

ONE shot COMPLETE CONCEPT



B.PHARMA | 5 SEMESTER

BASICS OF PHYTOCHEMISTRY

Spectroscopy | Chromatography | Electrophoresis



UNIT - 5

B. PHARMA 5TH SEM ONE SHOT NOTES

UNIT-5 BASICS OF PHYTOCHEMISTRY

EXTRACTION

- The process of separating medicinally active constituents of plant and animal tissue with the help of selective solvent & a standard procedure is called extraction.
- **Marc** after filtration of extraction the undissolved residue left behind is termed as marc.
- **Menstrum** the selective solvent is called menstrum.

Steps of extraction

- The solvent penetrates the drug.
- The drug constituents dissolved in solvent.
- The solution diffuses out.
- The dissolve portion separate from exhausted drug.

Choice of solvent

- Ideal solvent
- Economical
- Should be stable physically & chemically
- Non- toxic
- Should be selective in nature
- Not too volatile
- **Example-** H₂O, acetone, alcohol, HCl

MODERN METHODS OF EXTRACTION

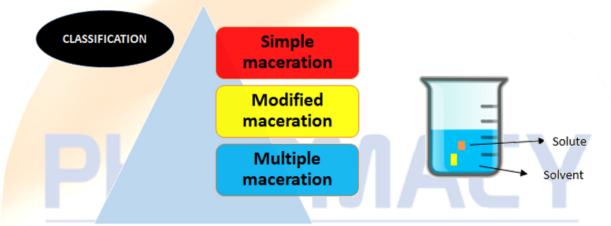
- Maceration
- Digestion
- Percolation
- Conventional methods
 - Continuous hot extraction
 - Super critical fluid extraction
 - Counter current extraction
- Modern method
 - Microwave assisted extraction
 - Ultrasonic assisted extraction
 - o Infusion & decoction

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- Pressure, cooker extraction
- Extraction by passing through colloidal
- Use of surface-active agents in drug extraction
- Expression

MACERATION

- The maceration process is used for producing tinctures, extracts, and concentrated infusions.
- It is the simplest method of crude drug extraction which was official in I.P. 1966.



DIGESTION

- It is the modified maceration process.
- It involves extraction at a high temperature which do not affect the active ingredient
- Temperature enhances the solvent action.

PERCOLATION

- The term percolation has been derived from Greek word percolare which means to pass through.
- It involves extracting the constituents of Granulated or powdered drug by slowly passing down through it a suitable menstrum.
- The menstruum while travelling down the drug Column under the influence of gravity, extract the Drug particle layer-wise.
- Percolation method achieves drug extraction.

Principle

- No fine powder
- No large powder
- Medium particle are:
 - \circ More contact



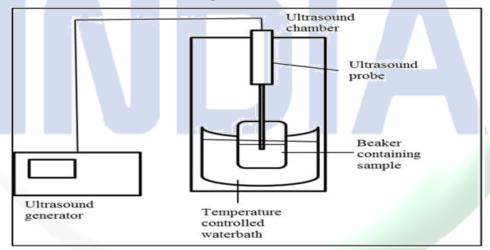
- More surface
- $\circ \quad \text{More extraction} \quad$
- Before percolation drug must be moist by solvent.

CONVENTIONAL METHODS

- The apparatus for continuous hot extraction used on a laboratory scale, consists of a flask, a Soxhlet extractor, and a reflux condenser.
- This apparatus will use a slightly higher extraction temperature Than the Soxhlet in which a small fraction of heat from the material bed is lost in the surroundings.
- The condensed menstruum percolates through the drug and Drops into the flask without collecting until syphoning.
- The maceration period of the drug with the hot menstruum is lost in this apparatus.

ULTRASONIC ASSISTED EXTRACTION

- The range of human hearing is form 16-18KHz.
- Ultrasound is the sound waves whose frequencies are higher than those to which the human ear can Respond.
- There are many natural products which are thermally Unstable and which degrade during thermal extraction.
- Due to this reason, recently UAE is considered as an Efficient alternative to various conventional thermal Extraction processes.



MICROWAVE ASSISTED EXTRACTION

- Microwave-assisted extraction is an efficient method that involves deriving natural compounds from raw plants.
- Microwave extraction allows organic compounds to be extracted more rapidly, with similar or better yield as compared to conventional extraction methods.

Procedure

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- ✓ When plant material is immersed inside a microwave transparent solvent, the heat of microwave radiation directly reaches to the solid without being absorbed by the solvent, resulting in instantaneous heating of the residual moisture in the solid.
- ✓ Heating causes the moisture to evaporate and creates a high vapour pressure that breaks the cell wall of substrate and releases the content into solvent.
- ✓ The extracting selectivity and the ability of the solvent to interact with microwaves can be modulated by using mixtures of solvents.
- ✓ One of the most commonly used mixtures is hexane-acetone.

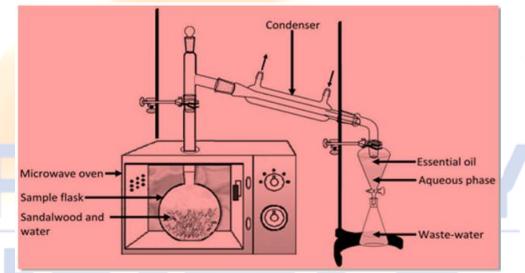


Figure: Instrument used for microwave assisted extraction

INFUSION & DECOCTION

- These methods are now rarely used.
- Infusions were prepared from vegetable drugs with water- soluble and easily extractable constituents
- And decoction process was used for extracting vegetable drugs with water-soluble and heat – soluble constituents.
- In decoction the drug was boiled with water, cooled, expressed, the liquid was strained and desired volume was made
- In infusion the drug was moistened with water, macerated with boiling water, the liquid was strained and desired volume was made

PRESSURE, COOKER EXTRACTION

- This method, the drug is initially macerated with the menstruum and then is held for 5-15 min. in a pressure cooker at 151b/sq.inch pressure.
- The cooker is then cooled and the extract is removed by straining and pressing the marc.

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This method achieves complete drug extraction in comparatively less time.

TECHNIQUES USED IN THE ISOLATION, PURIFICATION AND IDENTIFICATION OF CRUDE DRUGS

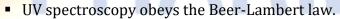
- □ Spectroscopy
- □ Chromatography
- **D** Electrophoresis

SPECTROSCOPY

- Spectroscopy is the study of the interaction between matter and electromagnetic radiation
- Spectroscopy can involve any interaction between light and matter, including absorption, emission, scattering, etc.

UV-VISIBLE SPECTROSCOPY

 Ultraviolet & visible absorption technique involve the analytical methods which measure the light absorption lying in the wavelength region from 190-900nm by different substances.



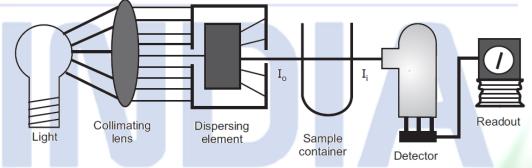


Figure: Diagrammatic representation of UV -Visible spectrophotometer

IR SPECTROSCOPY

 The IR spectroscopy is used for the identification of many functional groups present in the compound, as different functional groups have specific absorbance in the electromagnetic spectrum.

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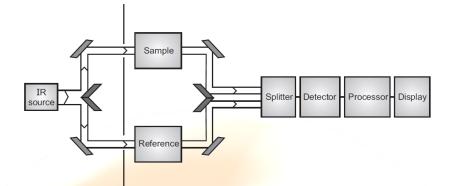


Figure: Diagrammatic representation of Infra-red spectrophotometer apparatus

CHROMATOGRAPHY

- It is a technique which are used in the isolation, purification & identification of crude drugs.
- Basic principle-
- Chromatography=from Greek chroma "color and graphein "to write".
- The mixture is dissolved in a fluid called the mobile phase, which carries it through a structure holding another material called the stationary phase.
- The separation is based on differential partitioning between the mobile and stationary phases.

Application-

- ✓ To identify the actual components in complex mixtures.
- ✓ To isolate separate the component form the mixture.
- ✓ To determine the quantity of active constituent present in mixture.
- ✓ Also helps to check the quality of drugs by comparing with standard.
- ✓ Overall, the main role of chromatography is to purity the drugs.

Types of chromatography

- 1. Paper chromatography
- 2. Thin layer chromatography
- 3. High performance thin layer chromatography
- 4. Gas chromatography
- 5. High performance liquid chromatography

PAPER CHROMATOGRAPHY

- In this technique sheet of paper is used to analysis of unknown substance
- Cellulose layer in filter paper contain moisture which acts as stationary phase & organic solvents are used as mobile phase.

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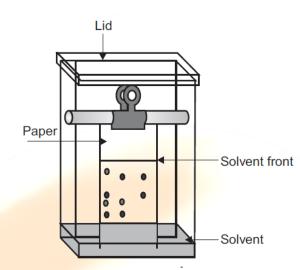


Figure: Diagrammatic representation of paper chromatography

THIN LAYER CHROMATOGRAPHY

- It is used to identify the phytoconstituents & also used for qualitative determination of plant extracts.
- It is widely used technique & is similar to paper chromatography.

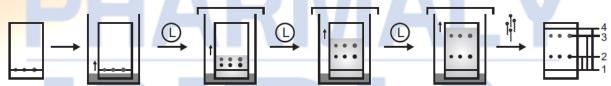
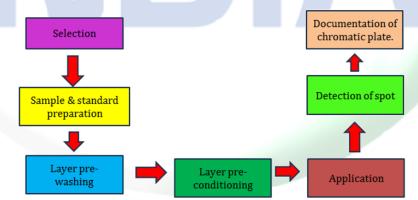


Figure: Process involved in Thin layer chromatography

HPTLC [HIGHPERFORMANCE THIN LAYER CHROMATOGRAPHY]

• It has similar approach & employed the same physical principles of TLC [adsorption chromatography].



HPLC [HIGH PERFORMANCE LIQUID CHROMATOGRAPHY]

• High performance liquid chromatography (HPLC) was used to analyze the phytochemical profile of the extracts.

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- One of the most convenient and comprehensive analytical technique for separating individual components in plant extracts.
- The HPLC technique has potential importance for quantification, quality control of herbal products.

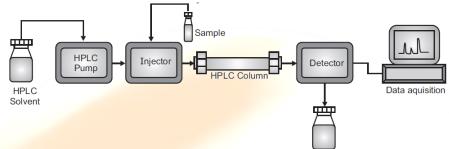


Figure: Diagrammatic representation of High-performance liquid chromatography **ELECTROPHORESIS**

- Electrophoresis is a technique used to separate macromolecules in a fluid or gel based on their charge, binding affinity, and size under an electric field.
- It takes a short time for analysis.
- It require less amount of sample and electrolyte.
- It is easy to operate.
- It generates less waste.

Types of Electrophoresis

- Zone Electrophoresis
- Moving boundary electrophoresis

ZONE ELECTROPHORESIS

- In zone electrophoresis the charged molecule or ion moves on the supporting media like gel, paper etc.
- Example of zone electrophoresis are:
 - ✓ Paper electrophoresis
 - ✓ Gel electrophoresis
 - ✓ Thin layer electrophoresis
 - ✓ Cellulose acetate electrophoresis

MOVING BOUNDARY ELECTROPHORESIS

- In the moving boundary electrophoresis the charged molecule can move freely in a free moving solution.
- There is no supporting media like gel or paper required.
- Examples are
 - ✓ Capillary electrophoresis

- ✓ Isotacto electrophoresis
- ✓ Isoelectric focusing
- ✓ Immuno electrophoresis

PAPER ELECTROPHORESIS

- This technique can be useful for the separation of amino acids, small proteins or small charged molecules.
- The paper strips moist with buffer and the end of this paper strip dipped into buffer solution which contain electrode.

GEL ELECTROPHORESIS

- Just like the other electrophoresis techniques it also involves the electric field.
- Those phytoconstituents which have to be separated are introduced into the gel under electric field.
- The gel contains the pore through which phytoconstituents have to move.

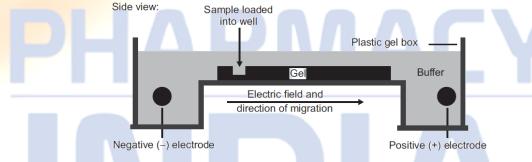


Figure: Diagrammatic representation of Gel electrophoresis

CAPILLARY ELECTROPHORESIS

- In the capillary electrophoresis separation takes place inside the capillary having internal diameter 10 to 100 μ m.
- The ends of fused silica capillary tube dip into the buffer solution.
- It contains platinum electrode (cathode and anode).
- Capillary tube contain optical window for detection which attached with UV detector.

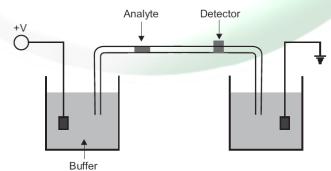


Figure: Diagrammatic representation of Capillary electrophoresis

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