1.3 Instruments used in Pharmacognosy

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Compound Microscope

- Used for the study of structural details of a cell/tissue/organ
- Provides high magnification upto 1500X
- The term compound applies to multiple/complex consists of 2 lenses
- Objective lens (4x, 10x, 40x or 100x) & Eyepiece or Occular lens (10x, 15X)

Zacharias Jansen created a compound microscope that used collapsing tubes and produced magnifications up to 9X.

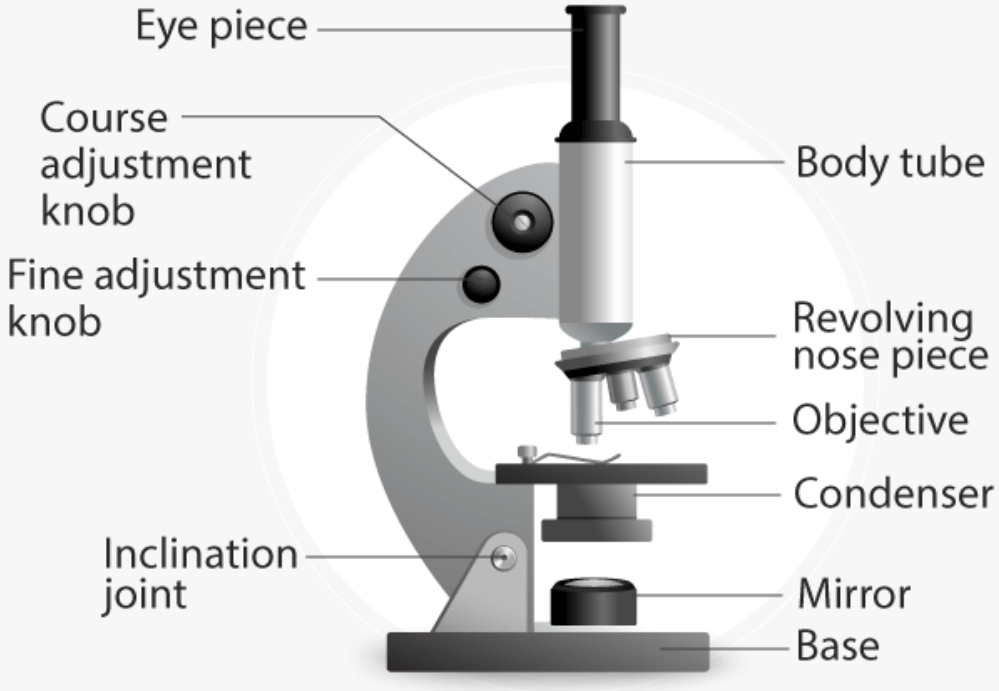
The three basic structural components -

Head/Body houses the optical parts in the upper part of the microscope

- **Base** of the microscope supports the microscope and houses the illuminator
- **Arm** connects to the base and supports the microscope head. It is also used to carry the microscope.

COMPOUND MICROSCOPE

BYJU'S The Learning App



Optical Components

1. **Eyepiece or Occular -** at the top of the microscope; varying powers from 5x to 30x.

1. **Eyepiece Tube** holds the eyepieces in place above the objective lens.

1. **Objective Lenses** are the primary optical lenses on a microscope. Standard objectives include 4x, 10x, 40x and 100x

1. **Nosepiece -** objectives are mounted on a rotating turret so that different objectives can be conveniently selected.

1. **Coarse and Fine Focus knobs -** are used to focus the microscope; they are built on the same axis with the fine focus knob on the outside. Coaxial focus knobs are more convenient since the viewer does not have to grope for a different knob.

Optical Components

6. Stage is where the specimen to be viewed is placed. A mechanical stage is used when working at higher magnifications where delicate movements are required.

7. Stage Clips are used when there is no mechanical stage. The viewer is required to move the slide manually to view different sections of the specimen.

8. Aperture is the hole in the stage through which the base (transmitted) light reaches the stage.

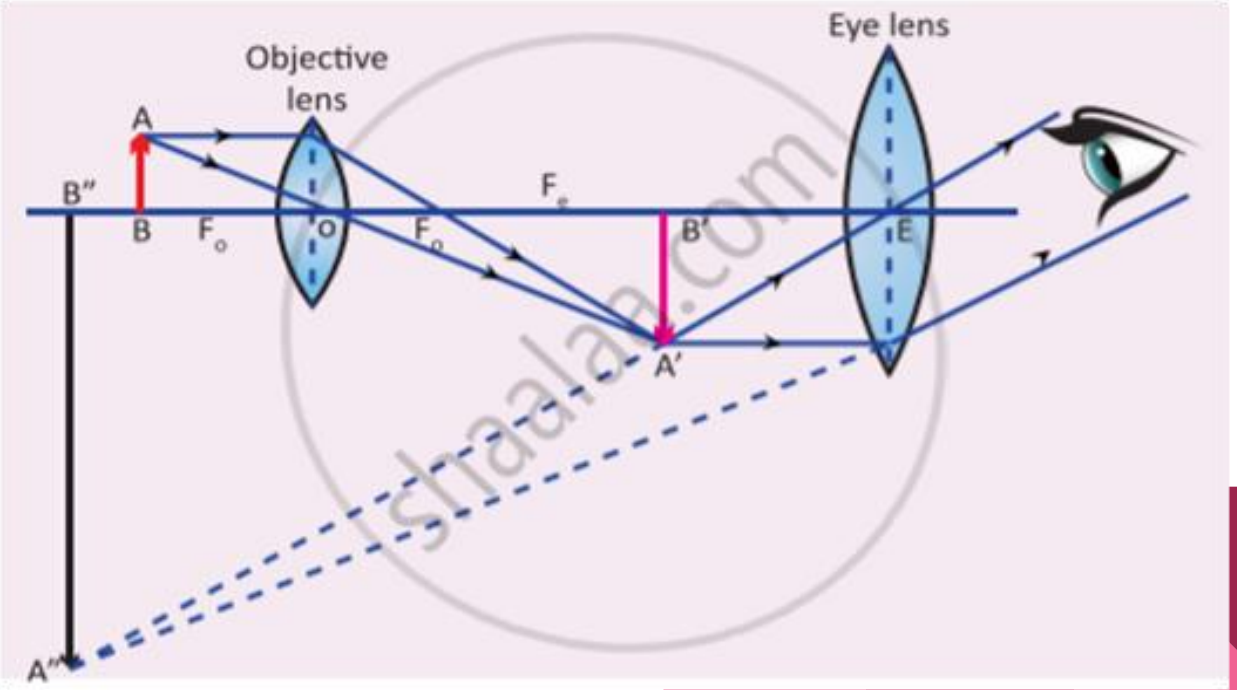
9. Illuminator is the light source for a microscope, typically located in the base of the microscope. Most light microscopes use low voltage, halogen bulbs with continuous variable lighting control located within the base.

10. Abbe's Condenser is used to collect and focus the light from the illuminator on to the specimen. It is located under the stage often in conjunction with an iris diaphragm.

11. Iris Diaphragm controls the amount of light reaching the specimen. It is located above the condenser and below the stage. Most high quality microscopes include an Abbe condenser with an iris diaphragm. Combined, they control both the focus and quantity of light applied to the specimen.

12. Condenser Focus Knob moves the condenser up or down to control the lighting focus on the specimen

Ray Diagram



Compound Microscope

WORKING PRINCIPLE –

- Compound microscopes have a combination of lenses that enhances both magnifying powers as well as the resolving power.
- The specimen or object, to be examined is usually mounted on a transparent glass slide and positioned on the specimen stage between the condenser lens and objective lens.
- A beam of visible light from the base is focused by a condenser lens onto the specimen.
- The objective lens picks up the light transmitted by the specimen and creates a magnified image of the specimen called the primary image inside the body tube.
- This image is again magnified by the ocular lens or eyepiece.

Working Principle

When higher magnification is required, the nose piece is rotated after low power focusing to bring the objective of a higher power (generally 45X) in line with the illuminated part of the slide.

Occasionally very high magnification it required (e.g. for observing bacterial cell). In that case, an oil immersion objective lens (usually 100X) is employed.

The common light microscope is also called a bright-field microscope because the image is produced amidst a brightly illuminated field.

The image appears darker because the specimen or object is denser and somewhat opaque than the surroundings. Part of the light passing through or object is absorbed.

Muffle Furnace

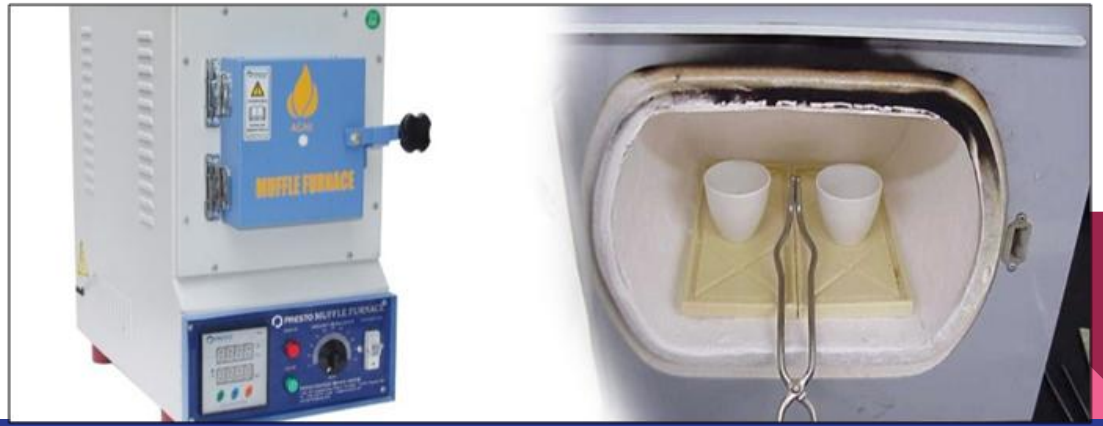
Lab muffle furnace is also known as a retort furnace is generally used to heat the air inside its chamber using the basic fundamentals of *thermal convection and thermal radiation*.

In Pharmacognostic study, it is required to determine ash values which act as important parameter to judge purity of natural or crude drugs.

The name muffle furnace is derived from the fact that the internal ceramic chamber of the furnace which is also known as muffle is wrapped up in vast layers of insulation so as to prevent heat loss and achieve high temperatures.

The Lab Muffle Furnace constitutes of 4 vital parts:

1. The Outer chamber.
2. The Inner Chamber.
3. Digital Controller cum indicator.
4. Insulation Material.



Parts of Muffle Furnace

1. Outer chamber of the muffle furnace is made up of steel sheet because it has better malleability, weld ability and ductility properties than cast iron along with corrosion resistance. Outer chamber of the muffle furnace also has a thick epoxy paint coating to give it an aesthetic look and more shelf life.

2. Inner chamber of the muffle furnace is made of either pre fabricated ceramic molds or firebricks depending upon the size and temperature requirements of the equipment.

3. & 4. Digital temperature controller cum indicator and Insulation material are the most important factors in working of a muffle furnace and hence extra care is taken while designing this part of the

furnace. The insulation being used in our muffle furnace or combination of ceramic wool and mineral wool which provide better thermal insulation and less heat loss.

It can generate heat up to 1200°C. Laboratory furnaces are generally used to heat small materials or test specimens over 1000°C. It also helps in rapid ashing of samples. Thus its an important instrument in material research and development and become useful in Ceramics, Semiconductors, Petro chemistry, Glass, Plastic, Medicines and other material Testing and Quality Control.

TLC
Thin Layer Chromatography

CHROMATOGRAPHY

(**1906)** Russian Biochemist **Michael Tswett -** separation of plant pigments

.**‘Chromatography’ -** Greek words, viz., **‘Chromo’ i.e. colour** and **‘Graphy’ means to ‘Write’.**

Principles of Tswett could be applied in different ways, resulted in development of various types of Chromatography techniques.

Two basic techniques of chromatography –

1. Planar Chromatography – In this technique, stationary phase is coated on plane surface. E.g paper in Paper Chromatography or silica gel plate in Thin Layer Chromatography.
2. Column Chromatography – In this technique, stationary phase is packed in glass or plastic column. E.g. Adsorption/ Partition/ Ion-Exchange/ Molecular Sieve Chromatography

THIN LAYER CHROMATOGRAPHY (TLC)

TLC is one of the techniques of plane chromatography.

In the pharmacognostic methods, TLC contributes as the primary reliable method of separation of compounds

It helps in detection of impurities in the crude drug.

It involves two phases -

Stationary Phase

Mobile Phase

TLC

Stationary phase - A uniform thin layer of adsorbents such as silica gel, alumina, cellulose, etc. form stationary phase. For preparing TLC plate, the slurry of adsorbent binding agent (Calcium carbonate) and solvent is prepared.A thin layer of this slurry is applied over a inert glass plate or plastic plate. The plate is activated at 110C by keeping it in an oven for few minutes. The normal thickness of slurry layer is 0.25mm.

Mobile phase – In TLC mobile phase is in the form of solvents. The choice of solvent depends on the compounds to be separated as well as nature of the stationary phase or adsorbent. For commonly used adsorbents such as alumina, silica gel, starch, mobile phase in the form of carbon tetrachloride (CCl4), cyclohexane and petroleum ether are used respectively.

TLC

Loading of Sample – the activated TLC plate is used for loading.

The sample mixture is loaded with the help of micropipette or capillary tube as a spot just above 2.5cm form the bottom end of TLC plate.

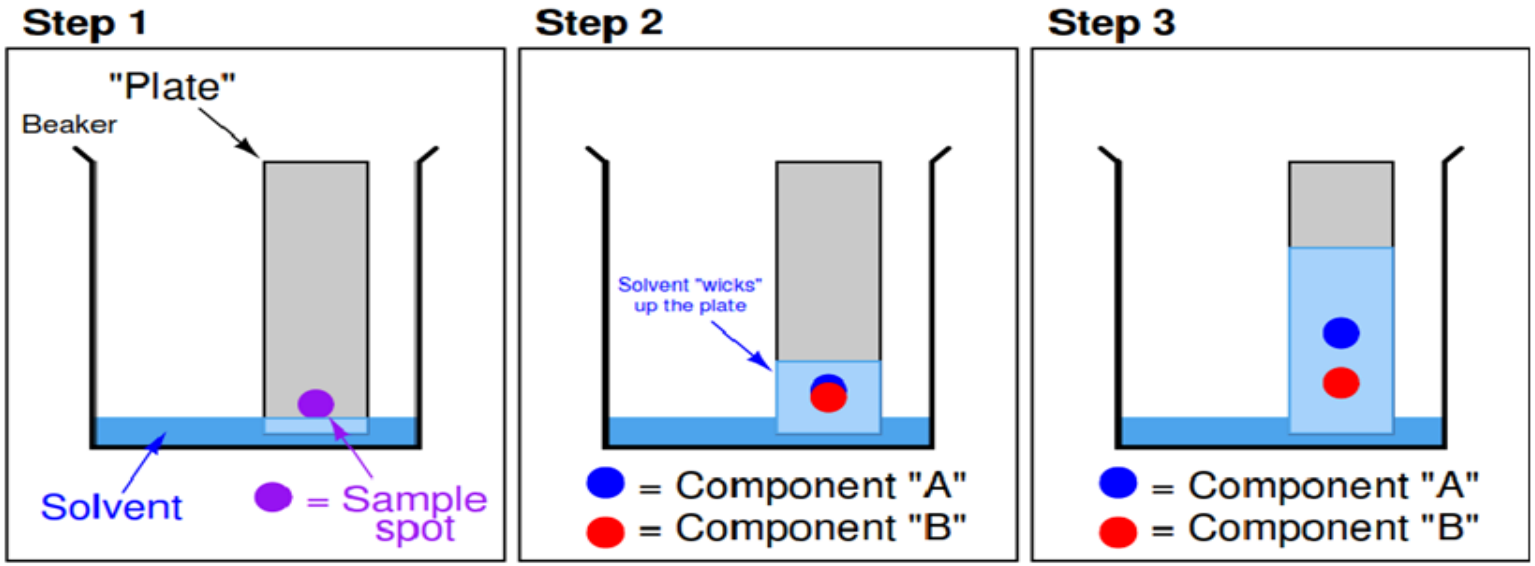
The spot is allowed to dry.

The process is repeated 6-7 times to concentrate the mixture of the spot.

Development of chromatogram –

- The glass chamber is filled with small quantity of mobile phase (solvent) as the bottom layer.
- It is allowed to saturate by the vapours of mobile phase.
- The loaded TLC plate is placed vertically in such a way that loaded spot should not dip in mobile phase
- As the solvent travels up on the plate, different components of sample interact with stationary and mobile phase differently.
- The components of the mixture (sample) which adsorbed strongly on the plate, travel less with mobile phase and remain close to the loaded spot.
- While the components of the mixture (sample) which adsorbed loosely on the plate, travel more with mobile phase and occupy position away from the loaded spot.

Principle of TLC



The component which interact least with stationary phase & more with mobile phase, migrates fast

The component which interact more with stationary phase & least with mobile phase, migrates very slow

TLC

- This interaction leads to separation of compounds from the mixture and development of chromatogram.
- When solvent covers 2/3rd distance on the plate, the plate removed from the chamberand solvent front is marked.

- The plate is dried. The separated spots are visualized by heating or by spraying a suitable staining reagent.

- The Rf value i.e. relative front / Retardation factor of separated components calculated by following formula

  Rf = Distance traveled by Solute/ Distance traveled by Solvent.

- The identification of the components of the given sample performed on the basis of their Rf values.

Principle of TLC

Chromatography is strong separation technique which involves two phases -

Stationary Phase – immobile material (Solid)

Mobile phase – moving material (Liquid)

Each component of Sample mixture interacts differently with these two phases

The component which interact least with stationary phase & more with mobile phase, migrates fast

The component which interact more with stationary phase & least with mobile phase, migrates very slow

The rate in difference of migration lead to separation of compounds from mixture

TLC

Advantages of TLC

1. It is widely used technique for separation and detection of very small fraction of compounds from a mixture.
2. The technique is comparatively faster than paper chromatography
3. It gives better separation of solutes compared to paper chromatography
4. It provides choice of different adsorbent materials unlike paper chromatography.
5. The modified form of TLC i.e. HPLC High Performance Liquid Chromatography allows even better resolution as well as quantifications of separated components.

TLC

Disadvantages of TLC

1. The results obtained from the experiment are difficult to reproduce.
2. It is applicable for soluble mixture components only.
3. TLC is not an automatic process.
4. The humidity and temperature can affect the results as Thin layer chromatography works in an open system.
5. Separation process takes place only to a certain length as plate length is limited

Principle of TLC