried product with minimal damage to its structure and activity.

3. Steps Involved in Freeze Drying:

   - Freezing: The biological material is rapidly frozen to form ice crystals. Controlled freezing helps minimize damage to delicate structures.
   - Primary Drying (Sublimation): The frozen material is placed in a vacuum chamber, and the temperature is raised slightly. Ice sublimes under reduced pressure, removing water vapor.

Secondary Drying (Desorption): Remaining bound water is removed by raising

   - the temperature further, ensuring complete drying without re-absorption of moisture.

4. Components of Freeze Drying Equipment:

   - Vacuum Chamber: Provides controlled pressure conditions for sublimation.
   - Condenser: Collects and traps water vapor released during sublimation.
   - Heat Source: Provides gentle heating to facilitate sublimation and secondary drying.
   - Controlled Cooling System: Allows precise temperature control during freezing and drying phases.

5. Applications of Freeze Drying in Biological Systems:

   - Pharmaceuticals: Preserves vaccines, antibiotics, proteins, and other drugs without loss of potency.
   - Biological Samples: Extends shelf life of enzymes, antibodies, cell cultures, and tissues for research and clinical use.
   - Food Preservation: Produces lightweight, shelf-stable food products (e.g., instant coffee, freeze-dried fruits) with preserved nutritional quality.
   - Diagnostic Kits: Stabilizes reagents and biological components in diagnostic tests for accurate and reliable results.

- Cosmetics: Enhances stability and shelf life of active ingredients in skincare and beauty products.

X-RAY DIFFRACTION:

1.Definition:

- X-ray diffraction (XRD) is a technique used for analyzing the atomic and molecular structure of crystalline materials by measuring the diffraction patterns produced when X-rays interact with the crystal lattice.

2.Principle:

- X-rays are electromagnetic waves with wavelengths comparable to atomic spacings in crystals. When X-rays strike a crystalline material, they are scattered by the atoms in the crystal lattice, producing constructive and destructive interference patterns (diffraction patterns).
- The angles and intensities of these diffraction patterns are related to the atomic arrangement within the crystal lattice, allowing determination of crystal structure, phase identification, and crystallographic orientation.

3.Components of XRD Instrumentation:

- **X-ray Source:** Typically a copper (Cu) or cobalt (Co) X-ray tube that generates X-rays with a specific wavelength.
- **Sample Holder:** Holds the powdered or crystalline sample in a fixed orientation for X-ray exposure.
- **Detector:** Records the intensity and position of diffracted X-rays.
- **Goniometer:** Rotates the sample and detector to collect diffraction data at different angles.
- **Computer System:** Controls instrument operation, collects data, and performs data analysis.

4.Types of XRD Techniques:

- **Powder X-ray Diffraction (XRPD):** Analyzes powdered samples to determine crystal structure and phase composition.
- **Single-Crystal X-ray Diffraction:** Determines the complete three-dimensional atomic structure of a single crystal.
- **Thin-Film X-ray Diffraction:** Analyzes the crystal structure and orientation of thin films deposited on substrates.

5. Applications of XRD:

- **Phase Identification:** Determines the crystal structure and phase composition of materials (e.g., minerals, metals, ceramics).
- **Quantitative Analysis:** Measures the relative amounts of phases present in asample.
- **Crystal Structure Determination:** Provides precise atomic positions and unit cell parameters of crystalline materials.
- **Texture and Grain Orientation:** Studies the orientation and texture of crystalline grains in polycrystalline materials.
- **Stress and Strain Analysis:** Measures lattice parameters and assesses residual stresses in materials.

6. Advantages of XRD:

- Non-destructive technique that requires minimal sample preparation.
- Provides detailed information about crystal structure, phase purity, and atomic arrangements.
- High sensitivity to changes in crystallographic parameters.

7. Limitations of XRD:

- Requires crystalline samples; amorphous materials do not produce diffractionpatterns.
- Sensitivity to sample orientation and surface roughness.
- Resolution limitations for closely spaced peaks in complex diffraction patterns.

8. Recent Developments:

- Advances in high-resolution X-ray detectors and synchrotron radiation sources for enhanced data collection.
- Development of in-situ and operando XRD techniques for studying dynamic structural changes in materials under different conditions.
- Integration with computational methods for more accurate interpretation and modeling of diffraction data.

X-ray diffraction is widely used in materials science, geology, chemistry, and physics for its ability to provide detailed structural information about crystalline materials, aiding in fundamental research, materials characterization, and technological development.