PHARMACOGNOSY-II

UNIT 5 NOTES

BASIC OF PHYTOCHEMISTRY

- Modern Methods of Extraction
- Applications of Spectroscopy
- Chromatography
- Electrophoresis
- Identification of Crude Drugs

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PHYTOCHEMISTRY

- Phytochemistry is the branch of chemistry concerned with the study of chemical compounds produced by plants, including their structures, functions, biosynthesis and roles in plant growth, metabolism and defense.
- It focuses on identifying and analyzing bioactive compounds such as Alkaloids, Flavanoids, Terpenes, tannis etc which have application in agricultural & pharmaceutical industry.

EXTRACTION

- Extraction is the process of isolating bioactive compounds from plant material using solvents like water, ethanol, hexane etc.
- Two major component of extraction :

  Marc : Solid residue obtain after extraction
  Menstruum : Solvent used for extraction

Importance Of Extraction

- Isolation of active ingredients.
- Purification of compounds
- Concentration of solutions
- Removal of Impurities
- Improvement of Product Quality.

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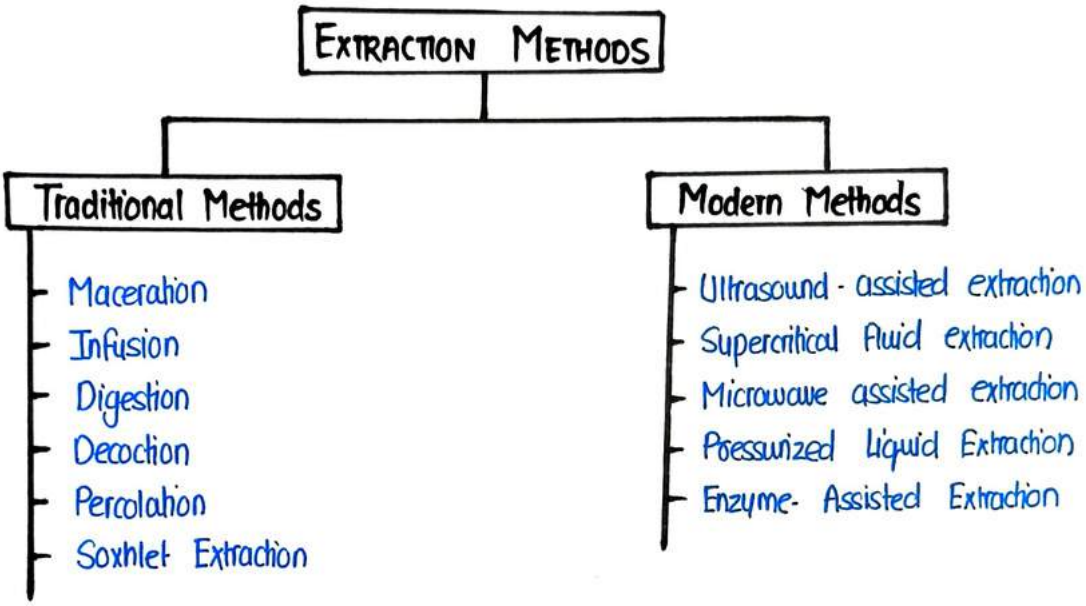
Methods Of Extraction

It involves following methods



Properties Of Solvents Used For Extraction

- Be highly selective for compound to be extracted
- Not react with extracted compound or with other compound in the plant material.
- Have a low price.
- Harmless to the environment
- It should be stable.

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MACERATION

- Maceration is the process of extraction where solid material, such as plant tissue, is soaked in a solvent, such as water or ethanol to extract the desired compounds.
- The goal of maceration is to break down the cell wall & release the compound of interest into the solvent.

Process of Maceration

- The plant material is cleaned, dried and crushed or chopped to increase its surface area.
- A suitable solvent is selected based on type of compounds to be extracted.
- The plant material is soaked in the solvent for at least three to seven days in a warm place with frequent shaking.
- The mixture is filtered to separate the solvent from the plant material
- The solvent is concentrated through evaporation or distillation to obtain a concentrated extract.

Application Of Maceration

- Pharmaceutical Industry
- Food Industry
- Cosmetics Industry.

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PERCOLATION

- Percolation is a widely used method for extracting soluble compounds from solid materials, particularly in the herbal and pharmaceutical industry.
- The process involves passing a solvent through solid material to dissolve and carry out the desired constituents.
- The process involves use of an instrument known as Percolator

PROCESS OF PERCOLATION

It involves following steps:

- Preparation of the Material
- Packing the Percolator
- Addition of Solvent
- Percolation Process
- Collection of Percolate
- Post - Processing

① Preparation of the Material

- The solid material (i.e., plant material) is first reduced to a specific size to increase surface area and enhance solvent penetration.
- The material is moistened with a small amount of the solvent to soften and swell the particles, ensuring uniform solvent flow.

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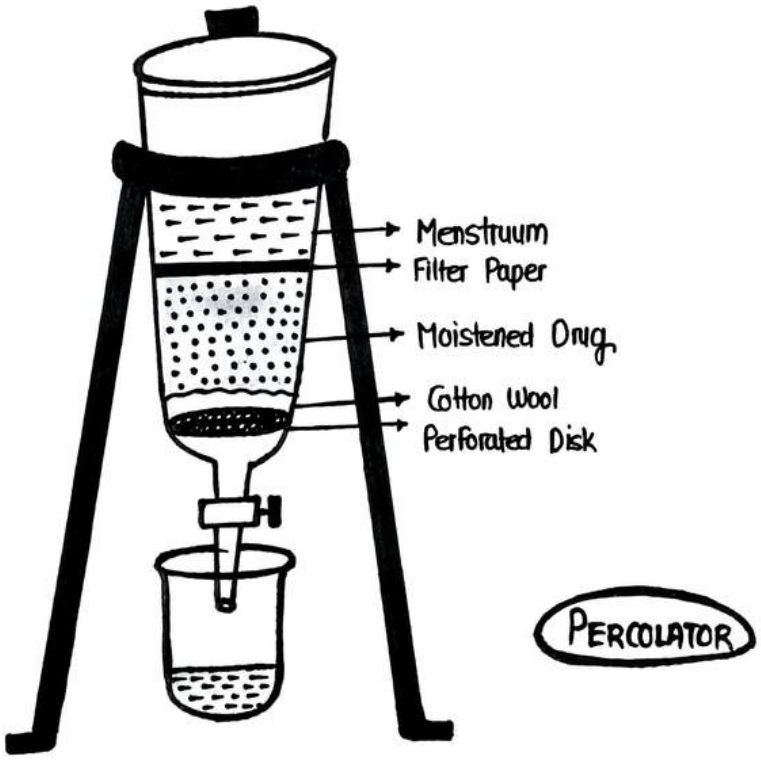
② Packing the Percolator

- The moistened material is packed into a cylindrical vessel called Percolator.
- Care is taken to ensure the material is packed evenly and moderately to avoid excessive compaction.



③ Addition of Solvent

- A suitable solvent (mainstruum) is added from the top of percolator.
- Common solvents include water, alcohol or hydroalcoholic mixtures depending on solubility of target compound.

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④ Percolation Process

- The solvent percolates through the material under gravity, extracting soluble compound ast it moves down.
- The solvent continuously dissolves the desired constituents from the material.
- The process is influenced by nature of the solvent, temperature and solubility of compounds.

⑤ Collection Of Percolate

- The extract is collected at the bottom of the percolate.

⑥ Post - Processing

- The collected percolate may undergo further processing, such as concentration, evaporation or filtration to isolate active ingredient

APPLICATION OF PERCOLATION

- Pharmaceuticals
- Food Industry
- Perfume Industry

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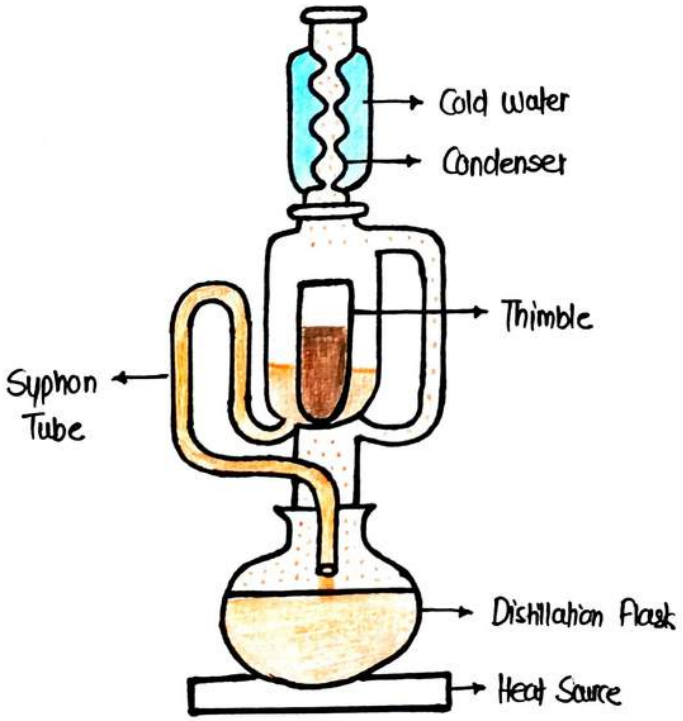
SOXHLET EXTRACTION

- Soxhlet Extraction is a widely used technique for extracting specific compounds from solid materials using a solvent.
- It is particularly effective when the desired compound has limited solubility in solvent or solid contains a mixture of compounds with different solubilities.

PRINCIPLE

- The methods works on the principle of repeated extraction and condensation.
- The solvent dissolves the desired compound from solid matrix and this process is repeated multiple times to ensure complete extraction.
- The solvent is continuously recycled, minimizing its usage.



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Process Of Soxhlet Extraction

It involves following steps :

- Preparation of sample
- Assembly of Apparatus
- Heating
- Condensation
- Extraction
- Recycling
- Post Processing

Application

- Pharmaceuticals
- Food Industry
- Environmental Studies
- Material Science

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INFUSION

- Infusion extraction is a method of extracting soluble components from plant material by soaking them in a hot or cold water for a short period of time.
- This process is commonly used to prepare herbal teas or medicinal preparations

DECOCTION

- Decoction extraction is a method of extracting water-soluble, heat stable compounds from tough plant materials such as roots, barks or seeds by boiling them in water.
- It is mainly used to prepare teas or extracts rich in alkaloids, tannins or glycosides.

DIGESTION

- Digestion is a process of extracting soluble compounds from solid materials by using a solvent at moderate temperature below boiling point for prolonged period.

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ENZYME - ASSISTED EXTRACTION

- Enzyme Assisted Extraction is an advanced extraction method that uses enzymes to break down cell walls, membranes or complex molecules in plant or animal materials, enhancing the release of desired bioactive compounds into a solvent.

PRINCIPLE

Enzymes such as cellulases and proteases selectively degrade structural components (e.g. cellulose, lignin, proteins) of the material, making the intracellular compounds more accesible for extraction.

STEPS

- Preparation : The material is pre treated to increase surface area.
- Enzyme Treatment :
  - A suitable enzyme or enzyme mixture is added to the material in a solvent.
  - The mixture is incubated under optimal conditions.
- Extraction: Enzymatic action breaks down the structural barriers, releasing bioactive compounds into the solvent.
- Filtration : The extract is separated from solid residue.

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ADVANTAGES

- Eco friendly & uses mild conditions.
- Increases extraction efficiency.
- Reduces solvent and energy consumption.
- Preserves heat - sensitive compounds.

APPLICATION

- Extraction of phenolics, flavanoids and polysaccharides from plants.
- Recovery of proteins, lipids and bioactives in food and neutraceutical industries.
- Extraction of essential oils.

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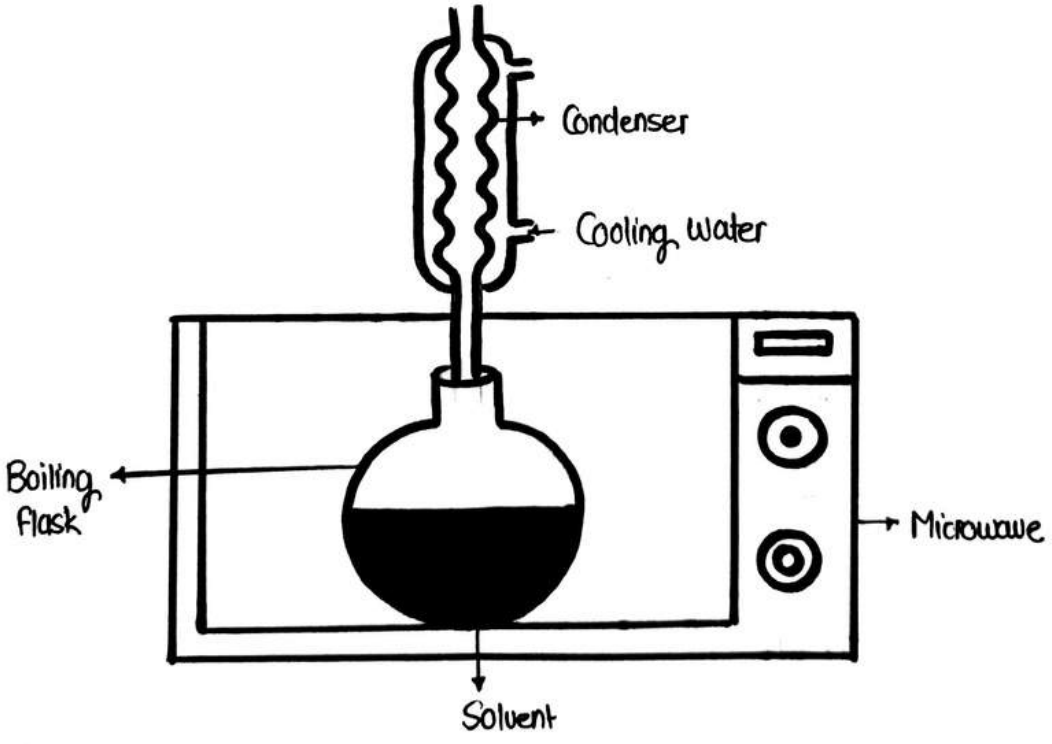
MICROWAVE - ASSISTED EXTRACTION

- Microwave- Assisted Extraction is a technique that uses microwave energy to heat the solvent and the material being extracted.
- This accelerates the release of bioactive compounds by breaking down cell walls and improving solvent penetration.

PRINCIPLE

Microwave- Assisted Extraction is a technique that uses microwave energy to enhance the extraction of compounds from solid matrix such as plant material.



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STEPS IN MAE

It involves following steps :

- **Preparation :** The material is dried and ground to desired size.
- **Mixing :** The material is mixed with a suitable solvent in a microwave - safe container.
- **Microwave Irradiation :**
  - The mixture is exposed to microwave radiation for a specific time & power level.
  - The solvent absorbs the heat, enhancing extraction.
- **Filtration :** The mixture is filtered to separate the extract from solid residue.

ADVANTAGES

- Rapid extraction
- High Efficiency
- Preserves Heat sensitive compounds due to controlled temperature.

APPLICATIONS

- Extraction of polyphenols, essential oils.
- Isolation of bioactive compounds.
- Extraction of lipids & flavours.

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CHROMATOGRAPHY

- Chromatography is a laboratory technique used to separate mixtures of substances into their individual component.
- In pharmacognosy, it is an essential technique used for isolation, identification and analysis of bioactive compounds derived from natural sources, such as plant, fungi or microorganisms.

TYPES OF CHROMATOGRAPHY

Some most common chromatographic techniques used in pharmacognosy are as follows:

- Thin-Layer Chromatography
- High Performance Thin Layer Chromatography
- High Performance Liquid Chromatography
- Gas Chromatography
- Column Chromatography

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THIN LAYER CHROMATOGRAPHY

- Thin Layer Chromatography is a simple and versatile analytical technique used in pharmacognosy to separate and identify components in a mixture.
- It is crucial for evaluating quality, purity and consistency of herbal products.

PRINCIPLE

The principle of Thin Layer Chromatography relies on the differential affinity of components for stationary phase & mobile phase.

PROCEDURE

- Sample Preparation: The plant material or extract is dissolved in a suitable solvent.
- Stationary Phase: The TLC plate is coated with a thin layer of adsorbent material (typically silica gel or alumina) This acts as the stationary phase where compounds in the sample will interact & separate.
- Application of Sample: A small amount of prepared sample is applied as a spot or band on TLC plate
- Development: The TLC plate is placed in a developing chamber with a solvent that moves up the plate via capillary action.
- Visualisation: After development, plate is dried and separated compounds are visualized using a suitable detection method.

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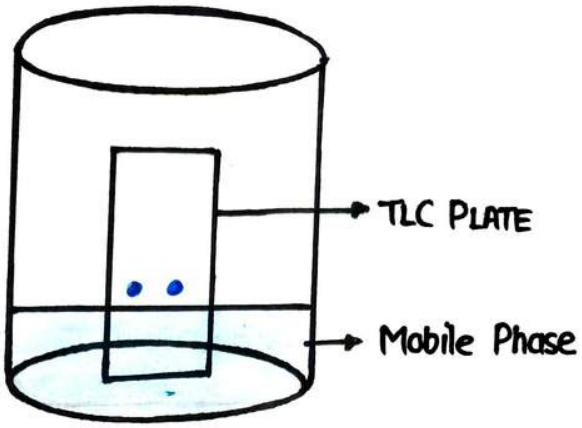

ADVANTAGES OF TLC

- Simplicity
- Low Cost
- Versatility
- Quick Results

LIMITATIONS OF TLC

- Less Quantification
- Less Sensitivity

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HPTLC

- HPTLC (High Performance Thin Layer Chromatography) is an advanced form of traditional Thin-Layer Chromatography used for separating and analyzing complex mixture of chemical substances.
- It is a highly efficient, reproducible and automated technique widely used in various industries, including pharmaceuticals, food cosmetics & environmental sciences.

PRINCIPLE

HPTLC is based on principle of affinity of sample towards Mobile Phase and Stationary Phase.

CHARACTERISTICS

- High Resolution Separations: HPTLC provides high-resolution separations of complex mixtures, allowing for detection & quantification of multiple components.
- Improved Sensitivity: HPTLC is more sensitive than traditional TLC, allowing for the detection of smaller amounts of substances.
- Increased Accuracy: HPTLC provides more accurate results than traditional TLC, due to use of precise instrumentation and standardized methods.

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Components Of HPTLC

- Automatic Sample Applicator
- Automatic Development Chamber
- Detection Chambers
- Densitometer [scanner]
- Photo Documentation Unit

Advantages

- High-resolution separations
- Improved sensitivity & accuracy
- Faster- analysis times
- Cost- effective
- Easy to use

Disadvantages

- Limited Automation
- Requires specialized instrumentation.

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HPLC

High-Performance Liquid Chromatography (HPLC) is a powerful analytical technique used to separate, identify and quantify individual components within a complex mixture.

Importance Of HPLC In Pharmacognosy

- Compound Identification : It helps in identifying active phytoconstituents like alkaloids, flavanoids, glycosides, terpenes & phenolic compounds.
- Quantitative Analysis : Accurate quantification of these compounds is essential for standardization and quality control of herbal medicines.
- Purity Assessment : Determines the purity of extracts and isolates from natural products.

Key Features Of HPLC

- High sensitivity & precision
- Versatility
- Reproducibility
- Speed & Efficiency

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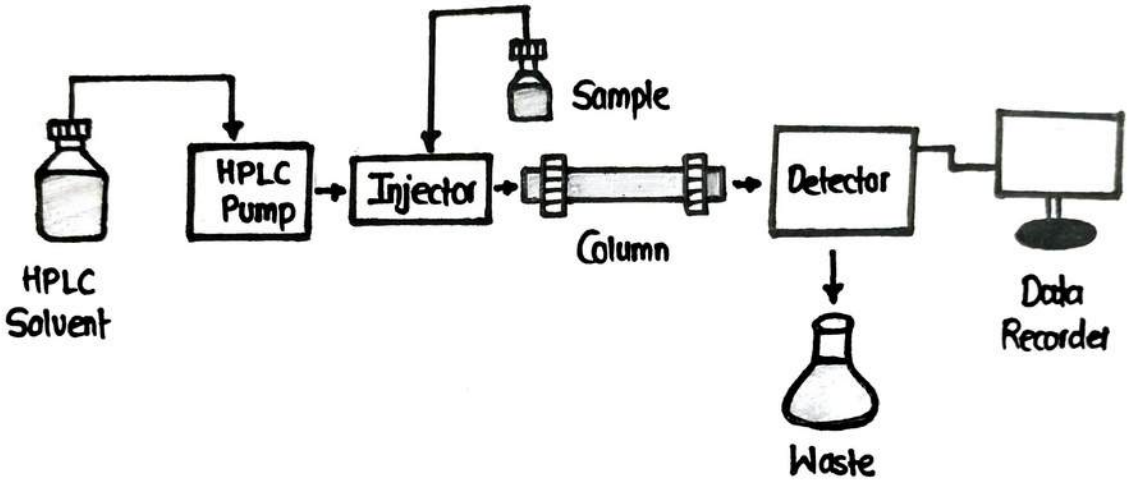
Components Of HPLC System

- Pump : Delivers the mobile phase at high pressure
- Injector : Introduces the sample into the system
- Column : Packed with stationary phase for separation of compounds
- Detector : Identifies & quantifies the separated compounds.
- Data System : Software for data collection and analysis.



Application In Pharmacognosy

- Standardization of herbal formulations.
- Identification of bioactive compounds.
- Quality control of raw plant materials & finished products.
- Monitoring chemical stability during storage.

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SPECTROSCOPY

- A Spectroscopic method of analysis is a technique used to study and identify substances by examining how they interact with light.
- Different substances absorb, emit or reflect light in unique ways, depending on their chemical structure.
- By measuring these interactions, scientists can figure out what a substance is made of & how much of each component is present.

TYPES OF SPECTROSCOPY

There are various types of spectroscopic techniques as follows:

- UV Visible Spectroscopy
- IR Spectroscopy
- Mass Spectroscopy
- NMR Spectroscopy
- Atomic Absorption Spectroscopy

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UV-VISIBLE SPECTROSCOPY

- UV- Visible Spectroscopy is an analytical technique used to analyze the interaction of Ultraviolet & visible light with sample.
- It is a simple & widely used analytical technique in analysis of phytoconstituents in pharmacognosy.

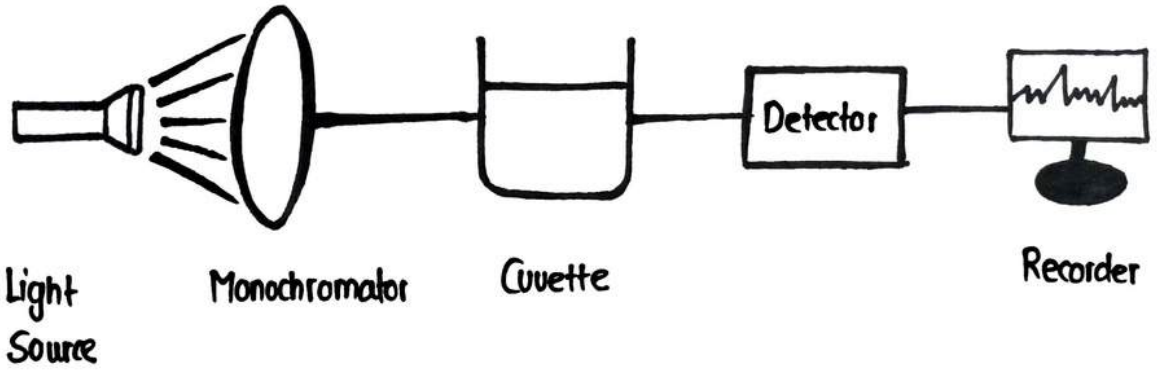
PRINCIPLE

- When a molecule absorbs UV or visible light, it undergoes electronic transition, means its electrons move from a lower energy level to a higher one.
- The specific type & amount of light absorbed by the sample is the measure of its concentration & qualitative properties.

INSTRUMENTATION IN UV-VISIBLE SPECTROSCOPY

- Light Source : UV lamps (for UV range) and tungsten lamp (for visible range) are used to provide light across the required spectrum
- Monochromator : It is a device that selects a specific wavelength of light from the source.
- Sample Holder : A cuvette, typically made of quartz, hold the sample.
- Detector : It measures the intensity of light that passes through the sample.

- Data Output : Device that shows the absorbance of UV & visible light.



APPLICATION OF UV VISIBLE SPECTROSCOPY

- Identification & characterization of Active compounds.
- Quantification of bioactive compounds
- Standardization of Herbal Medicines
- Analysis of Plant Extracts

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ELECTROPHORESIS

- Electrophoresis is a laboratory technique used to separate charged molecules, such as proteins, DNA or RNA based on their size, charge and shape by applying an electric field.
- When an electric current is applied to a sample in a medium the molecules move towards the electrode with the opposite charge.
- Molecules with greater charge or smaller size move faster, while those with less charge or larger size move slowly.
- This results in the separation of different components of the sample.

APPLICATION OF ELECTROPHORESIS

DNA Fingerprinting
Forensic Science
Medical Diagnosis
Protein Analysis

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