

Practical pharmacognosy

Second year

**UNIVERSITY OF BAGHDAD/PHARMACY COLLEGE
2014**

Done by: Dhuha al-Shammaa

Supervised by: L. Maha.Nori

INTRODUCTION

Of all forms of life, plants are the most vital to all others. The connection we have with plants is critical and life giving. In addition to providing the oxygen we breathe, plants often have complex molecular structures that are able to heal our bodies. Every ancient culture known to man has had some form of medicinal relationship with plants. The World Health Organization (WHO) states that the practice of herbal medicine should be safe and efficacious. . It is estimated that 70-80% of people worldwide rely on traditional herbal medicine to meet their primary health care needs.

Pharmacognosy is the study of medicines derived from natural sources. The word " pharmacognosy "is derived from the Greek words pharmakon(drug), and gnosis(knowledge). The term " pharmacognosy "was used for the first time by the Austrian physician Schmidt in 1811.

According to the American society of pharmacognosy, **pharmacognosy** "is the study of natural product molecules (typically secondary metabolites) that are useful for their medicinal, ecological, or other functional properties".Although most pharmacognosy studies focus on plants and medicines derived from plants, other types of organisms are also regarded as pharmacognosy interesting, in particular, various types of microbes(bacteria, fungi,...etc.),and recently various marine organisms.

Plant preparations are said to be medicinal or herbal when they are used to promote health beyond basic nutrition.

The study of drugs from plants includes the subjects of botany, chemistry and pharmacology.

Botany includes the identification (taxonomy), genetics and cultivation of plants.

Chemistry includes the isolation, identification and quantification of constituents in plant materials.

Pharmacology is the study of the biological effects that the chemicals in medicinal plants have on cell cultures, animals and humans.

Classification of vegetable drugs for study:

- Alphabetical: using either Latin or English names.
- Taxonomic: Families, Genera, and Species.
- Morphological: either organized drugs (leaves, flowers and seeds) or unorganized (extracts, gums, oils....etc.).
- Pharmacological or therapeutic use.
- Chemical e.g. alkaloids, glycosides, V.O....etc.

The most important points you have to recognize for plant drugs:

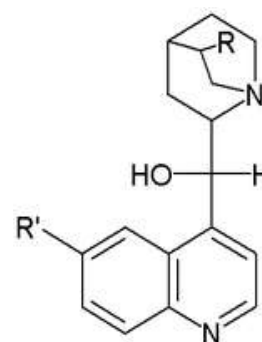
- The Botanical name.
- Family name.
- Local name.
- Part used.
- Active compounds.
- Basic structure.
- Dosage form.
- Therapeutic use.



Cinchona

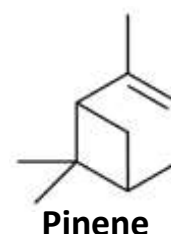
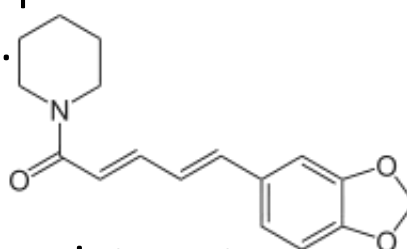


- Botanical name: Cinchona succirubra
- Family name: Rubiaceae
- Local name: الكينينة
- Part used: bark
- Active compound: Alkaloid quinine
- Basic structure:
- Dosage form: Tablets
- Therapeutic use: Malaria



Black pepper

- Botanical name: Piper nigrum Linne
- Family name: Piperaceae
- Local name: الفلفل الاسود
- Active compound: Piperine alkaloid and terpenes volatile oil.
- Basic structure:
- Dosage form: powder, ointment.
- Therapeutic use: Stimulant, febrifuge.

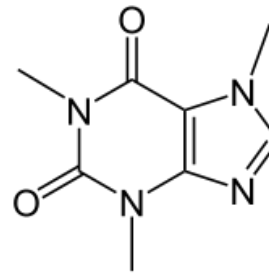




Coffee



- Botanical name: Coffea arabica
- Family name: Rubiaceae
- Local name: القهوة
- Part used: Coffee seeds
- Active compound: caffeine
- Basic structure:

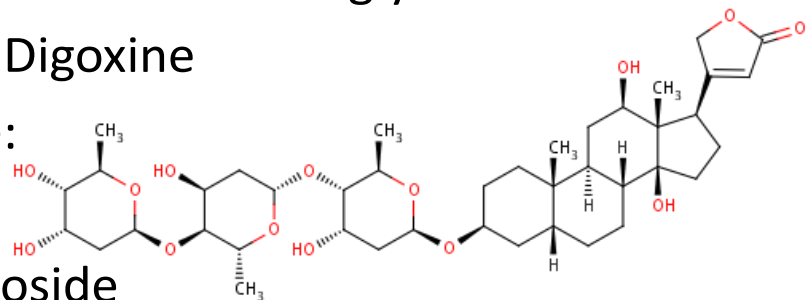


- Therapeutic use: Central stimulant

Digitalis



- Botanical name: Digitalis lanata
- Family name: Scrophulariaceae
- Local name: زهرة الكشتبان
- Part used: Dried leaves
- Active compound: Cardiotonic glycoside
- Basic structure: Digoxine
- Therapeutic use:



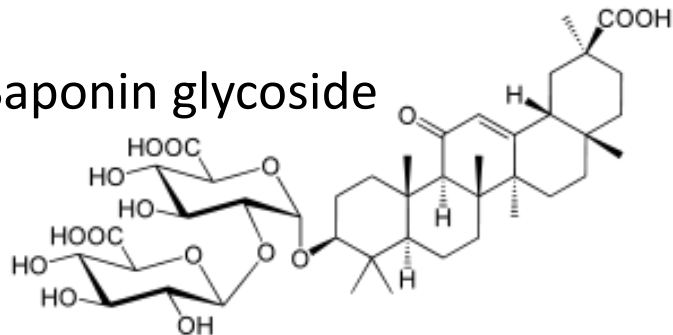
- Cardiotonic glycoside
- (increase the tone of heart muscle).

Glycyrrhiza

Botanical name: Glycyrrhiza glabra



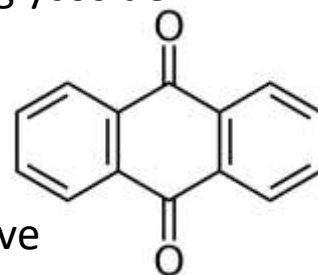
- Family name: Leguminosae
- Local name: السوس
- Part used: Root
- Active compound: Saponin glycoside (glycyrrhizin).
- Basic structure:
- Therapeutic use: Demulcent, expectorant, laxative.



Senna



- Botanical name: Cassia acutifolia
- Family name: Leguminosae
- Local name: السنمكي
- Part used: leaves and pods.
- Active compound: Anthraquinone glycoside
- Basic structure:



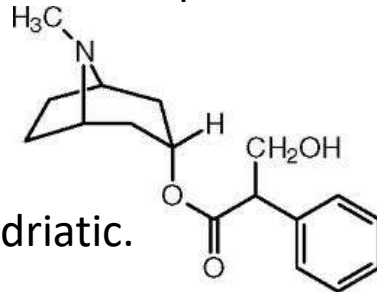
- Therapeutic use: cathartic or laxative



Belladonna



- Botanical name: Atropa belladonna
- Family name: Solanaceae
- Local name: ست الحسن
- Part used: Leaves
- Active compound: Hyoscyamine, hyosine, atropine.
- Basic structure:

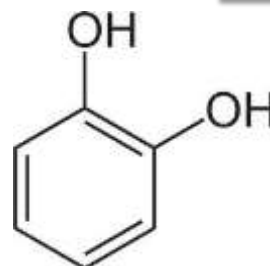


- Dosage form: tablet, drops, inj.
 - Therapeutic use: antispasmodic, mydriatic.
-

Hamamelis(Wilch hazel)



- Botanical name: Hamamelis virginiana
- Family name: Hamamelidaceae
- Local name: عصا الساجر
- Part used: leaves
- Active compound: Tannins
- Basic structure: catechol



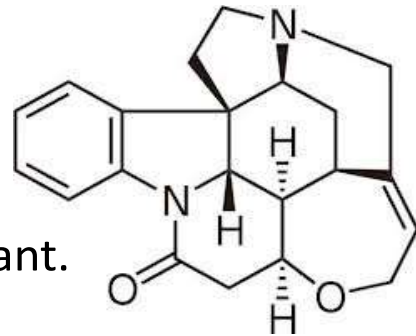
- Dosage form: decoction or infusion.
 - Therapeutic use: astringent homeostatic.
-



Nux vomica



- Botanical name: Strychnos nux vomica
- Family name: Loganiaceae
- Part used: dried ripe seed
- Active compound: alkaloid (Strychnine and brucine)
- Basic structure:



- Therapeutic use: central stimulant.
-

Exp.No.1

Micro measurement and Magnification:

Drawing-To calibrate the eye piece micrometer for L.P.

Magnification

Two scales are required, known, respectively, as a *stage micrometer* and an *eyepiece micrometer*. The stage micrometer is a glass slide 7.6×2.5 cm (3×1 inch) with a scale engraved on it. The scale is usually 1 or 1.1mm long and is divided into 0.1 and 0.01 parts of a millimeter. The value of one eyepiece division is determined for every optical combination to be used, a note made in each case of the objective eyepiece and length of draw- tube.

To do this, unscrew the upper lens of the eyepiece, place the eyepiece micrometer on the ridge inside, and replace the lens. Put the stage micrometer on the stage and focus it in the ordinary way. The two micrometer scales now appear. For example, when the 7 line of the stage micrometer coincides with the 0 of the eyepiece, the 10 of the stage coincides with 7.7 of the eyepiece. As the distance between 7 and 10 on the stage scale is 30 small stage micrometer divisions (since 1 small stage micrometer division equals 10 microns), 77 of the small eyepiece divisions equal 0.3 mm or 300 μ m; therefore, 1 eyepiece division equals $300/77$ or 3.9 μ m.

General Notes on Micro measurement:

- 1- Carry out the micro measurements using the highest power possible for the greatest accuracy in results for most determinations, but not all, you will find it correct to use the H.P. objective.
- 2- Only calibrate the microscope for the power which is going to be used. Every microscope has a different factor for low and high power. It is therefore necessary to calibrate the eye piece micrometer on each occasion that micro measurements are made.
- 3- Approximate calibration factors to one place of decimals.

To measure the diameter of starch grains:

Since the eyepiece micrometer has been calibrated, it may now be used to determine the linear measurement of microscopic objects and the stage micrometer is no longer required.

Using the high power objective, record the dimension of the long axis of 25 starch grains in terms of small eyepiece micrometer divisions.

Record the average figure and calculate, from the power calibration factor, the average size in microns.

Don't select large grains only, but measure the sizes of both large and small grains at random as they appear in the field being viewed. Then tabulate your results.

Exp. No. 2

Morphological and microscopically examination of crude drugs and cell inclusions

In standard works such as the European Pharmacopoeia, the British Herbal Pharmacopoeia and similar publications of other countries one will find detailed morphological and anatomical descriptions of those plant drugs.

Microscopically characteristics of the powdered drugs are also given. Such descriptions form the basis for the identification of drugs.

Plants form ranges from unicellular plants –for example, yeast and some green algae-to the strongly differentiated higher plants. For convenience of study, drugs may be arranged not only according to families and chemical constituents, but also into such morphological groups as barks, roots, leaves, seeds, etc. in another word drugs can be arranged into organized and non-organized drugs.

• ORGANIZED DRUGS:

1-Leaves and tops (herbs)

These consist of stems and leaves often associated with flowers and young fruits.

2-Barks

Barks consist of all tissues outside the cambium.

3-Woods

Wood consists of the secondary tissues produced by the cambium or its inner surface.

4- Leaves or leaflets.

5- Inflorescences and flowers.

6- Fruits.

7- Seeds.

• **UNORGANIZED DRUGS:**

These include: fixed oils, fats and waxes; volatile oils; resins, oleoresins, oleo-gum-resins, balsams and gums. To these must be added dried juices (e.g. aloes), lattices (e.g. opium) and extracts (e.g. agar and catechu).

Cell differentiation:

- The cell wall
- Parenchymatous tissue
- The epidermis
- Epidermal trichomes
- The endodermis
- Cork tissue
- Collenchyma
- Sclereids
- Fibers
- Xylem
- Secretory tissue

1-The cell wall

There are different types of cell wall:

- Cellulose wall
- Lignified wall
- Chitinom wall
- Cutinized wall
- Mucillaginum wall

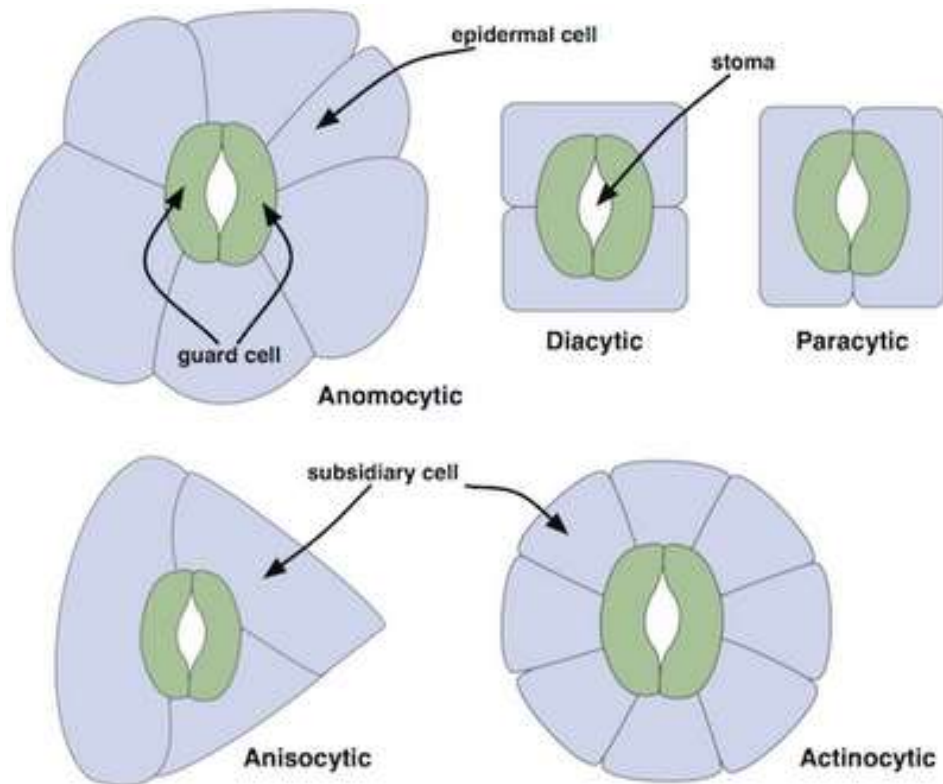
2-Paranchymatous tissue

3-The epidermis

Single layer of cells covering the whole plant, the structure of the epidermis and stomata are of first important in the microscopically identification of leaves. e.g. Strait – walled epidermis in Senna leaves, waxy walled in Belladonna leaves and beaded wall in digitalis.

Types of Stomata:

- ❖ Anomocytic stomata: Cells resembling the other epidermal cells may surround stomata. e.g. Digitalis purpurea leaves.
- ❖ Anisocytic stomata: With the stomata surrounded by 3 or 4 subsidiary cells, one of which is markedly smaller than other. e.g. Hyoscymus niger and Atropa belladonna leaves.
- ❖ Paracytic stomata: With two subsidiary cells with their long axis parallel to the pore. e.g. Cassia acutifolea (Senna leaves).
- ❖ Diacytic stomata: With two subsidiary cells with their long axis at right angles to the pore of the stomata. e.g. Mentha piperita (Peppermint).
- ❖ Actinocytic stomata: Subsidiary cells are arranged along the radii of the circle. e.g. Pilocarpus jaborandi.



4- Epidermal Trichomes:

(I) Covering Trichomes – example

1- **Unicellular**

From Senna leaves.

2- **Multicellular Unbranched, Uniseriate**

From Digitalis leaves.

3- **Multicellular Branched**

Stellate. From Witch – Hazel leaves.

4- **Multicellular Branched, Candelabra**

From Mullein.

5- **Multicellular Branched, T-Shaped**

From Pyrethrum.

6- **Cystolytic Trichomes**

From Cannabis.

(II) Glandular Trichomes – Example

1- **Unicellular stalk** with

a) Unicellular head

From Digitalis leaves.

b) Bicellular head

From Digitalis leaves.

c) Multicellular head

From Peppermint leaves.

2- **Multicellular Uniseriate Stalk**

a) Unicellular head

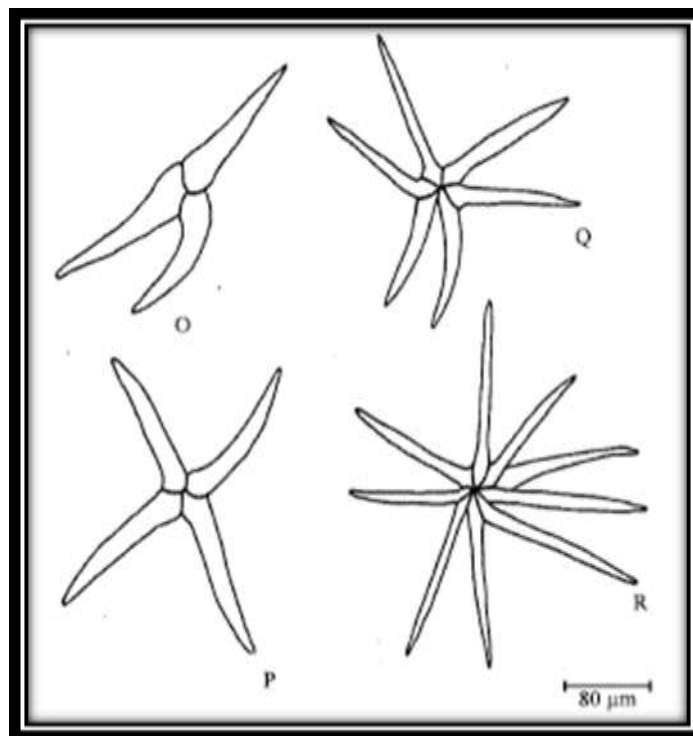
From Digitalis leaves.

b) Muticellular head

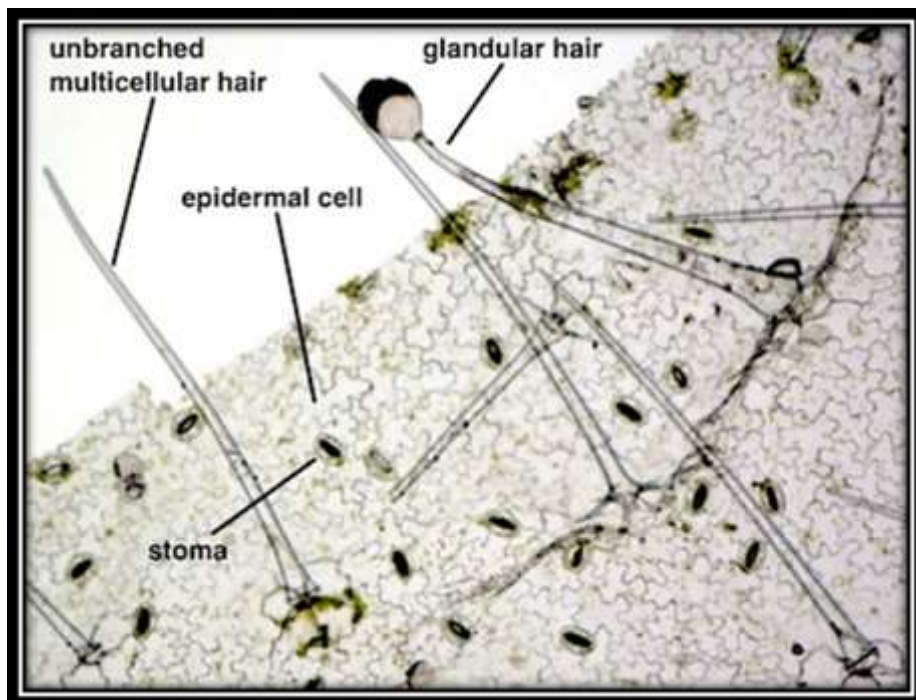
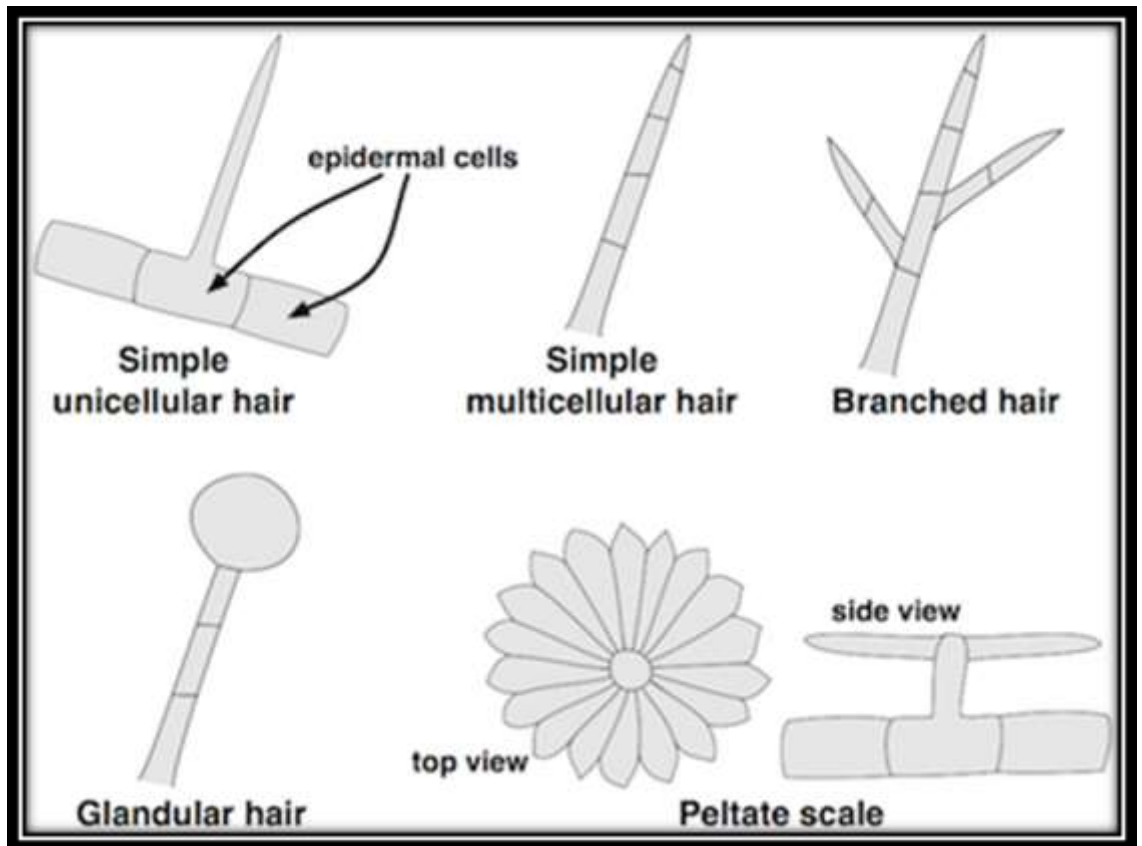
From Hyoscymus niger.

3- **Multicellular Multiseriate Stalk**

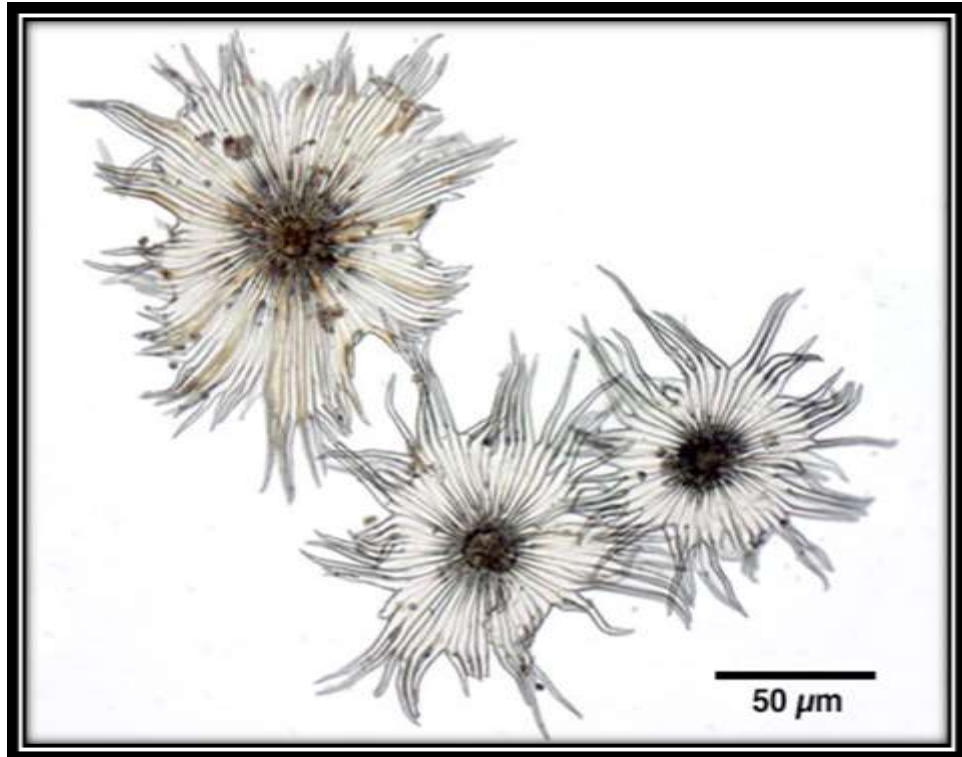
With multicellular head



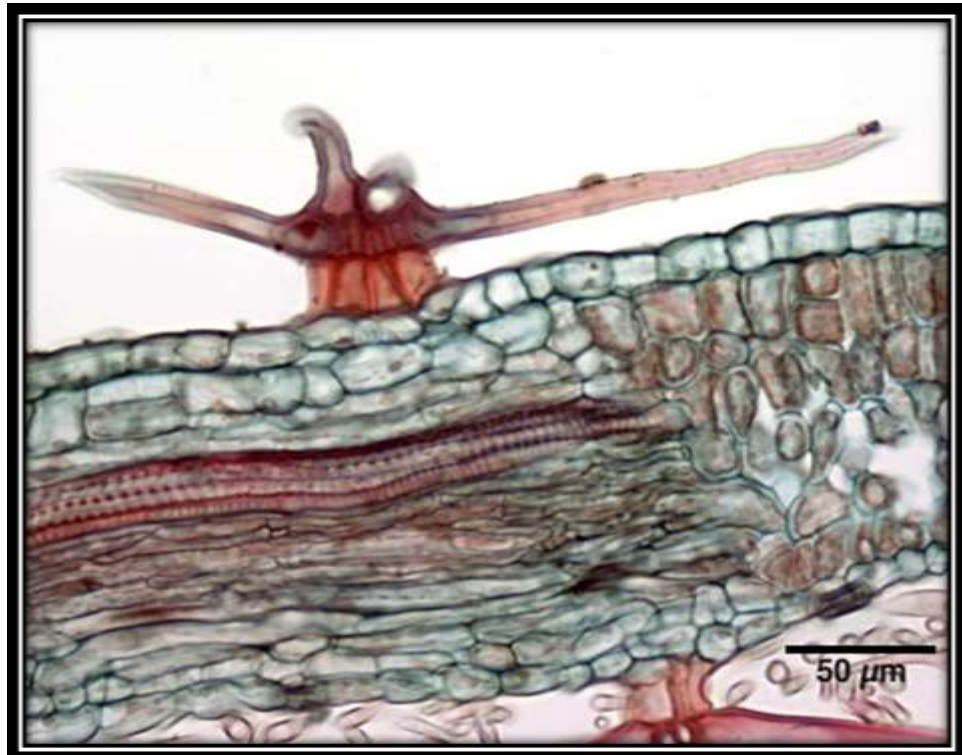
Non-glandular stellate trichomes



Complex trichomes are sometimes called scales:



- Peltate scales on leaves of Elaeagnus (whole mount)



T-shaped trichome

5- Cork tissue

6- Collenchyma: Is the tissue frequently found underneath the epidermis of many stems and leaf stalks. The term collenchyma is generally given to those cells where the thickening is laid down particularly in the corners of the cells.

7- Fibers: Tissue composed of spindle- shaped or elongated cells with pointed ends and thick walled. The cell wall may be composed of cellulose, lignification of sclerotic or sclerenchymatous fiber. Most mature fibers are unicellular, but occasionally transverse septa develop. e.g. ginger.

Fibers are best differentiated on the basis of the tissue in which they occur.e.g. Cortical fibers, pericyclic fibers, xylem fibers and phloem fibers.

Lignified fibers are moderately thick walled pericyclic fibers, accompanied by a parenchymatous sheath of cells containing prisms of calcium oxalate.e.g. senna leaf.

Phloem fibers occur in isolated or in irregular rows in the barks of Cinnamon, Cassia and Cichona. Cichona fibers large in diameter, fusiform in shape, thick walls striated and traversed by funnel shaped pits.

8-Xylem: Elongated water- conducting cell with lignified and thickened – pitted cell wall.

9-Vessels: Are the fundamental conducting elements of the xylem of the angiosperms. There are different types of vessels:

a-Spiral (Senna and Belladonna).

b- Annular (Senna and belladonna).

c- Reticulate (Gentian, Ginger, Rhubarb).

d- Pitted vessels.

Exp. No.3

Cell Contents

Cell contents which concerned in pharmacognosy are those which can be identified in vegetable drugs by microscopic examination or by chemical and physical tests. These cell contents represent either food storage products e.g. starch or by products of metabolism and these include carbohydrates, proteins, fixed oils, fats, alkaloids, purines, glycosides, V.O., gums, mucilage resins, tannins, calcium oxalate, calcium carbonate, and silica.

Starch:

Starch occurs in granules of varying sizes in almost all organs of plants, found in roots, rhizomes, fruits, and seeds. Starch granules may be simple or compound. Compound granules formed by aggregations of a large numbers of simple granules e.g. rice starch.

Hilum is the starting point of formation of starch granules, the position of the hilum either central or eccentric. There are different shapes of hilum (dot, curved, multiple clefts).

Concentric rings or striations (deposition of successive layers around the hilum) also appear in starch granules.

Types of starch	Shape	Size (um)	Hilum
Maize starch	Simple, polyhedral or sub spherical	10-30	Central triangles or 2-5 stellate clefts without striations.
Rice starch	Compound granules, polyhedral with sharp angles	4-6	Central point without striations.
Potato starch	Simple granules, mussel shapes.	45-65	Point eccentric with well-marked striations.

Calcium Oxalate:

Calcium oxalate is a dimorphous salt and both types occur in plants, these are tetragonal or monoclinic systems, these both types differ in the amount of water of crystallization.

- **Tetragonal system ($\text{CaC}_2\text{O}_4 \cdot 3\text{H}_2\text{O}$)**
e.g. **Prism (Senna, Hyoscymus), Rosset (Rhubarb, Strmonium).**
- **Monoclinic system ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$).**
Excess oxalic acid shine in polarized light.
e.g. **Raphide (Squill), Single needle crystal , Monoclinic prism.**

Exp. No.4

Extraction Methods

Extraction involves the separation of the medicinally active constituents of plants or animal tissues from the active or inert component by using solvent (s) and by using one of the standard extraction procedures.

The products that obtained from plants are relatively impure liquids, semisolid or powders, intended only for oral or external use. These total extractive products are called Galenical, Which came from the name Galen, the 2nd century Greek physician.

Methods of extraction can be divided into:

- 1- Cold Methods.
- 2- Hot methods.

Cold extraction methods:

Is the process whereby a substance is extracted from a mixture via cold solvent. The procedure carried out at room temperature (15-25 °C).

1- Maceration:

- This simple widely used procedure involves leaving the pulverized plant to soak in a suitable solvent in a closed container .simple maceration is performed at room temperature by mixing the ground drug with the solvent (drug solvent ratio : 1:5 or 1:10) and leaving the mixture for several days with occasional shaking or stirring. The main disadvantage of maceration is that the process can be quite time-consuming, taking from a few hours up to several weeks.

2- Percolation:

Percolation (from Lat. *percōlāre*, to filter) concerns the movement and filtering of fluids through porous materials. The powdered plant material is soaked initially in a solvent. In a percolator, additional solvent is then poured on top of the plant material and allowed to percolate slowly (drop wise) out of the bottom of the percolator. Additional filtration of the extract is not required because there is a filter at the outlet of the percolator.



Hot Extraction Methods:

1-Infusion:

Infusion is the process of extracting chemical compounds or flavors from plant material in a solvent such as water, oil or alcohol by allowing the material to remain suspended in the solvent over time. In this procedure we have special container called ‘Infusion pot’ which contain sieves and cover with heavy lid. After the addition of the solvent ,boiling water, left for a while for the extraction of active constituent during that time the volatile oil evaporated with steam and condenses on the lid, after that we take the solvent which contain the active constituent.



2-Decoction:

The term dates back to 1350–1400, from present participle stem of Latin decoquere (meaning to boil down), de "from" + coquere "to cook". Decoction is a method of extraction by boiling, of dissolved chemicals, from hard plant material, which may include stems, roots, bark and rhizomes on a source of heat or direct flame then agitating until the active constituents will be dissolved in the solvent. Here the solvent used depend on the active constituent and source of heat e.g. chloroform and ether can't be used because we used direct source of heat. In addition to that the active constituent should be heat stable.

3-Digestion:

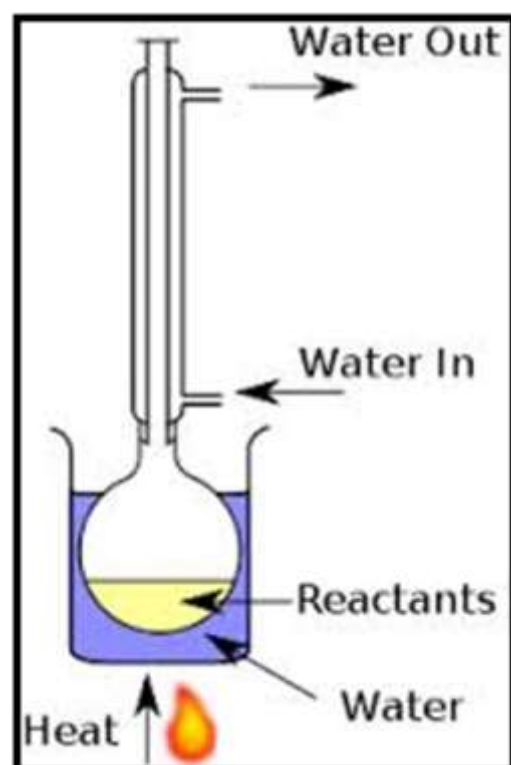
In this method the plant material is placed together with the solvent and application of gentle heat, so that the solvent will increase its power for extraction and this method is used in cases where moderately elevated temperature is required. e.g. Tea is the brew made from the leaves of the Camellia sinensis plant. It is the beverage most consumed worldwide, after water.



4-Continuous hot extraction methods:

a) Reflux condenser:

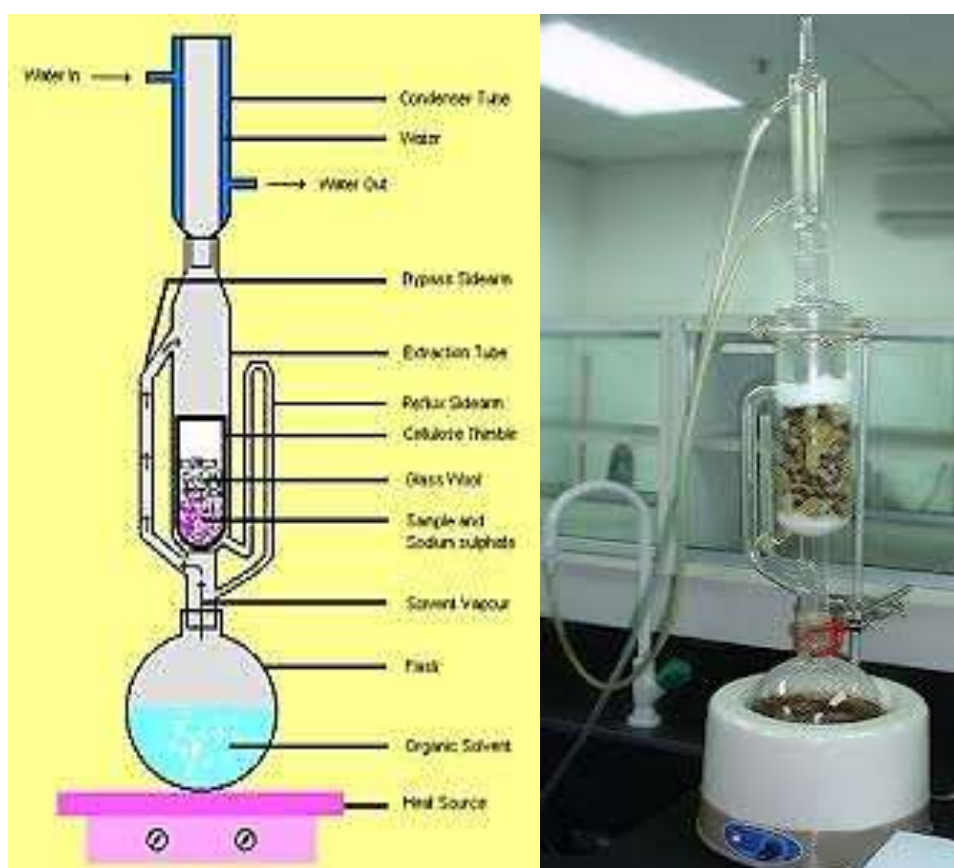
Plant material is immersed in a solvent in a round-bottomed flask, which is connected to a condenser. The solvent is heated until it reaches its boiling point. As the vapor is condensed, the solvent is recycled to the flask.



b) Soxhlet apparatus:

The plant powder is placed in a cellulose thimble in an extraction chamber, which is placed on top of a collecting flask beneath a reflux condenser. A suitable solvent is added to the flask, and the setup is heated under reflux. When a certain level of condensed solvent has accumulated in the thimble, it is siphoned into the flask beneath.

-The main advantage of Soxhlet extraction is that it is a continuous process for the extraction of active constituents decomposed by direct heat.



b) Clavenger:

In this method we used a special apparatus which is called ‘Clavenger’, it is used mainly for extraction of volatile compounds, e.g. orange peels has been used for the extraction of orange oil.



Clevenger Apparatus
(Oil heavier than Water)



Clevenger Apparatus
(Oil lighter than water)

Exp. No.5

Chromatography

History:

Mikhail Tswett, Russian Botanist (1872-1919).

In 1906 Tswett used the chromatography to separate plant pigments

He called the new technique chromatography because the result of the analysis was 'written in color' along the length of the adsorbent column. Chroma means “color” and graphein means to “write”.

Importance:

Chromatography has application in every branch of the physical and biological sciences. 12 Nobel prizes were awarded between 1937 and 1972 alone for work in which chromatography played a vital role.

The main uses of chromatography involve: Analytical procedures, scientific research and Purification.

Definition:

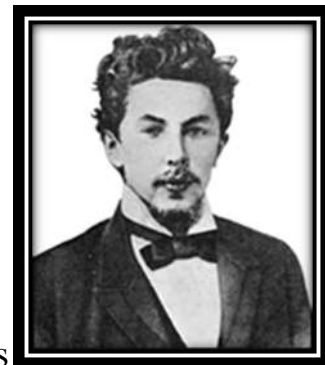
Chromatography is a physical method of separation in which the components to be separated are distributed between two phases.

One of which is stationary (stationary phase) while the other (the mobile phase) moves through it in a definite direction.

The chromatographic process occurs due to differences in the distribution constant of the individual sample components. It is used for large and small quantities so it is used quantitatively and qualitatively and proved to be more effective from the other means of separation and identification.

The separation of a mixture of compounds in chromatography to its components depends on the action of two forces:

- 1- Mobile force (driving force) that will try to move the components of mixture.



2- Opposing force (stationary or retardation force) that will try to keep components in their places depending on many factors:

- a) Solubility in mobile phase.
- b) Adsorption ability of component to be separated.
- c) Ionic forces.

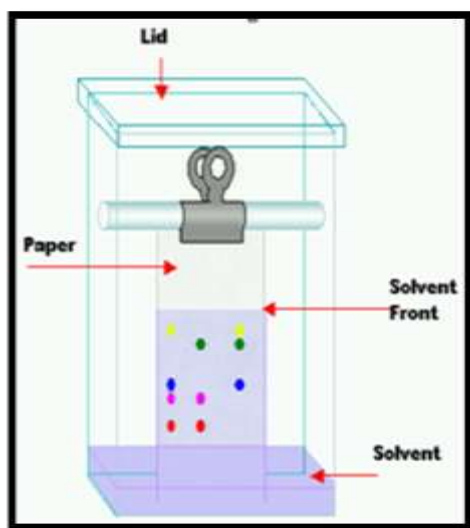
Classification:

There are different types of chromatography classification.

- Classification of chromatography according to mobile phase:
 - 1- Liquid chromatography: mobile phase is a liquid. (LLC, LSC).
 - 2- Gas chromatography: mobile phase is a gas. (GSC, GLC).
- Classification according to the packing of the stationary phase:
 - 1- Thin layer chromatography (TLC): the stationary phase is a thin layer supported on glass, plastic or aluminum plates.
 - 2- Paper chromatography (PC): the stationary phase is a thin film of liquid supported on an inert support.
 - 3- Column chromatography (CC): stationary phase is packed in a glass column.
- Classification according to the force of separation:
 - 1- Adsorption chromatography.
 - 2- Partition chromatography
 - 3- Ion exchange chromatography.
 - 4- Gel filtration chromatography.
 - 5- Affinity chromatography
 - 6- Electrophoresis.

PYPER CHROMATOGRAPHY

Paper chromatography is a method of partition chromatography using filter paper strips as carrier or inert support. The factor governing separation of mixtures of solutes on filter paper is the partition between two immiscible phases. One is usually water adsorbed on cellulose fibers in the paper (stationary phase). The second is the organic solvent flows past the sample on the paper (stationary phase).



Partition occurs between the mobile phase and the stationary aqueous phase bound by the cellulose. The isolation depends on partition coefficient of the solute.

$$K = \frac{c(\text{stationary})}{c(\text{mobile})}$$

General Procedure :

- 1- Choice of paper and solvent to be used.
- 2- Desalting of sample.
- 3- Application of the sample.
- 4- Equilibration of paper.
- 5- Development.

6- Detection.

7- Identification of substances.

Techniques of development with various flow directions:

Ascending development

The paper will be dipped in the solvent mixture so that the solvent front travels up the paper.

Descending development

When the through of solvent will be supported at the top of the chamber. In this case the solvent travels down the paper.

Radial development

Circular or horizontal paper chromatography is another technique used, in which circular filter paper bearing a wick at the center of the paper is placed in a petri dish and the solvent system supplementation is through the central wick.

Multiple developments

Multiple chromatography includes all procedures in which the development is repeated after one development is completed.

A- Multiple developments: the chromatogram is repeatedly developed in the same direction and thus the complete resolution of two or more substances which have R_F values close together can be obtained.

As the mobile phase one can use either the same solvent system or different solvent systems.

B- two- dimensional chromatography:

When large numbers of substances are to be separated on a single chromatogram.

Development in a direction perpendicular to the first, and with a solvent system different from that used initially is often necessary.

The sample is applied on one corner of a square piece of paper and after development with the first solvent; the paper is dried, rotated 90° and developed in the second direction.

Usually, different types of solvents systems are used in each direction. It is essential that the first solvent be completely volatile.

Retardation factor can be defined as the distance moved or traveled by the compound to the distance moved by the solvent and it is constant for each compound when chromatography is carried out using the same technique. Mobile phase and the same conditions. Usually the R_F value is used for the identification of the separated compound by comparison with the R_F value of a standard. The R_F value is going to change if we:

- 1) Change the solvent.
- 2) Aging.
- 3) Impurities.
- 4) Temperature.
- 5) Saturation.
- 6) Solvent front must be uniform

Methods of detection:

- 1) Chemical detection by using chemical reagents.

- 2) Physical detection by using UV light.
- 3) Radioactive method: specific detection procedures when we use to detect separated compounds having some radioactivity or labeled' compounds.
- 4) Biological methods by using certain microorganisms and are especially used' for the detection of antibiotics.

Identification of isolated compounds:

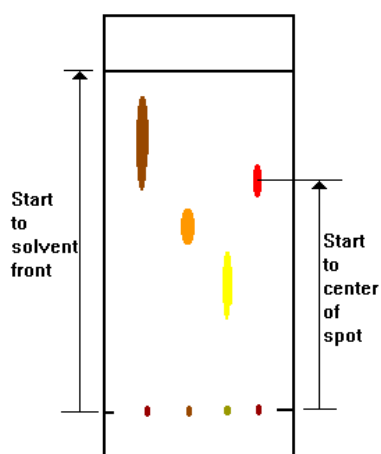
Identifying the Spots by visualization, if the spots can be seen, outline them with a pencil.

If no spots are obvious, the most common visualization technique is to hold the plate under a UV lamp. Then the R_F (retention factor) value for each spot should be calculated.

It is characteristic for any given compound on the same stationary phase using the same mobile phase for development of the plates.

Hence, known R_F values can be compared to those of unknown substances to aid in their identifications.

$$R_f = \frac{\text{Distance from start to center of substance spot}}{\text{Distance from start to solvent front}}$$



*Note: R_F values often depend on the temperature and the solvent used in the TLC experiment.

CIRCULAR FILTER PAPER CHROMATOGRAPHY

(HORIZONTAL PAPER CHROMATOGRAPHY)

Method:

- 1) Prepare a circular filter paper and insert a wick in the center of the paper. Mark four pencil dots (starting points), approximately 1 cm from the wick.
- 2) Apply the sample on pencil dot (3 different magic colors and ink).
- 3) Place a chromatographic paper over the dish that contains the mobile phase in such a way that develops to about 4-5cm.
- 4) Remove the chromatogram, mark the solvent front and dry at room temperature.
- 5) Examine the chromatogram by the daylight and calculate the R_F value for each separated spot.
- 6) Make full report.

Not: mobile phase is prepared by shaking n-butanol, acetic acid, and water (4:1:5) for 3min in a separatory funnel and collect the upper phase.

EXPERIMENT NO.6:

PAPER CHROMATOGRAPHY FOR THE SEPARATION OF NATURAL PRODUCTS

Method:

Separation of a mixture of natural products (Leucine and Cysteine) uses ascending paper and their identification.

Technique: one way ascending.

Paper: Whatman no.1.

Mobile phase (solvent): n-butanol: glacial acetic acid: water (4: 1:5)

Temperature: at lab. emperature.

Reference solution: 0.5% Leucine and 1% Cystein in aqueous isopropanol.

Examination: Day light after spraying and heating.

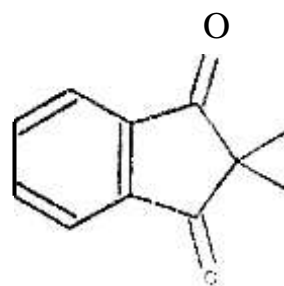
Spray: 0.1% Ninhydrine in n-butanol.

Requirement: calculate Rf values, note all colors and tabulate, the results.

What conclusions may be drawn from these results? The extracts provided contains amino acids, which are the building blocks for extracts provided contains amino acids, which are the building blocks for proteins and alkaloids, and which are readily separated by paper chromatography.

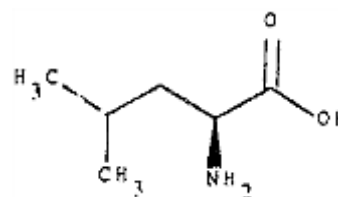
·*Note that amino acids and the spray reagent may produce different colors.

Draw the chemical reaction between Ninhydrin and amino acids.

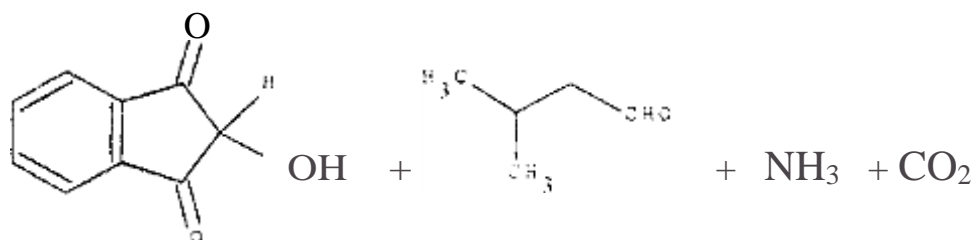


Ninhydrin

+



Leucine



Ninhydrine oxidatively dcarboxylate aminoacids to CO_2 and NH_3 and an aldehyde with less carbon atom than the parent aminoacids and reacted ninhydrine that react with liberated ammonia forming blue complex.

*Note: Cysteine is freely soluble in water, slightly soluble in alcohol, practically in soluble in ether. While Leucine sparingly soluble in water, practically insoluble in alcohol and in ether, it dissolves in dilute mineral acids and in dilute solutions of alkali hydroxide.

EXPERIMENT NO.7

THIN LAYER CHROMATOGRAPHY (TLC)

Is a method for identifying substances and testing the purity of compounds.

TLC is a useful technique because it is relatively quick and requires small quantities of material.

Separations in TLC involve distributing a mixture of two or more substances between a stationary phase and a mobile phase.

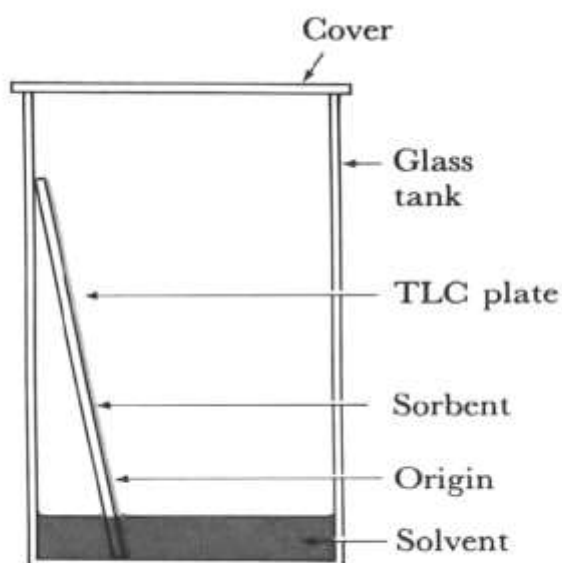
The stationary phase:

Is a thin layer of adsorbent (usually silica gel or alumina) coated on a plate.

The mobile phase:

Is a developing liquid which travels up the stationary phase, carrying the samples with it.

Components of the samples will separate on the stationary phase according to how much they adsorb on the stationary phase versus how much they dissolve in the mobile phase.



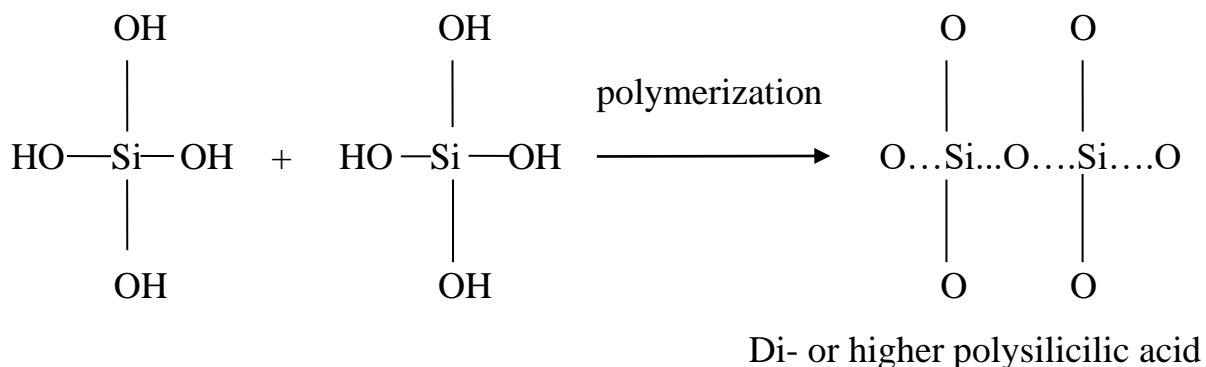
ADVANTAGES OF TLC OVER PC:

- 1) Fractionations can be effected more rapidly with smaller quantities of a mixture.
- 2) The separated spots are usually more compact and more clearly Identified from one another.
- 3) The nature of the film is often such that drastic reagents such as H_2SO_4 which would destroy a paper chromatogram, can be used for the location of separated substances.

TLC ADSORBENTS:

The grain sizes of most TLC adsorbent lie between 5-50 μm . There are different types of adsorbents that have been used in TLC:

- 1) Silica gel is amorphous porous substances formed from polysillicic gel.



- 1) Alumina oxides used in chromatography containing either $-Al_2O_3$ or $X Al_2O_3$.

- 2) Kieselguhr: Naturally occurring amorphous silicic acid of fossil origin referred to as diatomaceous earth. It has a lot of impurities, water, and organic substances consist of small only slightly active surface and relatively large pore volume (used for partition chromatography).
- 3) Kieselguhr G: Finally divided powder of grain size less than 60 μm , use in TLC with Gypsum used as binder.

The stationary phase in TLC is a solid stationary phase, used as a thin film and we can use plastic or glass sheath as an inert support for coating material which does not involve in the separation technique.

We can use Silica gel GF (G = Gypsum and F=Flourescence). In addition to that Alumina can be used as a coating material in TLC depending on the type or the chemical nature and the solubility of the separated compounds.

The mobile phase in TLC is a liquid and it could be a mixture of liquids or a single liquid. We have to know the solubility of the compound and determine what type of stationary phase and mobile phase should be used.

We have different types of silica gel depending on the number of free hydroxyl groups left on the silica gel:

- 1- Activated.
- 2- Inactivated. By the addition of water to silica gel we block the active sites of silica gel. If the silica gel have a large content of water, the water content is considered as a stationary phase and the mechanism of separation is partition.

Development Technique in TLC:

Usually the same technique used in PC can be used in TLC but mainly we are going to use ascending technique in which the TLC plates are placed in a chamber contain the mobile phase.

Detection methods in TLC:

- 1- Physical detection.
- 2- Chemical detection.
- 3- Biological detection.
- 4- Radioactive detection.

Experiment No.8

TLC on microscope slides

1- Preparation of slides for TLC.

Thin layer slides are prepared from slurry of the adsorbent which after spreading and drying forms a powder film over the surface of glass slide. The slurry is prepared by mixing 35gm of silica gel G with 100 ml of acetone in a jar. Three clean slides are prepared by dipping in the slurry(make sure that the slurry is well shaken before each dipping process to ensure homogenous coating of the slurry).

2- Drying of TLC slides:

Number your slides by using fine needle at the top corner then:

- a- Leave slide no.1 to dry at room temp.
- b- Activate slide no.2 by heating in an oven at 110 C for 10 min.
- c- Hydrate slide no.3 by exposing it to water vapor on water bath and allow it to dry at room temperature for 5 min.

3- Application of test mixture:

The test mixture consists of 3 dyes (Crystal violet, Methyl red, Dimethyl yellow). Measure 0.5 cm. above the bottom the level of the mobile phase in the jar, this is the base line of the chromatogram. Spot the test dye mixture from a capillary tube to the base line. Repeat the same spotting procedure on slide no. 2 and slide 3.

4- Preparation of tanks:

The developing solvent used is chloroform occupy about 0.5-1 cm depth of the tanks provided, then seal the tanks with a ground glass lid and leave for 15 min. to ensure saturation of atmosphere, mark the solvent front about 3/4

length of the slide and place the slides in the developing solvent. Allow the solvent to travel to the front line, then remove from the tank and allow drying at room temp.

5- Measurement of chromatographic data:

- a- Making a permanent record by examine the slides and trace them on the paper. Label the color of each spot.
- b- Calculate the R_F value of the colored spots.
- c- Make a conclusion drawn from these results, state which adsorbent layer has higher order of activity? Which of the three slides give the best separation? Why?
- d- Essential experimental details of the chromatographic procedure used should be recorded on the chromatogram, i.e.:

Title: Thin layer chromatography.

Technique: One way ascending.

Adsorbent: Silica gel G.

Solvent system: Chloroform.

Time: Record the time required by the solvent to travel up the slide.

Temperature: Record lab. Temp.

Examination: e.g. in day light or in UV light.

- e- Draw the chemical structure of the test dye mixture.

Experiment No.9

EFFECT OF SOLVENT POLARITY UPON R_F - VALUES OF ALKALOIDS

Object: To demonstrate the effect of solvent composition upon solute migration by TLC.

Method:

-Slides: Silica gel adsorbent – prepare microscope slides using silica gel G slurry in acetone. The slides should be air – dried at room temp.

-Samples: 0.5% solution of the following alkaloids provided in methanol:

a- Strychnine.

b-Brucine.

-Solvent systems: The following solvent systems are provided:

- 1) Chloroform.
- 2) Ethyl acetate: Iso-propanol: Conc.ammonia (100:4:2).
- 3) Ethyl acetate: Iso-propanol: Conc.ammonia (80:15:5).
- 4) Ethyl acetate: Iso-propanol: Conc.ammonia (60:30:10).
- 5) Ethyl acetate: Iso-propanol: 5%.ammonia (45:35:20).

- Run chromatograms of the alkaloids in solvent systems from 1-5.

- Detection: Spray with Dragendorff's reagent.

* Ensure that ammonia is removed from a slide before spraying.

- Calculate the R_F values for each alkaloid in each solvent system.

-Construct a graph for strychnine and brucine alkaloids plotting R_F values against solvent system from 1-5.

-Conclusions:

Discuss the effect of solvent polarity upon R_F value.

Experiment No.10

Column Chromatography

Introduction:

This includes chromatographic methods in which: The stationary phase is packed into a column. The mobile phase is a moving liquid or gas.

According to the mechanism of separation of solutes, five major types of CC are distinguished. Usually, one mechanism predominates but does not exclude the others.

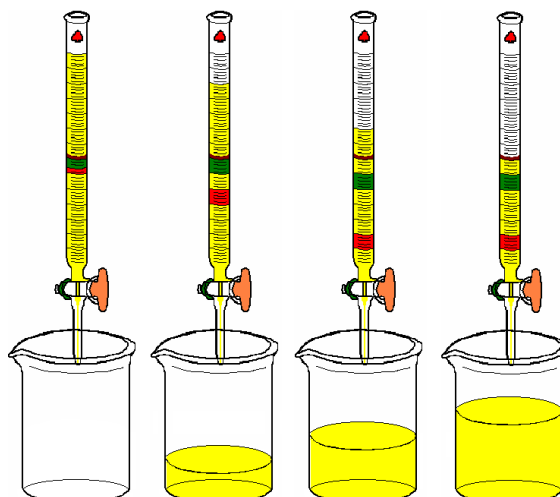
Different Types of Column chromatography

Mode or type	Stationary phase	Mobile phase	Mechanism
Adsorption Chromatography	Solid that attracts the solutes	Liquid or gas	Solutes move at different rates according to the forces of attraction to the stationary phase.
Partition Chromatography	Thin film of liquid formed on the surface of a solid inert support	Liquid or gas	Solutes equilibrate between the 2 phases according to their partition coefficients
Ion Exchange Chromatography	Solid resin that carries fixed ions & mobile counterions of opposite charge attached by covalent bonds	Liquid containing electrolytes	Solute ions of charge opposite to the fixed ions are attracted to the resin by electrostatic forces & replace the mobile counterions.

Molecular Exclusion Chromatography	Porous gel with no attractive action on solute molecules	Liquid	Molecules separate according to their size: <ol style="list-style-type: none"> 1. Smaller molecules enter the pores of the gel, and need a larger volume of eluent. 2. Larger molecules pass through the column at a faster rate.
Affinity Chromatography	Solid on which specific molecules are immobilized	Liquid or gas	Special kind of solute molecules interact with those immobilized on the stationary phase

Column chromatography

Stationary phase is held in a narrow tube through which the mobile phase is forced under pressure or under the effect of gravity.



Term	Definition
Solvent	Mobile liquid phase with no affinity to the stationary phase (i.e. inert towards it) & no effect on solutes.
Developer	Any liquid with more affinity to the stationary phase than the solvent but less than solutes and just capable to move them through the column.
Effluent	Any liquid that passes out of the column.
Eluent	Any liquid that has lesser affinity to the stationary phase than solutes but is capable to move them out of the column.
Eluate	Fraction of eluent containing a required specific substance.
Retention volume (V_R)	(or retardation volume): Volume of mobile phase that passes out of the column, before elution of a specific substance.

Packing & operating the column:

1- Packing

The selection of the method of packing depends mainly on the density of the solid. Techniques used are the wet, dry & slurry methods.

In all cases avoid inclusion of air bubbles.

2- Sample Application

Apply evenly & in a concentrated solution to the top of the column which is protected from disturbance (e.g. add glass wool or filter paper).

3-Elution techniques:

Technique	Procedure
Isocratic elution	Addition of solvent mixture of fixed composition during the whole process.
Gradient elution	<u>Continuous or linear elution:</u> in which there is continuous change in the composition of the mobile phase over a period of time (e.g. polarity, pH or ionic strength).
	<u>Step wise or fractional elution:</u> in which the change is not continuous i.e. a sudden change in the composition of the mobile phase is followed by a period where the mobile phase is held constant.

4- Detection:

On column detection for colored or fluorescent compounds directly after developing the chromatogram.

5- Monitoring of eluted fractions (PC or TLC). Using special detectors connected to the column such as refractive index, UV detectors, et

