

PHARMACOGNOSY LABORATORY I

Microscopy

PARTS OF THE MICROSCOPE

- A. OPTICAL PARTS
 - **Illuminating Parts**
 - ▶ Light Source
 - ▶ Mirror*
 - ▶ Diaphragm**
 - ▶ Condenser***
 - **Magnifying Parts******
 - ▶ Objectives
 - ▶ Ocular*****

* used to reflect light. (concave) In case the light source is closed to the microscope, concave mirror is used.

** regulates the amount of light reflected

*** **The condenser** bundles the rays from the light source, so they are projected equally on the object. Thus, every part of the object is illuminated on the same brightness level.

**** **Magnification** – ability of the microscope to magnify or enlarge an object (example: 4x, 10x, 40x)

***** fixed at 10X

PARTS OF THE MICROSCOPE

- **B. MECHANICAL PARTS**

- **Base**

- to keep microscope's position

- **Arm**

- **Body Tube**

- **Stage**

- place for putting object

- **Objective Revolver (Revolving Nosepiece)**

- **Macrometer (Coarse Adjustment)**

- to focus image quickly

- **Micrometer (Fine Adjustment)**

- to focus image slowly

Preparation of Samples

- ▶ Place 1 or 2 drops of reagent (water, Sartur or chloral hydrate TS) on a clean glass slide.
- ▶ Moisten the tip of a needle with water and dip into the powder. Transfer a small quantity of the material that adheres to the needle tip into the drop of fluid on the slide.
- ▶ Cover the sample using the cover slip, do this slowly and gently, with a 45 degree angle - this will help preventing the formation of air bubbles.
- ▶ In case a heat-induced reagent is used, carefully boil over a small flame of a micro burner until the air is completely removed.

Preparation of Samples

- ▶ Carefully replace the fluid that evaporates and ensure that the space beneath the cover slip is completely filled with fluid at the end of the operation.

Excess liquid should be removed by using blotting paper, if there is overflowing reagent.

Reagents in Microscopic Examinations:

Water, distilled

Chloral hydrate Solution

Sartur Reagent

Reagents in Microscopic Examinations:

Water, distilled: Used for observing of starches.

Chloral hydrate Solution: (chloral 50 g, water 50 ml) A valuable and widely used clearing agent. While using the solution add a few drops to the plant material, and boil briefly over a small flame. Chloral hydrate dissolves cellular contents (starches) and allows cell walls to be easily observed. It can be used to assist in the identification of cork, fibers, vessels, calcium oxalate crystals, trichomes, stomata, and pollens.

Sartur Reagent: (Sarım Çelebioğlu & Turhan Baytop)

Sartur reagent contains KI, I, aniline, Sudan III, lactic acid, alcohol, and water. It is a heat-induced reagent and does not damage calcium oxalate crystals.



Sartur Reagent

- **Lactic Acid:** Clarify sections and preparates.
- **Sudan III:** Stains oils and suberized walls (cork tissues) to orange-brown. It is also useful for the examination of secretory cells and ducts.
- **Aniline:** Reacts with lignin in acidic conditions and forms yellow color (stains the sclerenchyma tissues, xylem, stone cells and scleroids)
- **Iode:** Reacts with starch and stains yielding blue-purple color.
- **Potassium iodide:** It is essential to dissolve iode.
- **Alcohol 95% and water** are the supporting elements for the preparation of reagent.

Amylum Drugs, Starches

- ▶ Amyloplasts are plastids which function to **produce and store starch** within internal membrane compartments. Amyloplasts are derived from a group of plastids known as leucoplasts. Leucoplasts have no pigmentation and therefore appear colorless.
- ▶ Starch grains has classified into two groups, the simple and the compound grain:

Simple starch grain. The typical starch grain in this type has one starch granule in an amyloplast.

Compound starch grain. The amyloplast has a few of aggregated starch granules.

centric/concentric: Hilum is situated closed to the middle of the granule.

eccentric: Hilum located towards one edge of the granule.

Plant: *Solanum tuberosum* (Potato)

Sample: Amylum Solani (Potato Starch)

Reagent: Distilled water

Microscope Magnification: 10x40

- ▶ 10-100 μm in size, oval and pyramidal
- ▶ There are occasional compound granules having 2-4 components
- ▶ Eccentric hilum
- ▶ All granules have clearly visible striations

Plant: *Triticum vulgare* (Wheat)

Sample: Amylum Tritici (Wheat Starch)

Reagent: Distilled water

Microscope Magnification: 10x40

- ▶ Rounded or elliptical, lenticular in side view
- ▶ 2-10 μm or 10-60 μm in diameter
- ▶ Intermediate sizes are very rare
- ▶ The central hilum is invisible or barely visible

Plant: *Zea mays* (Corn)

Sample: Amylum Maydis (Corn/Maize Starch)

Reagent: Distilled water

Microscope Magnification: 10x40

- ▶ 2-23 μm
- ▶ Polyhedral with blunt angles
- ▶ Cleft or fissured centric hilum

Plant: *Oryza sativa* (Rice)

Sample: Amylum Oryzae (Rice Starch)

Reagent: Distilled water

Microscope Magnification: 10x40

- ▶ 2-10 μm , polyhedral and polygonal grains
- ▶ Aggregated from 2-150 component
- ▶ Rarely we can detect the presence of centric hila

Folia Sennae

- ▶ Senna Leaf
- ▶ P.N: *Cassia* sp.
- ▶ R: Chloral hydrate Solution

Diagnostic elements:

- ▶ Epidermal fragment with palisade parenchyma
- ▶ Nonglandular, one-celled, conical hairs
- ▶ Paracytic stoma
- ▶ Sclerenchyma fibers (vascular tissue with crystals)

Folia Digitalis

- ▶ Digitalis Leaf
- ▶ P.N: *Digitalis purpurea*
- ▶ R: Chloral hydrate Solution

Diagnostic elements:

- ▶ Anomocytic stoma
- ▶ Covering trichomes with a collapsed cell
- ▶ Glandular trichomes with unicellular heads
- ▶ Glandular trichomes with bicellular heads

Radix Liquiritiae (Licorice Root)

- Licorice
- *Glycyrrhiza* sp.
- R: Sartur Reagent

Diagnostic elements:

- ▶ Fragments of yellow thick-walled fibres accompanied by prisms of calcium oxalate
- ▶ Fragments of cork