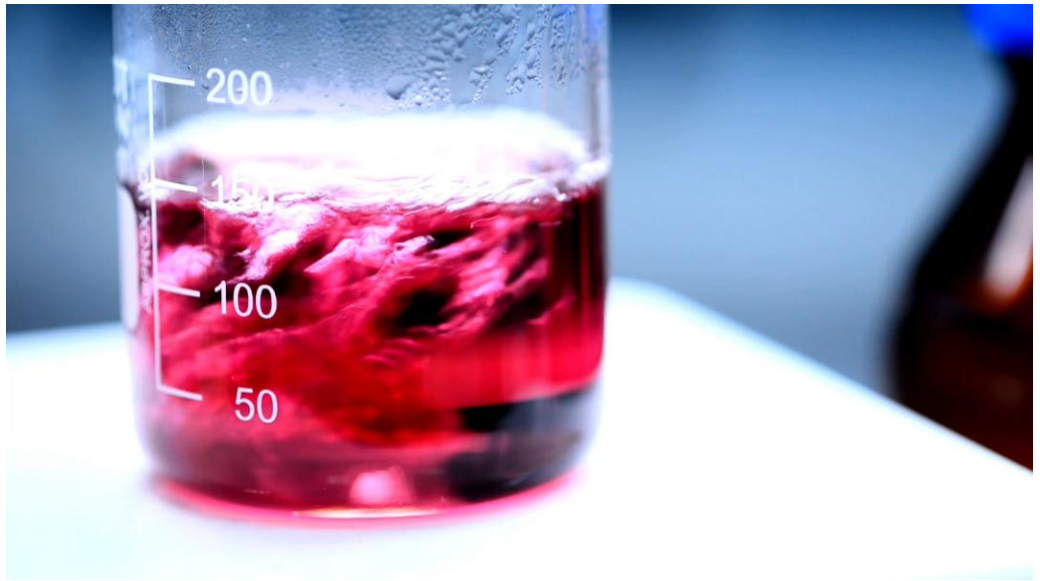


Chemistry of natural products
Methods of extraction

Assistant lecturer Dr. Ayah Al-Qrimli
MSc pharmacognosy

Pharmacognosy is dealing with natural chemicals those of certain pharmacological activity, thus consider them as **raw materials that used directly or indirectly as medicines.**



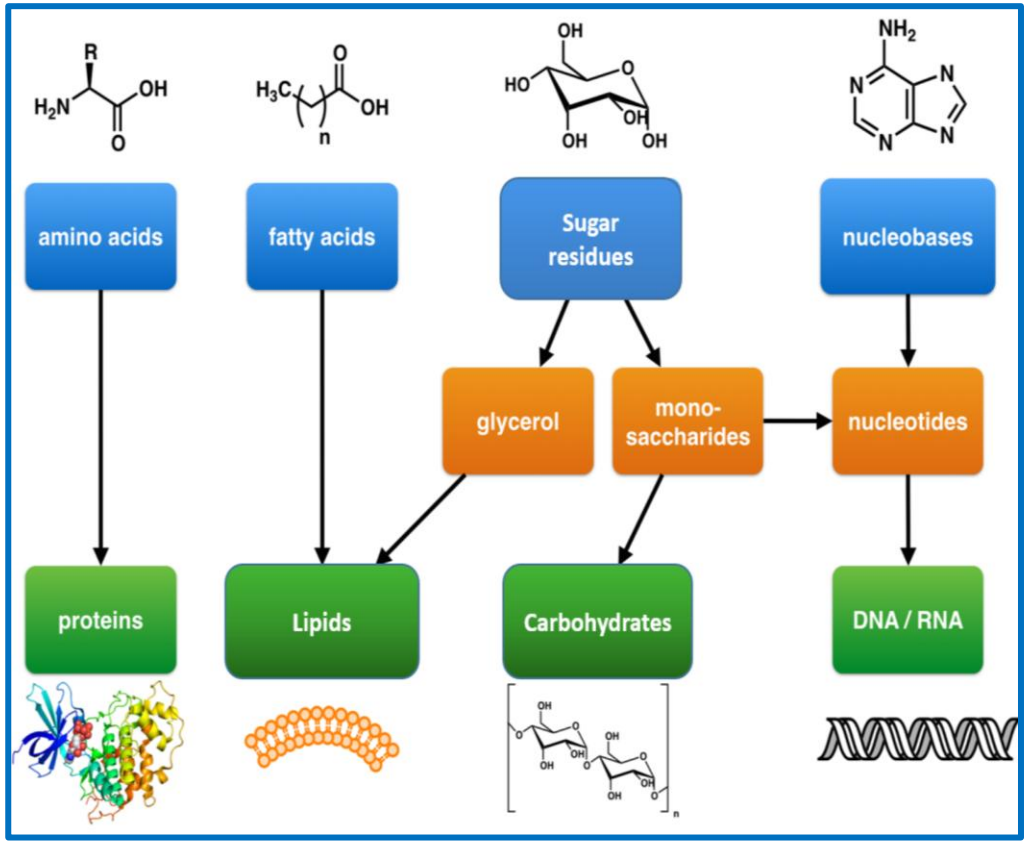
- The name (natural drug) means they can be produced by different biological sources like **plant, animal, bacteria, fungus** and others.
- Economically speaking **plants are the best** to considered as the main source of these **biological - organic natural chemical compounds.**



- **These organic natural drugs resulted from the metabolic reactions those take place inside the living cell.**
- The results of these different kinds of reactions are either what we call " primary metabolite" or " secondary metabolite".
- The scope of pharmacognosy mainly deals with **the secondary (2°)** metabolite those **posses a biological effect**. Which are also called **(phytochemicals).**

Primary metabolites

- Primary metabolites are components of basic metabolic pathways that are **required for life**.
- They are associated with **essential cellular functions** such as, **energy production, growth and development**.
- They have **a wide distribution.** Primary metabolites include the building blocks required to make the four major macromolecules within the body: **carbohydrates, lipids, proteins, and nucleic acids (DNA and RNA).**
- These are **large polymers** of the body that are built up from repeating smaller monomer units. The monomer units for building the nucleic acids, DNA and RNA, are the **nucleotide bases**, whereas the monomers for proteins are **amino acids**, for carbohydrates are **sugar residues**, and for lipids are **fatty acids**.



Properties of primary metabolites:

1-Have **high molecular weight**.

2-Inside plant cell, they are of **high concentration. (wide distribution)**

3-Plant depend on them as a **source of energy (essential for life)**

4-Produced by **photosynthesis process.**

5-Also have a functional and structural rule inside plants cell.

6- Example :**sugars, proteins, amino acids, fatty acids ,DNA, RNA and others**.

Secondary metabolites

- Secondary metabolites, in contrast to primary metabolites are dispensable and **not absolutely required for survival**.
- Furthermore, secondary metabolites typically have a **narrow distribution**. For example, *Atropa belladonna*, produces toxic hallucinogenic compounds, like scopolamine, but other plant species do not have this capacity.
- The **function** of many other secondary metabolites is unknown ; however, it is thought to have the following:

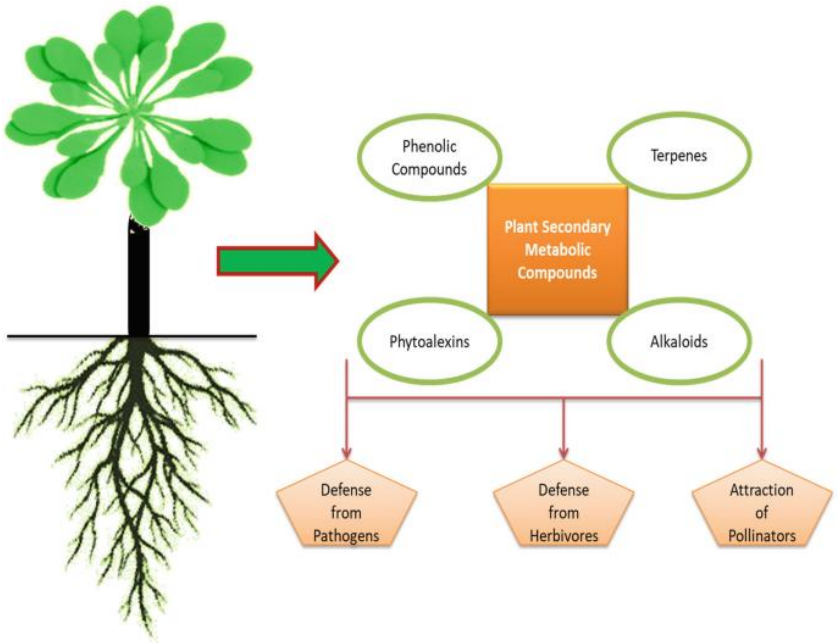
✓Competitive advantage to the organism that produces them.

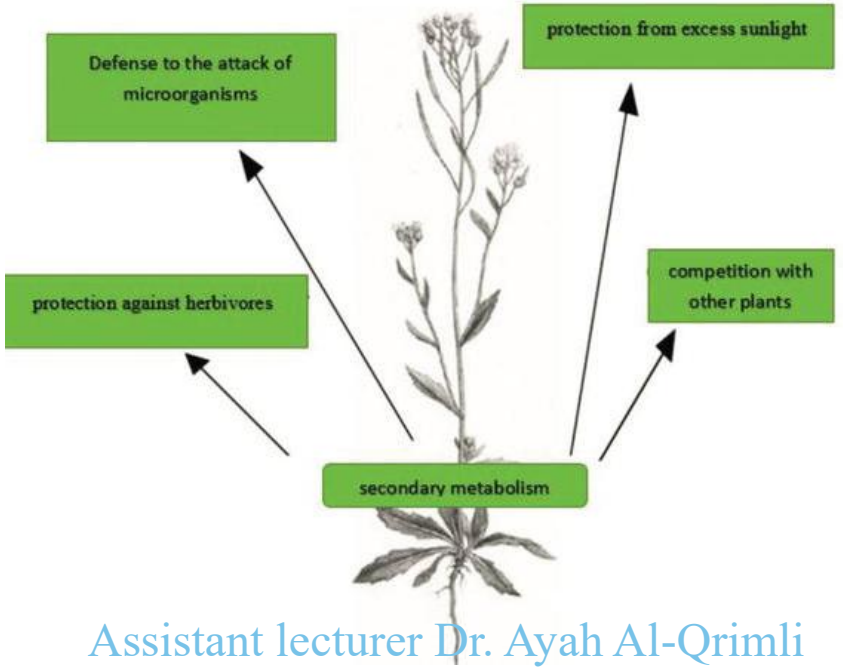
✓Protection :protect against predators (poisons/oils/toxins)

✓To attract insects (fertilization)

✓Adaptation (to environment/ disease/ climate)

- Secondary metabolites have a **smaller molecular weight,** and they include examples such as **alkaloids, , terpenoids, glycosides and sterols , etc.**.





Properties of secondary metabolites:

- Have **low molecular weight**.
- They are of **low concentration.** (narrow distribution.
- They are **not a source of energy (not essential for life).**
- **Biosynthesized from the primary metabolites** by two main pathways:

1. Shikimic acid pathway.
2. Acetate mevalonate pathway

- They have no apparent clear function in a plant, many of them are important for human.
- Example: digoxin, Hyoscine, Quercetin, Anthraquinones and others





Active metabolites Extraction Methods



- Each extraction process needs a **solvent system** to be **used as extractor media**, also needs a certain **method** to be followed **for plant compounds to be extracted**.
- **The choice of extraction procedure depends on:**

1) The **nature of the plant material.**

2) The **components to be isolated**.

- Before any extraction process, plant should be thoroughly **cleaned** with tap water and then let to **dry** in room temperature and in shade,
- This step followed by **crushing** and **homogenization**, these processes ensure **losing all water molecules that may prevent extraction solvent from being in contact with plant material**, on the other hand **grinding increases the total surface area to be attached with the solvent to get perfect extraction manner.**
- Sometimes plant material doesn't undergo the drying step, like plant **volatile oils which lost if not extracted immediately**.

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Factors considered in the selection of solvent for extraction:

- Extraction mainly a function of choosing the **efficient solvent**, where the:

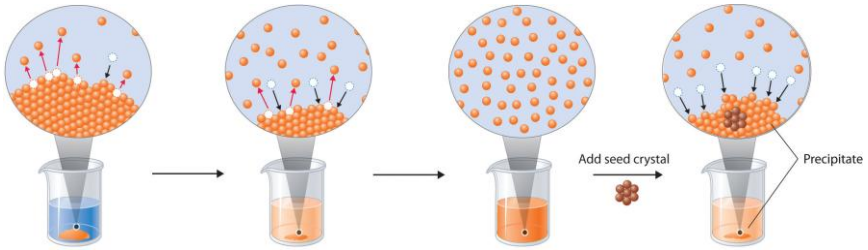
1) Polarity of the targeted compound is **the most important factor for solvent choice.**

2) Molecular affinity between solvent and solute. (solubility)

3) Environmental safety and **Human toxicity.**

4) Easy evaporation at low temperatures (boiling point).

5) Financial feasibility.



- **In most cases the solvent is a mixture of alcohol and water in different percentage.**

Extraction techniques:

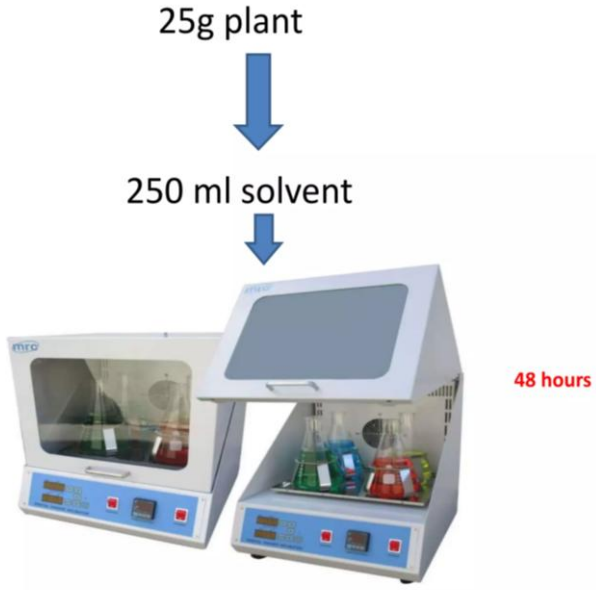
- Bioactive compounds can be extracted by various classical extraction techniques, most of these techniques are based on the **extracting power** of different solvents and the **application/non application of heat** according to plant material heat stability.
- So, extraction methods can be classified into **cold and hot**.

Cold extraction

Maceration: used for *heat sensitive compounds*



- It is an extraction procedure in which **coarsely powdered drug material** (leaves or stem, bark or root) is placed **inside a container**;
- The **menstruum (solvent)** is poured on top until completely covered the drug material (in a **1:3** percentage).
- The container is then **closed tightly** and kept for **at least three days with shaking** between while and another until the soluble matter has dissolved.
- The mixture then is strained, the marc (the damp solid material) is pressed, and the combined liquids are clarified by **filtration.**
- Sometimes in maceration, the **solvent changed every 24 hours** to **prevent solvent saturation** with active compounds and **decreasing extraction efficiency.**
- **Shaking** take place to **increase diffusion** and removing concentrated solution from the sample surface.
- Maceration is a **small-scale extraction** method, **time- solvent consumer** and **easy method.**



Hot extraction methods

used for compounds *not affected by heat*.

A) Infusion:

- Infusion is prepared by **macerating the crude drug** for a **short period** of time with **boiling water**.
- These are **diluted solutions** for **readily soluble** constituents of the crude drug.
- Very old, **non efficient** and used special infusion pots for extraction.
- Mainly used for **flavoring,** essential compounds that remain suspended in the solvent over time.
- It's used for **flowers and leave** plant parts.





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B) Decoction:

- The plant material is placed together with the solvent (cold) in a beaker on a **source of heat or direct flame** .
- This method is usually used for **hard plant parts** like **barks, stems and roots which contain a lot of fibers.**
- **In this method the crude drug is boiled with specified volume of solvent.**



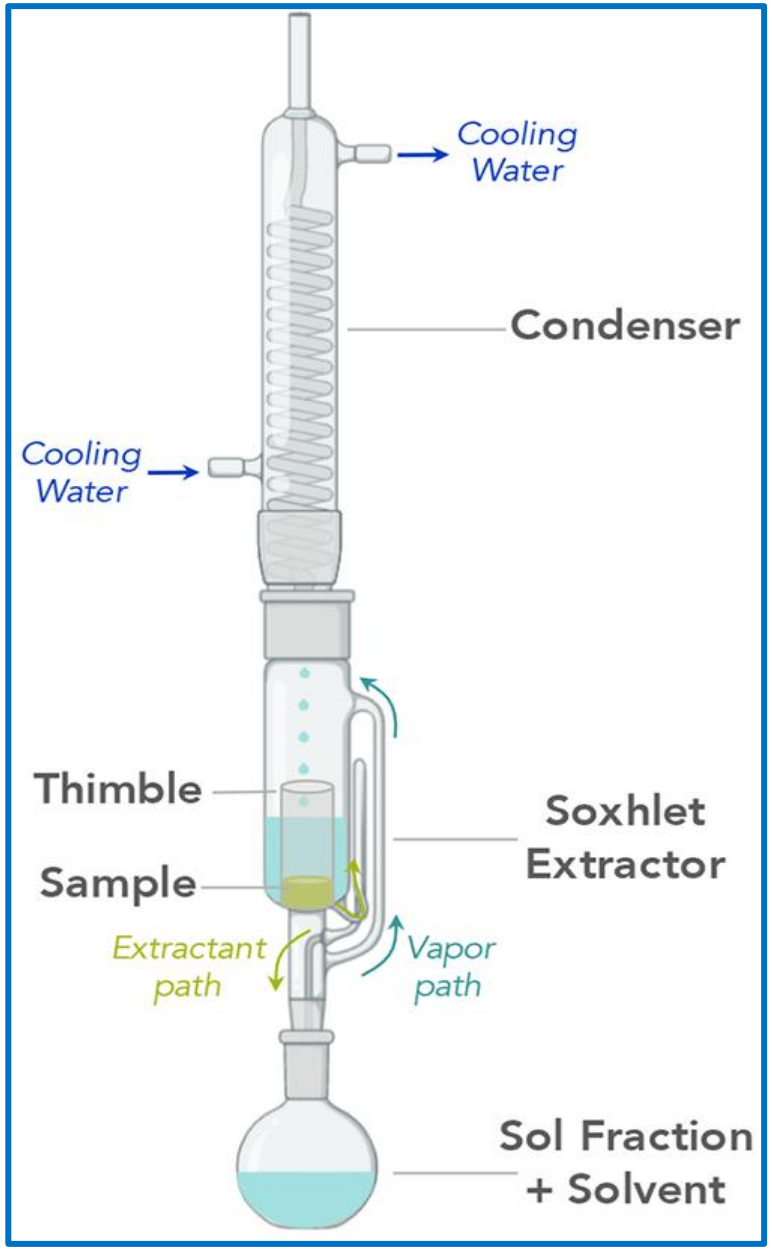
C) Digestion:

- This is a form of maceration in which **gentle heat** is used during the process of extraction.
- It is used when **moderately** elevated temperature is not objectionable.
- **The solvent efficiency of the menstrum is thereby increased**.
- **Note :** All previous methods are **noncontinuous** due to its **time limiting extraction**.



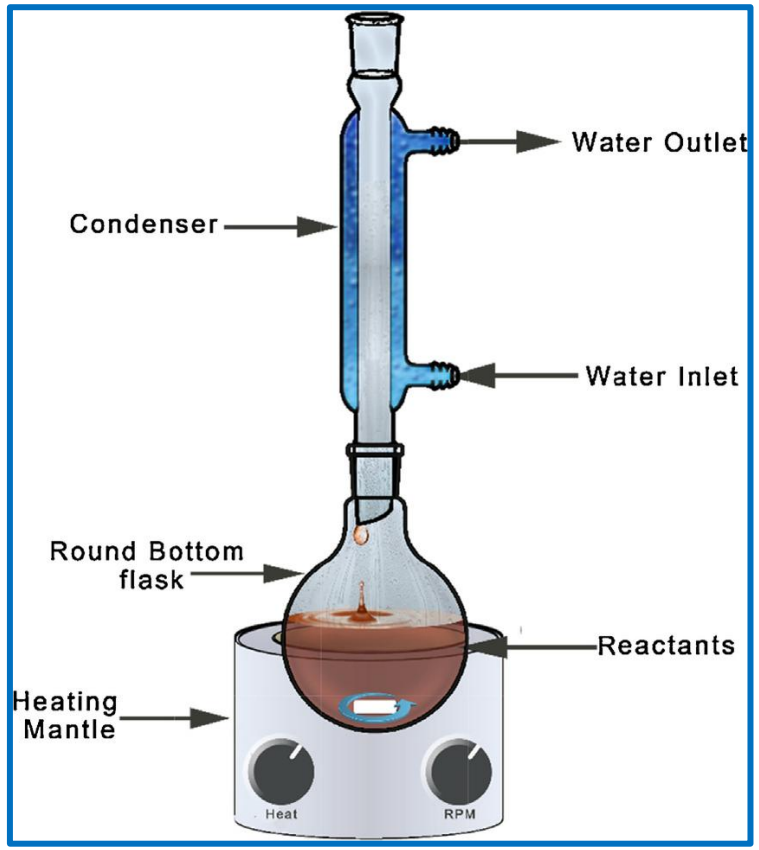
D) Soxhlet extraction:

- Soxhlet apparatus represents a **closed system** so it can be used for the extraction with **dangerous organic solvents.**
- In this method a **small amount of solvent** is required so it is **economic.**
- It can be used for the extraction of different active constituents by changing the solvent polarity, and it is suitable method when the **plant material is affected by direct heat**.
- In this method, the finely ground crude drug is placed in a porous bag or **"thimble"** made of cellulose, which is placed in **Soxhlet chamber** of the Apparatus,
- The solvent is heated in a **round flask**, and its vapors condense in attached **condenser** which drips into the thimble containing the crude drug and extracts it by contact.
- When the level of liquid rises to the top of siphon tube , the liquid contents of Soxhlet chamber siphon into the flask . This process is continuously repeated.



E) Reflex extractor:

- In this method the plant material is **placed together** with the solvent in a **round bottom flask** which is attached to a **condenser**.
- The round bottom flask is placed on a source of heat; the heat is applied and when the solvent reaches its boiling points it will evaporate to the condenser where it condenses and returns to the flask.
- This method has the same advantages as Soxhlet extractor, but the extract requires **filtration** and **not suitable if plant material is affected by direct heat.**
- Its also considered as a continuous extraction method.

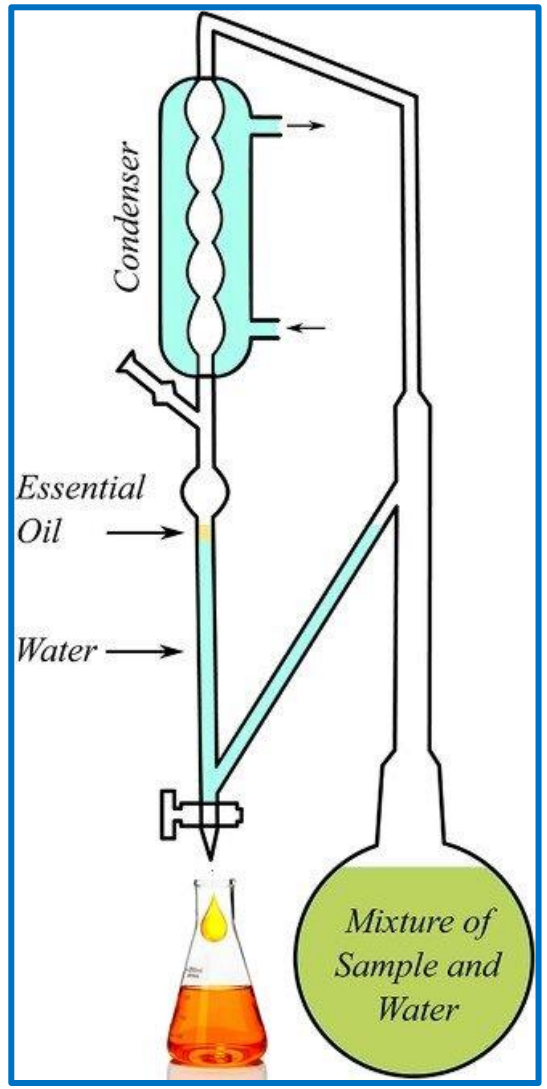


F) Hydro distillation:

- Is a method for extraction of **essential oils** (mainly)from plants.
- **Organic solvents are not involved,** and it can be performed on **fresh plant materials**.
- There are three types of hydro distillation:

1. **Water distillation,**
2. **Water and steam distillation**.
3. **Direct steam distillation**.

- The principle of all allow the plant materials to pack in a still **Compartment, water** is added in sufficient amount and then **brought to boil.**
- Alternatively, **direct steam is injected into the plant sample**.
- **Hot water and steam act as the main influential factors to free bioactive compounds of plant tissue.**
- **Indirect cooling** by water condenses the vapor **mixture of water and oil**, which flows from condenser to a separator, where oil and bioactive compounds separate automatically from the water.



Challenges of conventional extraction methods:

- The major challenges of normal extraction methods are :

1-**longer extraction time**. (weeks-days-several hours)

2-Requirement of costly and high purity **solvents**.

3-**Evaporation of the huge amount of solvent.**

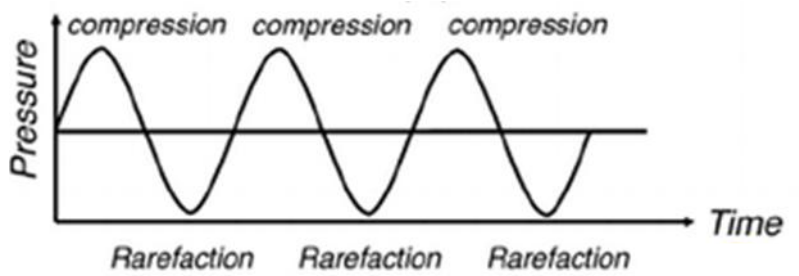
4-Low extraction **selectivity** .

5-Thermal decomposition of **thermolabile compounds**.

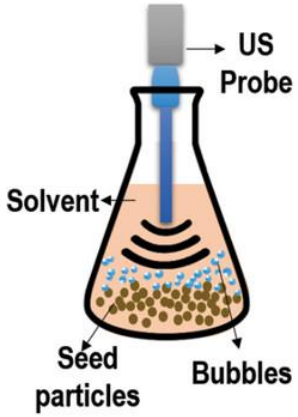
➢New and promising extraction techniques are introduced to overcome these limitations of conventional extraction methods. These techniques are referred to as **non-conventional extraction techniques.**

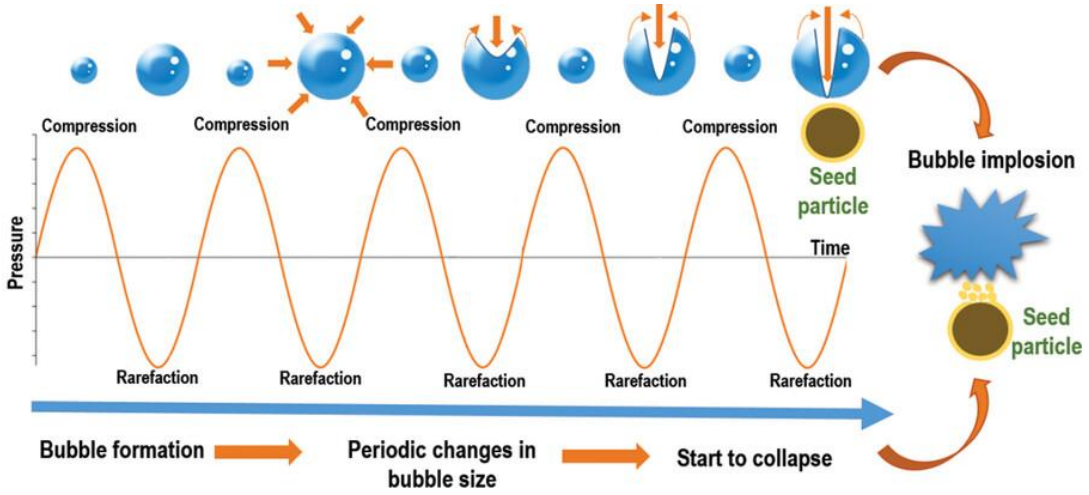
1.Ultrasound-assisted extraction (UAE)-Sonication

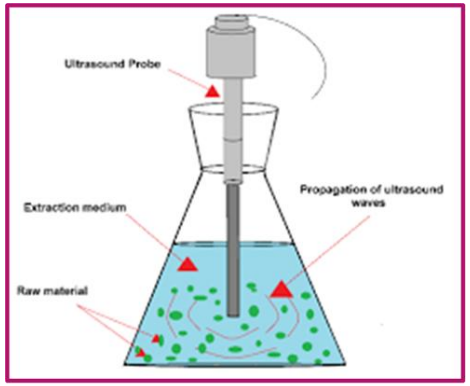
- This technique using a special type of **sound wave** that is **20 kHz to 100 MHz**
- They act by producing a **compression** and **expansion** through the medium it passes through.
- This process produces a phenomenon called **cavitation**, which means **production, growth and collapse of bubbles**.
- A **large amount of energy** can produce from the conversion of **kinetic energy** of motion into **heating the contents of the bubble**, the formed bubbles have :
- **These are the governing factors of UAE:**
- **Extremely high temperature about 5000 K,**
- **Extremely high Pressure 1000 atm,**
- **High heating rate** (high frequency) **.**

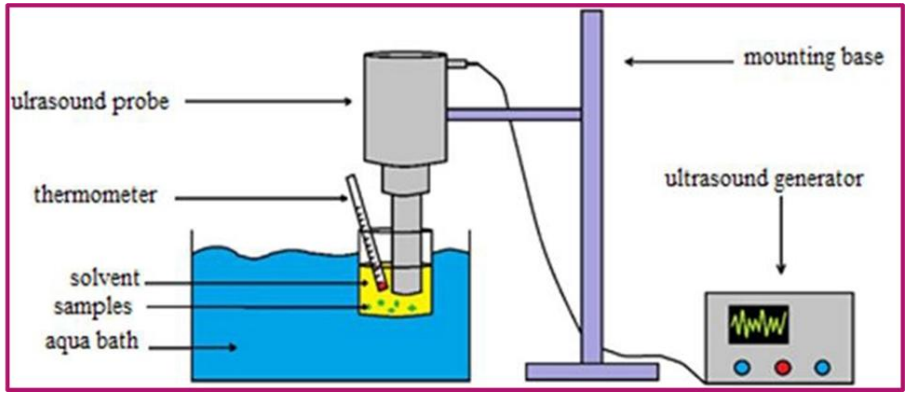


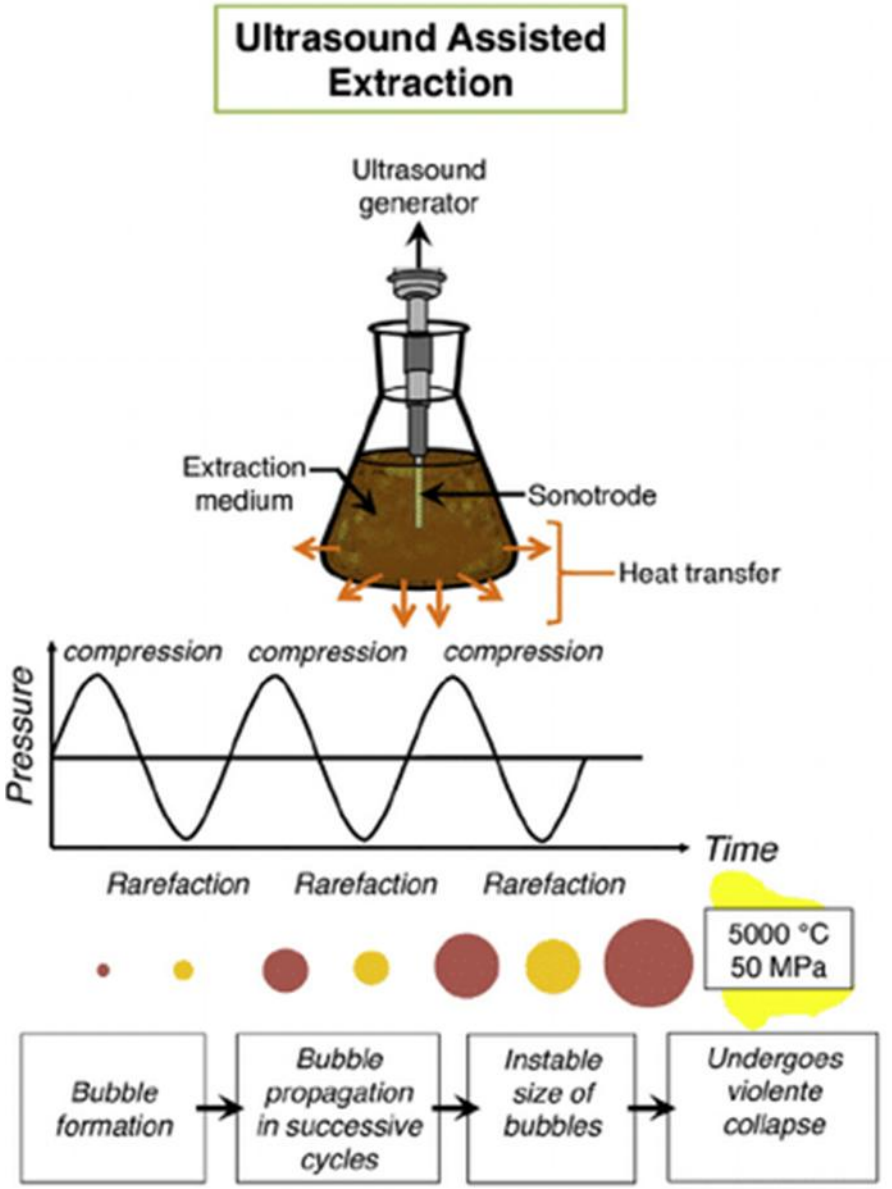
- **The resulting advantage of UAE technique is :**
- **Facilitating organic and inorganic compounds leaching from plant matrix (due to formation of microchannels)**
- **Accelerated access of solvent to cell materials of plant parts (fast and easy solvent penetration).**


US
Probe
Solvent
Seed
particles
Bubbles


Compression
Compression
Compression
Compression
Compression
Seed
particle
Bubble implosion
Pressure
Time
Rarefaction
Rarefaction
Rarefaction
Rarefaction
Rarefaction
Seed
particle
Bubble formation
Periodic changes in
bubble size
Start to collapse


Ultrasound Probe
Extraction medium
Propagation of ultrasound
waves
Raw material

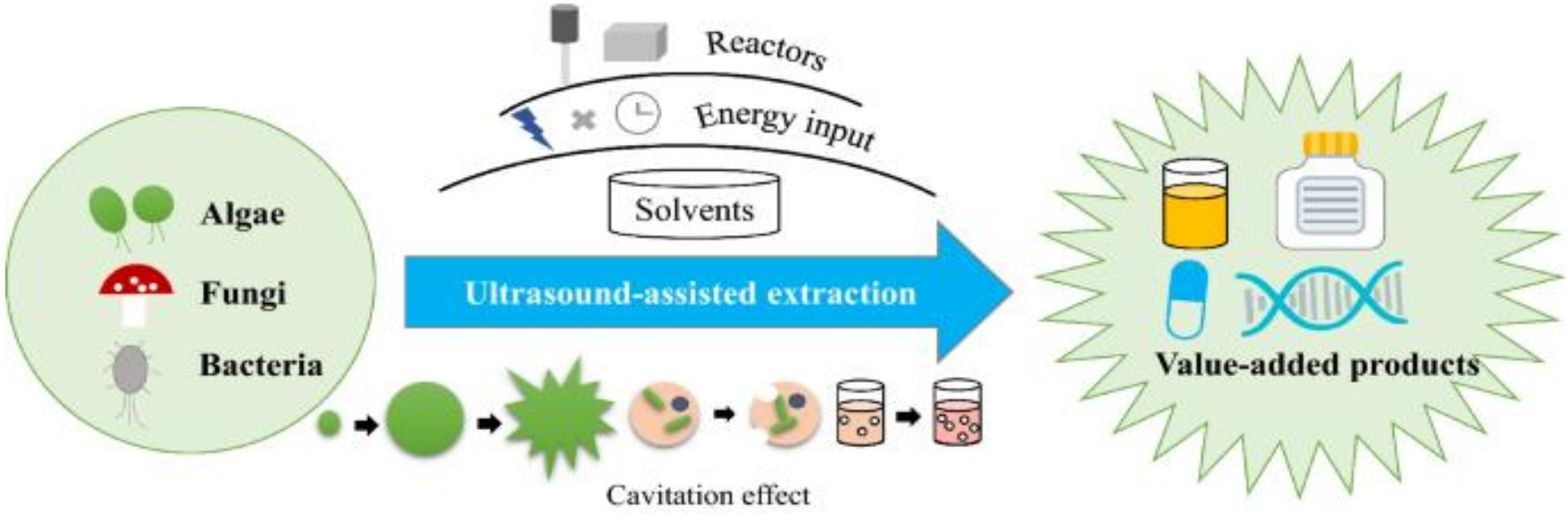

mounting base
ulrasound probe
thermometer
ultrasound generator
solvent
samples
aqua bath


Ultrasound Assisted
Extraction
Ultrasound
generator
Extraction
medium
Sonotrode
Heat transfer
compression
compression
compression
Pressure
Time
Rarefaction
Rarefaction
Rarefaction
5000 °C
50 MPa
Bubble
formation
Bubble
propagation
in successive
cycles
Instable
size of
bubbles
Undergoes
violente
collapse

Advantages of this method :

- **Reduction in extraction time, energy** and **use of solvent**,
- **Higher yield of crude compounds** and
- **selectivity in extraction process** .

- **Example for such extraction technique:**
- **Phenolic compounds** such as Rutin, and Kaempferol were extracted from strawberries.
- very effective for extracting three **alkaloids** (vindoline, catharanthine and vinblastine) from Catharanthus roseus .



2. Enzyme-assisted extraction (EAE)

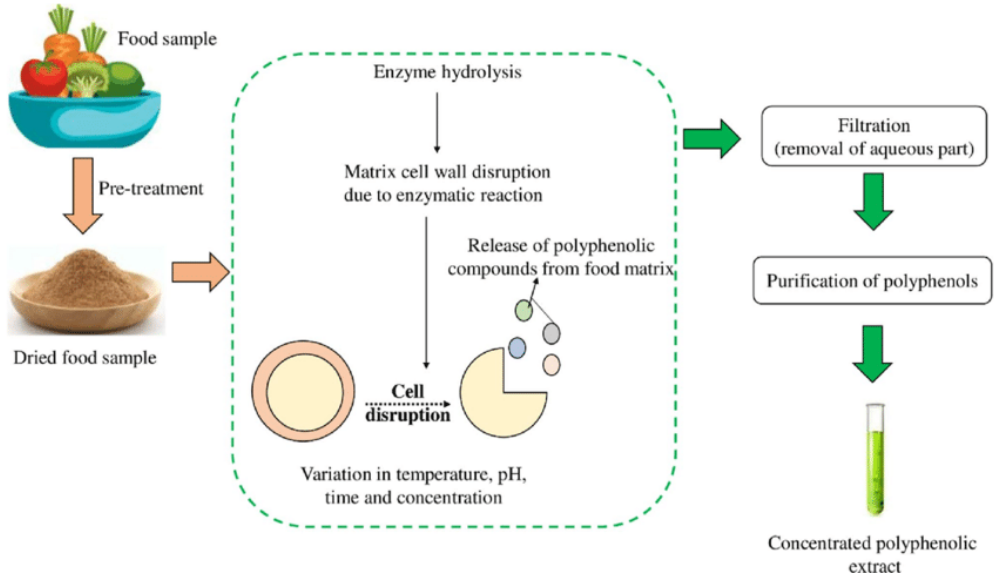
- Some phytochemicals in the plant are retained in the **polysaccharide-lignin** network by **hydrogen or hydrophobic bonding** making them **resistant to be extracted** with a solvent in a routine extraction process.
- A novel and an effective way to release bounded compounds and increase overall yield is the **Enzymatic pre-treatment technique** which has been considered.
- Enzymes like **cellulase, α-amylase,** and **pectinase** are used for this extraction purpose to **hydrolyze the structural polysaccharides and lipid bodies besides plant cellular wall.**

➢ There are two types of enzyme-assisted extraction:

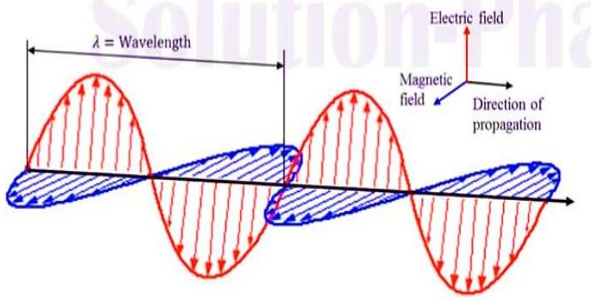
i. enzyme-assisted aqueous extraction (EAAE) .

ii. enzyme-assisted cold pressing (EACP)

➢ Enzymatic extraction considered as **eco-friendly technology for extraction of bioactive compounds.**



3. Microwave assisted extraction (MAE)

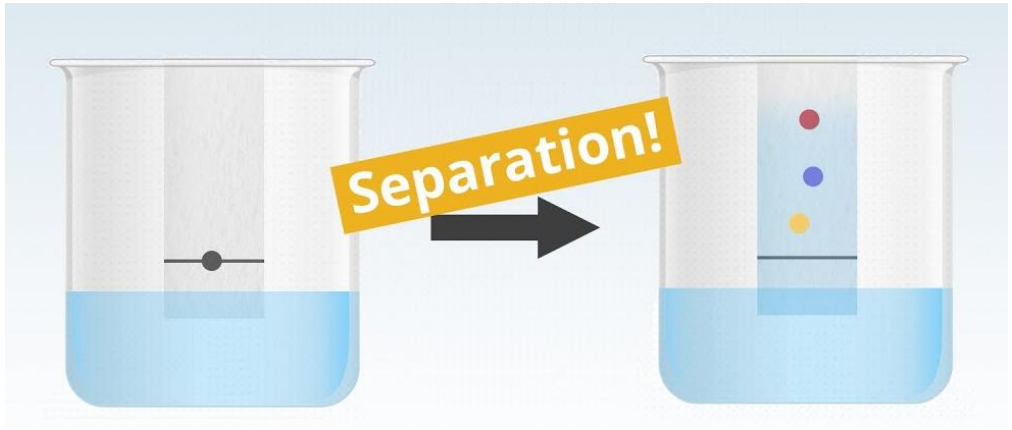


- Microwaves are **non-ionizing electromagnetic waves** (frequency of **300 MHz to 300 GHz**) which are useful in the extraction of Phytoconstituent.
- The prefix micro in microwave indicates that microwaves are the wave with **shorter wavelength** compared to radio waves.
- Microwaves are made up of two oscillating perpendicular fields which are **electric field and magnetic field**
- **Principle of Microwave Assisted Extraction:**
- Heat is generated directly within the solvent by **ionic conduction** of the **polar solvent's dipole rotation and dissolved ions,** therefore its principally used for **polar solvents**. Accordingly, substances with **low polarity are not heated while exposed to microwaves**.
- Irrespective of any drying method used, plants still do contain some moisture (microscopic traces of moisture that serve as a target for microwave heating). **When this moisture inside the plant material gets heated under the influence of microwaves get evaporated** and creates great pressure on the cell wall from inside because of **swelling of plant cells. This causes the rupturing of cell wall** (**electroporation effect**) thus facilitates the leaching of phytoconstituents (leakage and release of intracellular components) from the cells into the surrounding solvent.



Chromatography:

- Is a technique used **to separate and identify the components of a mixture**.
- Chromatography is a physical method of **separation in which the components of a mixture dissolved 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.
- Chromatography then is a mechanism for isolation, identification (**qualification**) and/or **quantification** of a single compound from a mixture of solutes.



- The **difference in distribution constant** of a solute in a mixture of compounds between the two phases control the isolation of that solute, also **solute solubility in Mobile Phase**, **adsorption on the stationary phase** and the **ionic forces** among the three components.
- **Chromatography types:** according to the **type of mobile and stationary phases**, chromatography can be divided to:

1- Liquid chromatography: mobile phase is a liquid. (LLC, LSC).

2- Gas chromatography : mobile phase is a gas. (GSC, GLC).

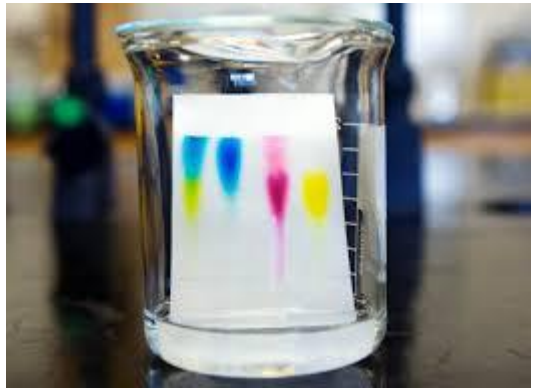
Mobile phase	Stationary phase	abbreviation
Liquid	Solid	LSC
Gas	Solid	GSC
Liquid	Liquid	LLC
Gas	Liquid	GLC

- The separation in both **LSC & GSC** depend on **adsorption of solute on the stationary phase**, while in **LLC & GLC** depend on **partitioning of solute between the 2 liquid phases.**

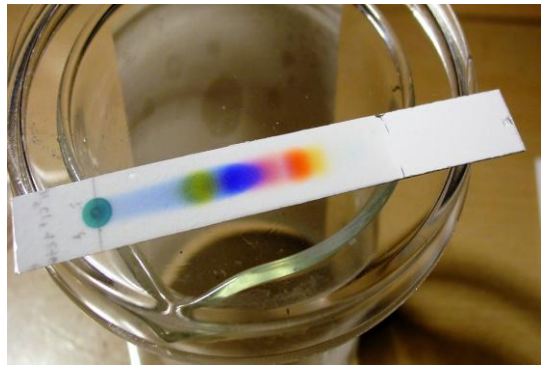
- **Classification based on the physical arrangement of the system**:

a) **Closed chromatography system**. Ex: column and gel chromatography.

b) **Open chromatography system**. Ex: paper and thin layer chromatography.



- In the closed system the stationary phase is packed in a **close container**, while in the open system the stationary phase **spread over a flat surface** like paper or glass plate.



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 mechanism of separation:

The basic mode of molecular interaction rolls this classification, sometimes more than one mechanism could be responsible for the separation and isolation of different compounds.

1- Adsorption chromatography.

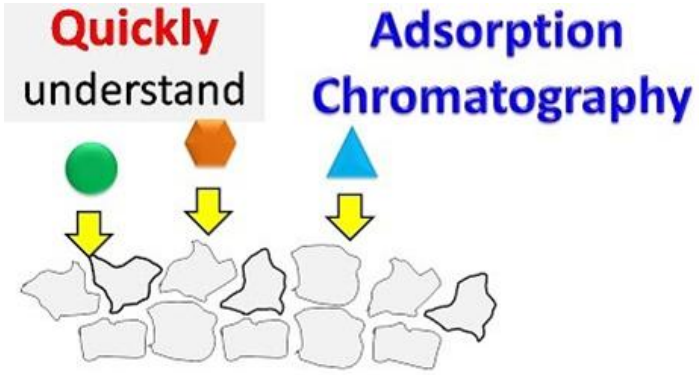
2- Partition chromatography.

3- Ion exchange chromatography.

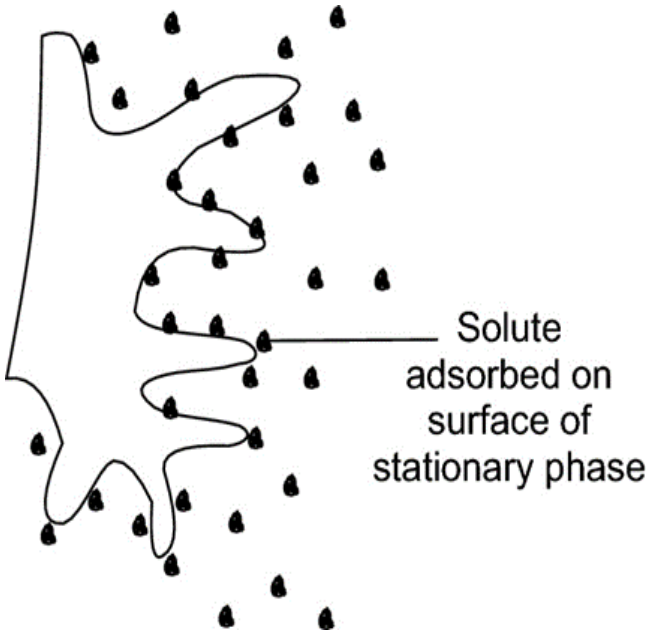
4- Gel filtration chromatography.

5- Affinity chromatography.

1) Adsorption chromatography:

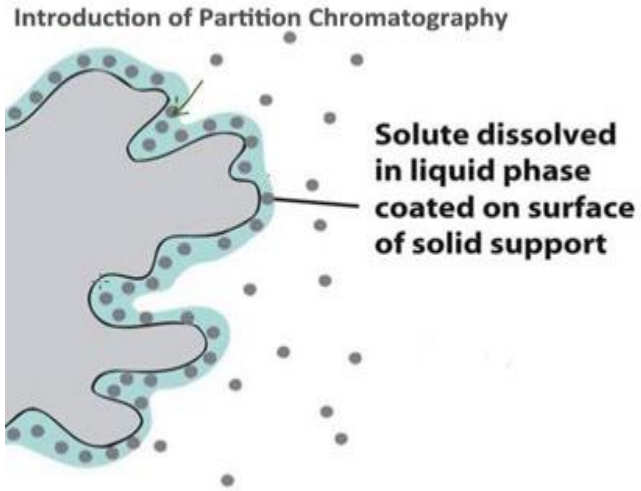


- It is a **physical process** that **doesn't involve any kind of chemical bond formation**, but only a sort of **weak forces of H-bonding, and Van der Waal forces**. In this way a state of sorption and desorption will affect the solute distribution between the stationary and mobile phases.
- To promote this process almost any **inert material** can be used as a **stationary phase (adsorbent phase),** so it **doesn't react** with the solute sample or the mobile phase and **doesn't dissolve** in the mobile phase like **silica, cellulose and alumina**.



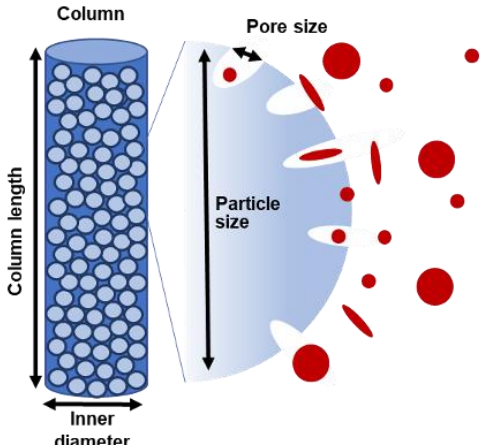
2) Partition chromatography:

- It is a **liquid-liquid isolation technique**, in which **both the solvent and the sorbent are liquids**.
- When one of the **liquids loaded on to a solid supporter** such as a **cellulose paper that coated with water**, then a separation of the solute carried out with an **immiscible (organic) mobile phase**, and the compound that is **more soluble in the mobile phase will migrate up the cellulose paper (plate/column) to a greater extent than those of higher solubility in the stationary phase.**
- **So, it depends on the solubility of the solute in these two liquids**



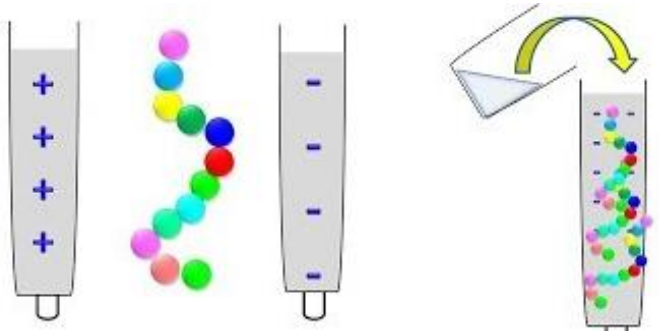
3) size-inclusion-exclusion chromatography:

- In this type of chromatography Separation depending on **relative sizes of solute and sorbent** take place. Ex: **gel chromatography.**



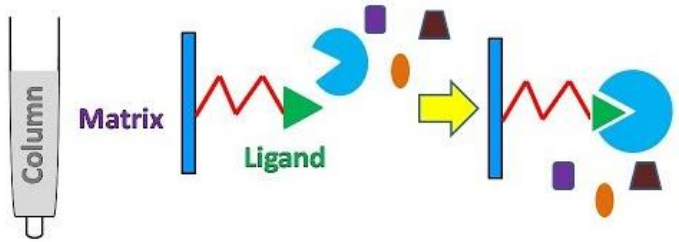
4) Ion-exchange chromatography:

- It is a specific and limited technique, its employed when the **compound to be separated carry a charge**.
- In this technique, the **stationary phase is composed of a polymer resin carrying a counter ion (charge).**
- This kind of separation controlled by the **difference in affinity between ionic compound and the stationary phase.**



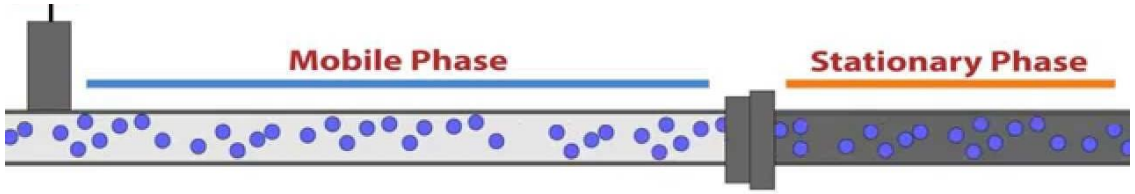
5) Affinity chromatography:

- it's a type of **biological molecule interaction**, like **antibody-antigen interaction, enzyme-inhibitor interaction, receptor-agonist/antagonist interaction and DNA-DNA interaction.**

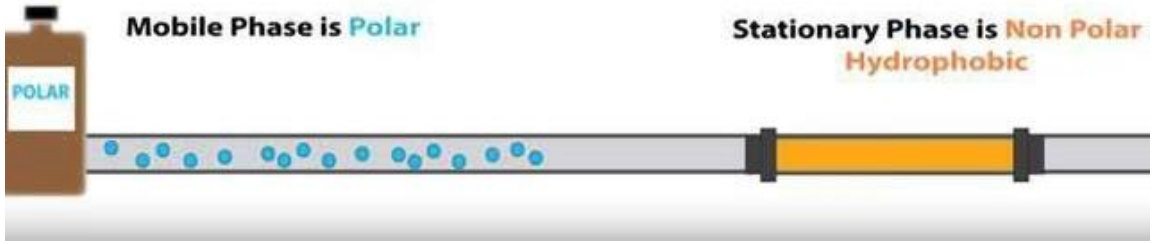


Normal phase and Reversed phase terms

- when the **stationary phase is more polar** than the mobile phase then it is a **normal phase chromatography**.
- Ex: column and TLC of **silica** with its **silanol group** as a stationary phase and an organic solvent/mobile system.



- while when the **stationary phase become less polar** than the mobile phase then is called a **reversed phase chromatography**.
- Ex: when **silica bonded to hydrocarbon** and the mobile phase is water or acetonitrile/water as in **HPLC** (high performance liquid chromatography).



Planar chromatography

- Is a separation technique in which the stationary phase is present as or **on a plane (open surface)**.
- The plane can be a **paper**, serving as such or impregnated by a substance as the stationary bed (**paper chromatography**) or a **layer of solid particles spread on a support such as a glass plate** (**thin layer chromatography**).
- Different compounds in the sample mixture travel different distances according to **how strongly they interact with the stationary phase as compared to the mobile phase**.
- The specific **Retention factor (Rf)** of each chemical can be used to **aid in the identification of an unknown substance**.

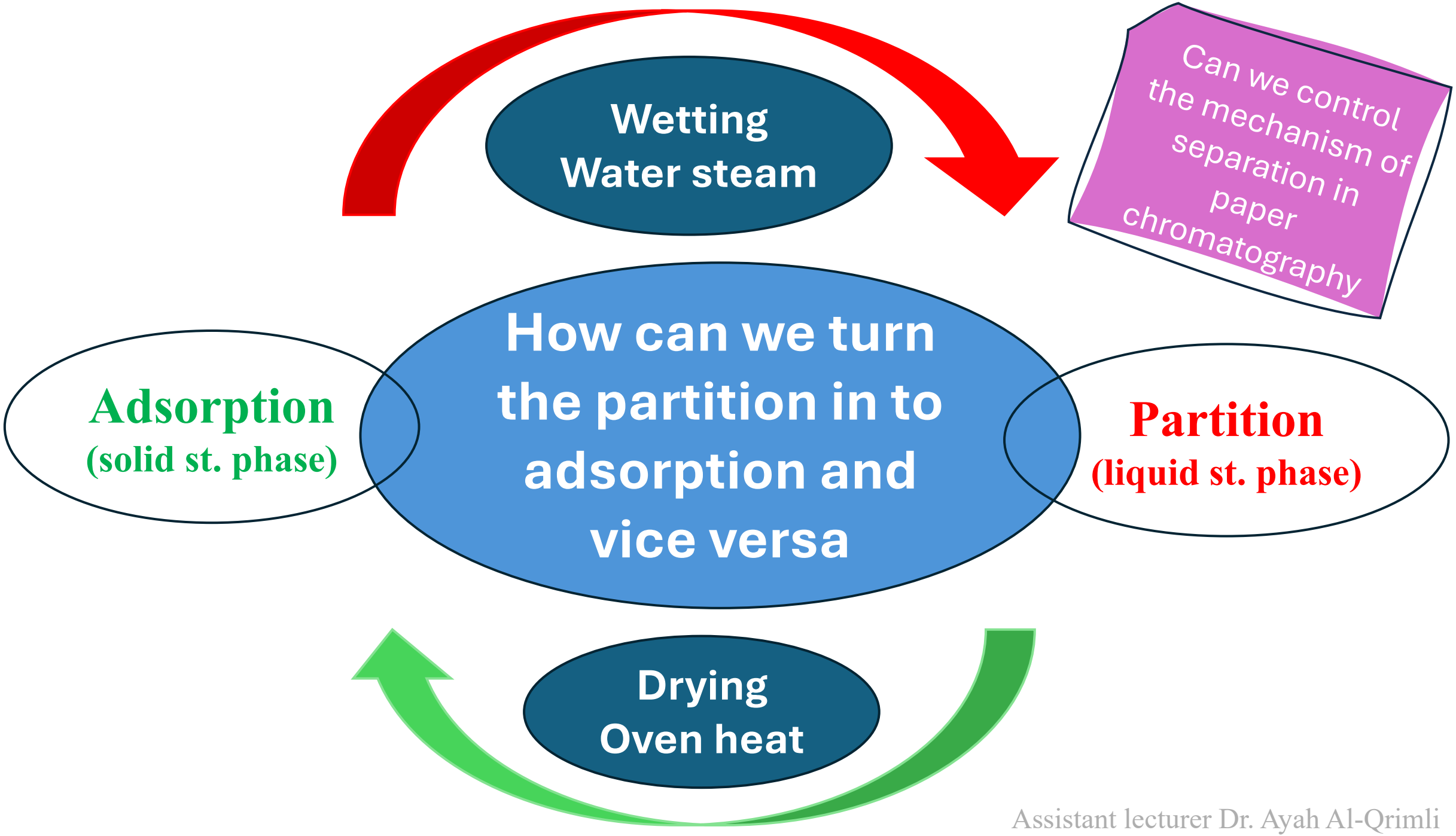
Paper chromatography



- It is a kind of **partition chromatography** .
- The paper used for chromatography are usually **Whatman No.1 filter paper**. The paper commonly used consists of **highly purified cellulose** .
- The principal of separation **is mainly partition** rather than adsorption since cellulose layers in filter paper **contains moisture** which **acts as stationary phase**.
- Apparatus :
- The apparatus required for paper chromatography consists of a **support for paper** , a **solvent** **and** an **air - tight chamber** in which the chromatogram is developed .
- The **closed chamber** is necessary to **prevent the evaporation of the volatile solvents** from the large exposed area of the paper.
- The size of the chamber vary according to the **size of the paper used** .
- The tank or chamber or jar used are usually round tanks which has ground glass lid & it should be **saturated with the solvent** before introduction of the paper .

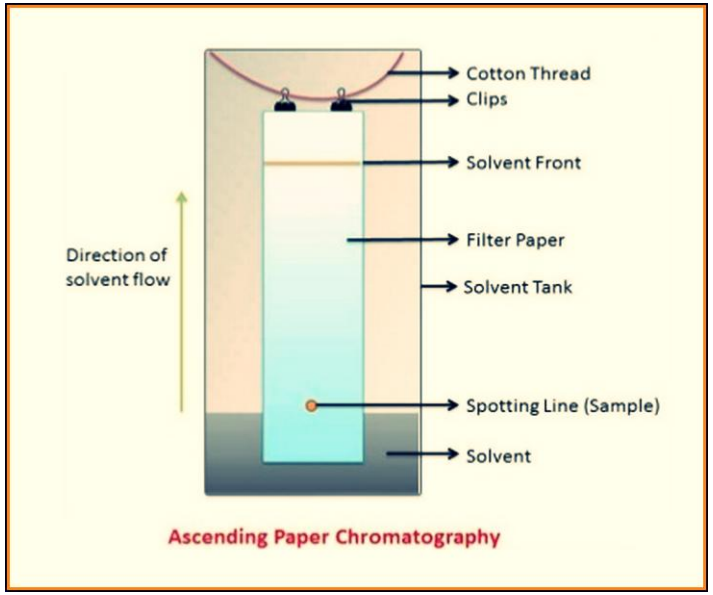


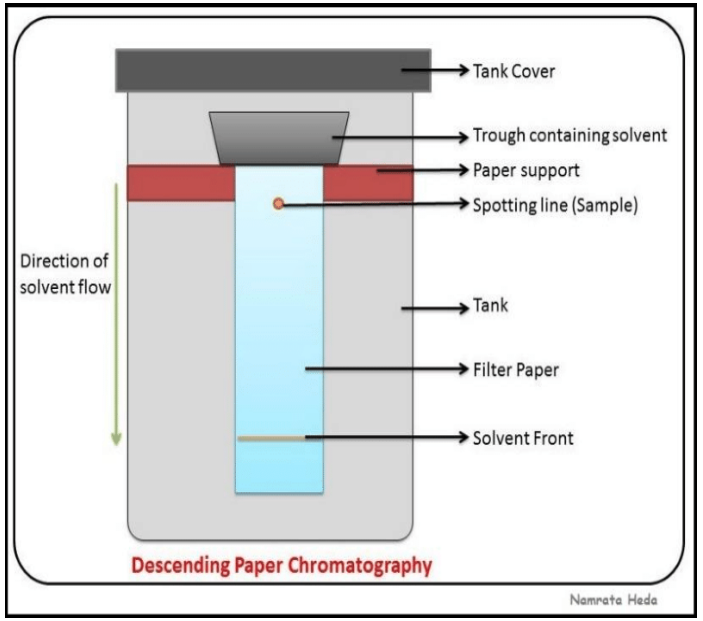



Wetting
Water steam
Can we control the mechanism of separation in paper chromatography
How can we turn the partition in to adsorption and vice versa
Adsorption
(solid st. phase)
Partition
(liquid st. phase)
Drying
Oven heat

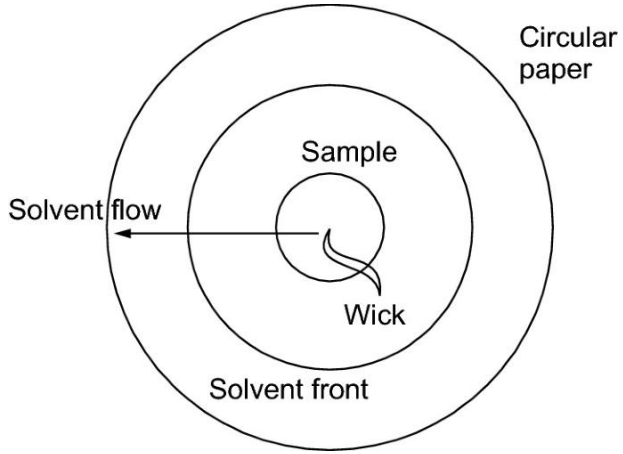
Development

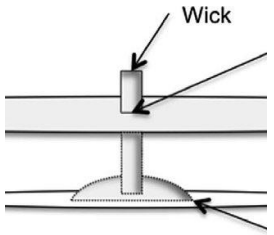
- Several developing techniques can be used in paper chromatography :
- **l-Ascending development:**
- This is the **simplest-most popular** type . The paper is suspended **vertically** with **the spot** about **1-2 Cm above the surface of the solvent** **to prevent diffusion of the sample downward into the solvent reservoir**.
- The solvent ascends through the paper by **capillary action** . The rate of ascent **is slow** & **decrease with time** **because of gravity** , however , the slow rate enhances the possibility of achieving partition equilibrium & often results in compact sharply defined spots .
- **2-Descending development :**
- This is done with a **solvent at the top of the tank or chamber** , the **direction of flow is downward,** **To prevent rapid syphoning , the paper is folded into a U-shape with a short initial rise from the solvent trough .**
- The method is **much faster** than the ascending development, **longer pieces of paper** may be used & **large amount of solvent** can be used if necessary for slow moving spots .

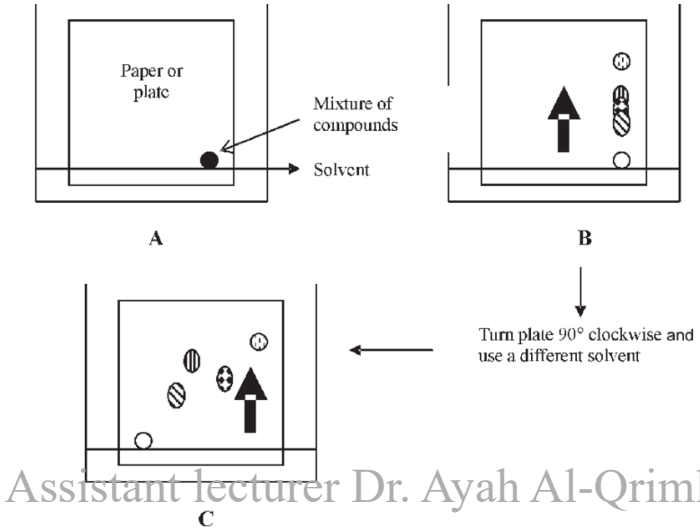




- **3-Radial or horizontal development:**
- In this method the sample is spotted around **the center** of the **horizontally placed filter paper** with a **wick in the center to supply the solvent to the center of the paper from a supply trough below .**
- The solvent move outward separating the components of the sample **along radial paths forming circles of increasing diameter** . A covered petri dish is an adequate container, or the paper can be sandwiched between two glass plates , with a hole in the center of the upper one for the introduction of the sample .
- **4-Two-dimensional development :**
- In all previously described methods of chromatography the eluting solvent could flow in one direction only across the paper . On a plane surface such as a piece of paper , it is possible to carry out a sequential development in **two directions .**
- **The sample is applied as a spot close to a corner . This is developed in the normal fashion by ascending or descending procedure until the fastest moving spot approaches the end of the paper** . Then the paper is removed & after evaporating the solvent it is **turned 90 degree** & developed a second time with another solvent having different eluting properties In this manner , samples which could be only partially separated with either solvent alone may be completely separated by the combination of solvents



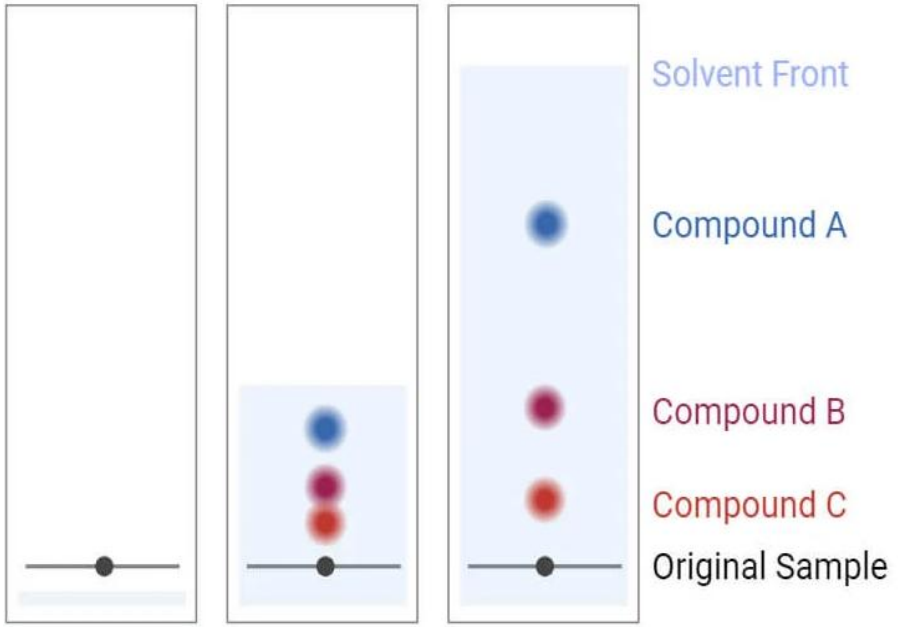


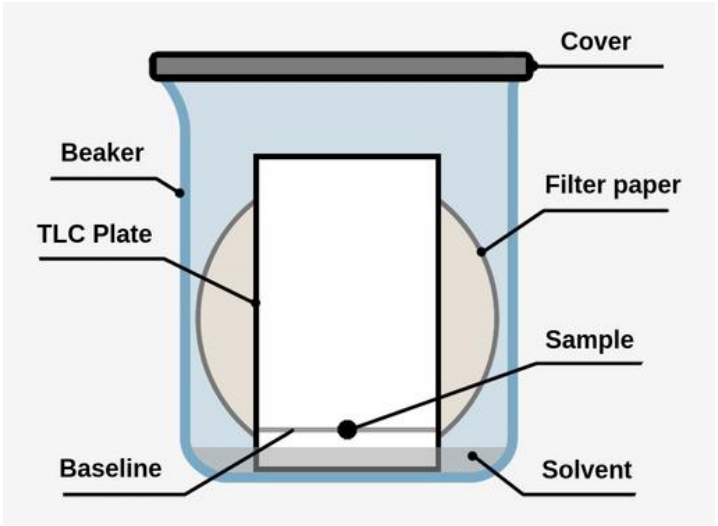


Assistant lecturer Dr. Ayah Al-Qrimli

Thin layer chromatography (TLC)

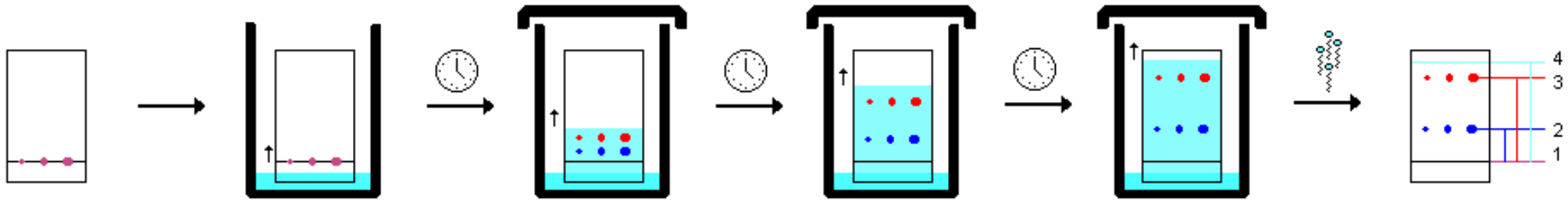
- It is a **simple** and **inexpensive** analytical technique that can quickly and **efficiently** separate quantities of less than ten micrograms of material.
- TLC has many applications in the organic laboratory.
- TLC is used for the **rapid analysis of reagent** and product **purity**, or to quickly determine the **number of compounds** in a mixture.
- Also, by comparing an **unknown compound's** behavior to the behaviors of known standard compounds, mixture compounds can be tentatively identified.





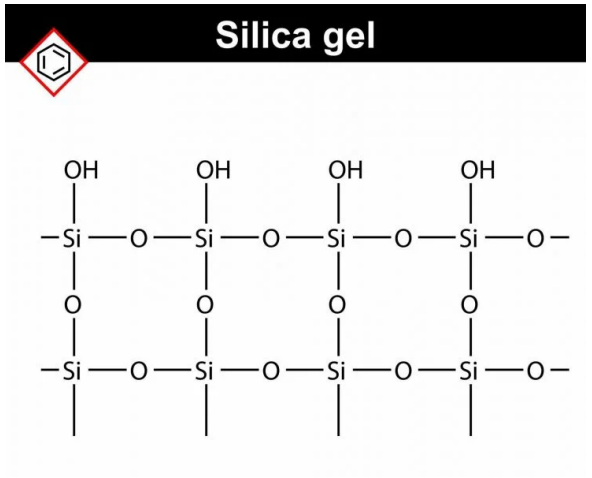
Principle of TLC is adsorption

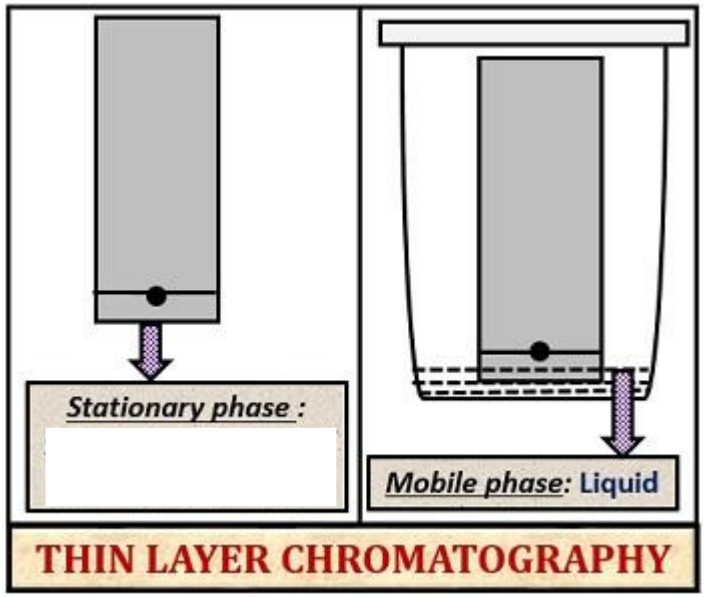
- The technique is based on a **polarity** interplay between the **sample** and the solid (or **stationary phase** which is **finely ground silica or alumina particles** coated on plastic or aluminum sheet or glass slide to produce TLC plate, and the **mobile phase**, which is a liquid called the **eluting solvent**.
- The sample to be separated first becomes **adsorbed** onto the surface of the solid phase through **polar interactions**.
- **More polar** samples adhere (or bind) **more strongly** than less polar ones.
- The **mobile phase** then comes in and sweeps across the stationary phase, competing for the sample.
- Different components with **different polarities will travel at different rates** as the solvent moves, causing their separation.



The components of TLC

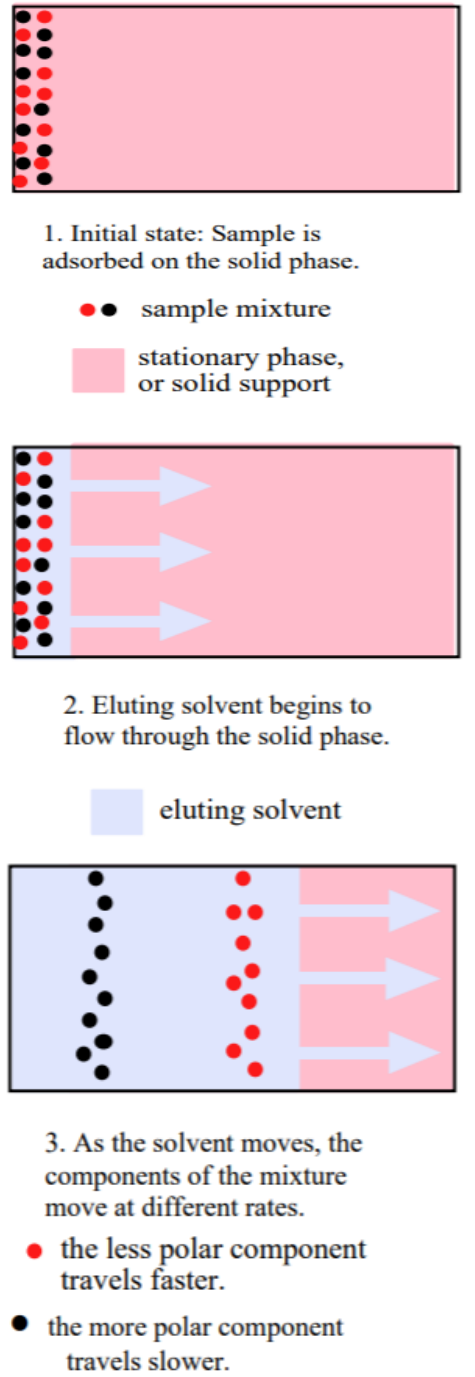
- In TLC, the **stationary phase** is typically **alumina** (Al203. XH20) n or **silica gel** (SiOz. xH2O) n.
- The **covalent networks** of these absorbents **create very polar materials (very polar stationary phase.)**
- Therefore, the **more polar** the molecule to be separated, the **stronger the attractive force to the stationary phase.**
- **Nonpolar** molecules will have a **lower affinity for the stationary phase and will remain in the solvent longer.**
- Although alumina and silica are the most common stationary phases used for TLC, there are many different types.
- They range from **paper to charcoal**, **nonpolar to polar,** and **reverse phase to normal phase.**





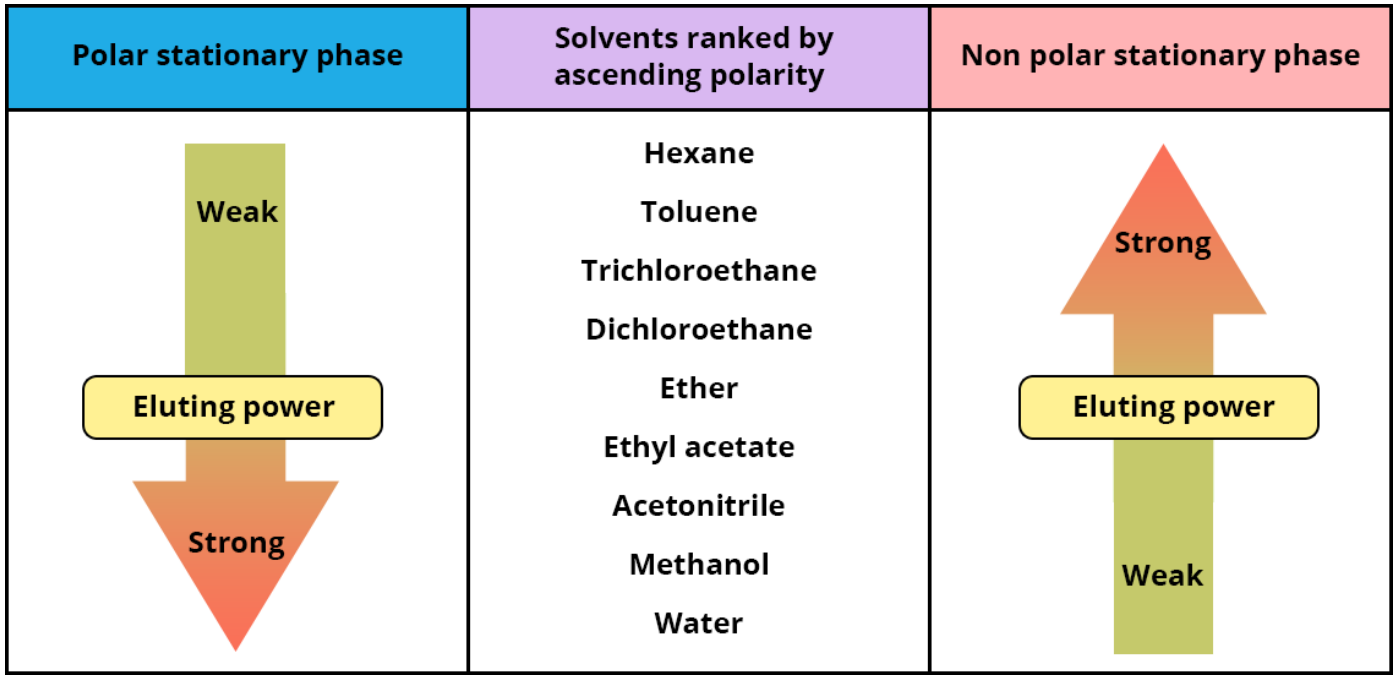
Elution/Mobile phase : Solvent effect

- **The most common factor that is adjusted to achieve good separation is the solvents used in the mobile phase.**
- There are many choices for solvents and **solvent mixtures** are quite common.
- The substances being separated are adsorbed onto the stationary phase, but polar solvent molecules are also adsorbed by the stationary phase.
- The eluting solvent should also show **good selectivity in its ability to dissolve the substances being separated.**
- The solubility of different compounds in the eluting solvent plays an important role in **how fast they move up the TLC plate.**
- However, a more important property of the **solvent is its ability to itself be adsorbed on the adsorbent.**
- To the extent that the solvent has affinity for the adsorbent, **it can displace the compounds being separated thereby "pushing" them up the plate.**
- If the solvent is **too strongly adsorbed**, it can **fully displace all compounds** causing them to move up the **plate together near the solvent front with no separation.**
- If the solvent is **too weakly adsorbed**, its solvating power alone may **be insufficient to move any compounds** fast enough to effect separation.



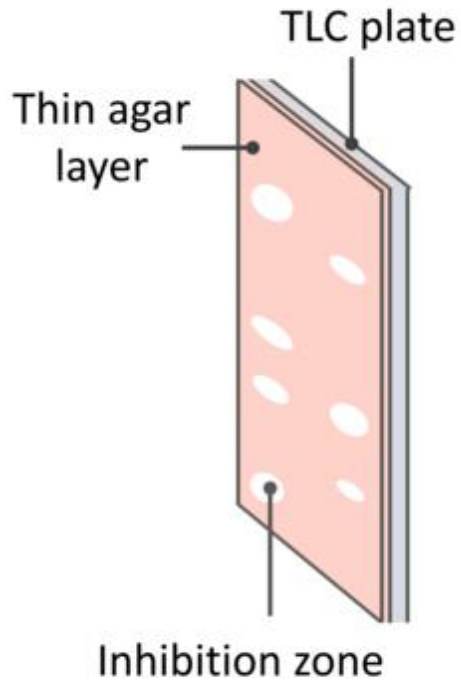
- The **eluting strength** of a solvent is primarily related to **how strong it adsorbs on the adsorbent** (stationary phase) and because typical adsorbents are **highly polar** thus the **eluting strength increases with solvent polarity.**
- **Mixtures of solvents** are employed to achieve optimum separation by TLC
- When using solvent mixtures, it should be kept in mind that addition a **minor amount of a polar solvent** can result in a **large increase in the eluting power of the mixture**.

Less Eluting Strength (less polar solvents)	Pentane, hexane, heptane
	Toluene, *p*-xylene
	Dichloromethane
	Diethyl ether (anhydrous)
	Ethyl acetate (anhydrous)
	Acetone (anhydrous)
	Acetic acid
	Ethanol (anhydrous)
	Methanol (anhydrous)
Greatest Eluting Strength (more polar solvents)	



Advantages of TLC

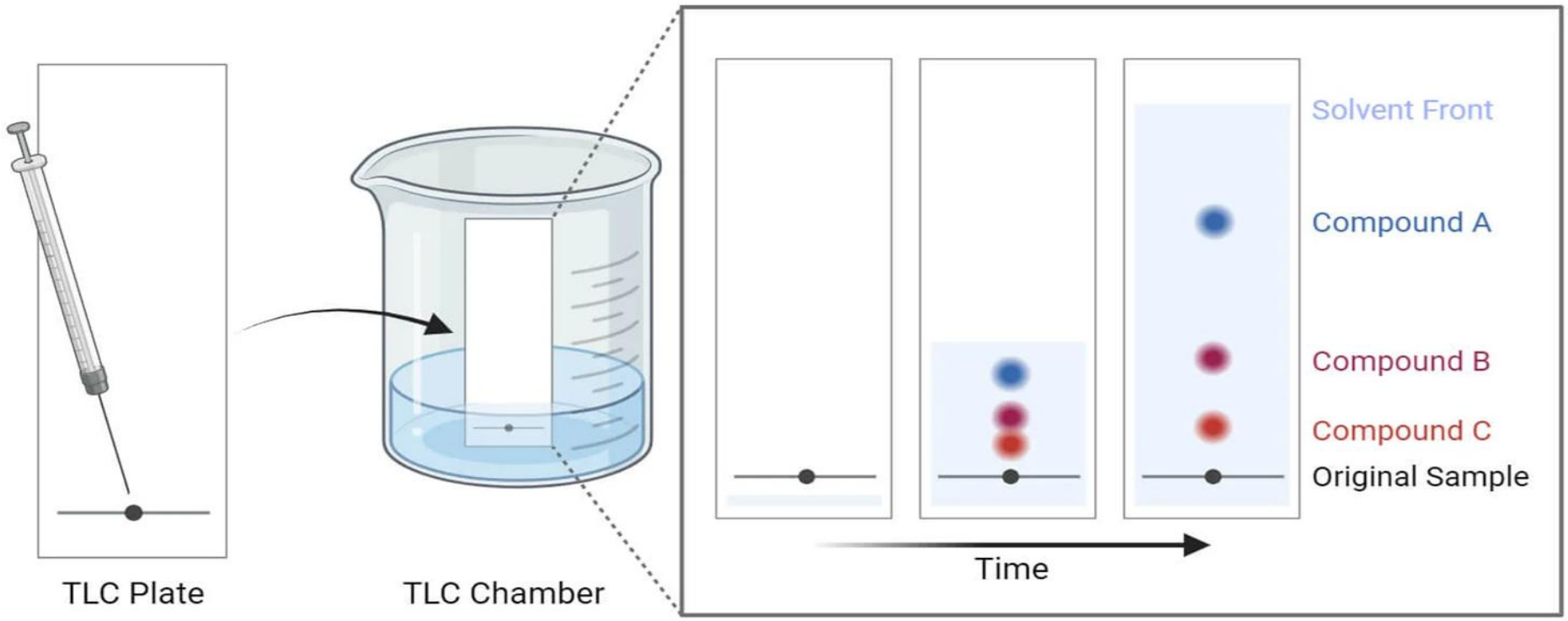
- **Microscale techniques**: only few milligrams of extracts are enough to run on TLC plate for analysis and identification.
- **Rapid identifier**: Sample identification in **short time** and **Easy determination**: Number of compounds in a mixture could be easily determined.
- **Easy to monitor** a chromatography separation reaction.
- **Bioautography**: Identification of antimicrobial compounds can be done readily of TLC profiled plates. Here pouring potential test microbes (E.coli and Staph. aureus) cultures along with agar on resolved TLC plates can yield the **inhibition band on TLC this will confirm an antibacterial activity.**
- Resolved TLC plates can **be stored for long duration time** .



Thin Layer Chromatography

Stationary Phase
Sample spotted on TLC plate

Mobile Phase
Compounds separated based on their polarity over time

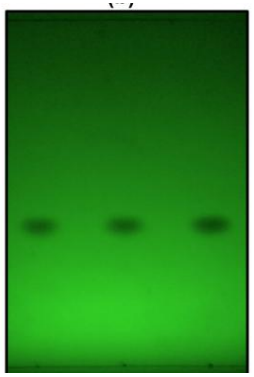


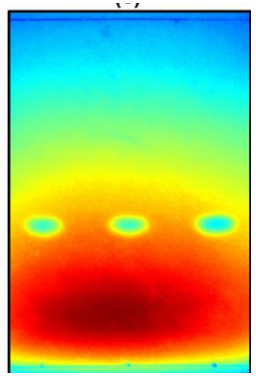
Visualization of TLC plate



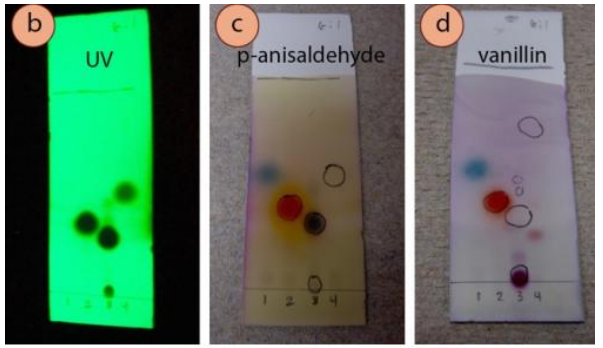


- If components of the reaction are **colored, no visualization method is required** (spots can be **seen directly on the silica layer**). However, since most organic compounds **are colorless**; therefore, one of the methods described below should be used to reveal the spots.
- **Non-destructive methods:**
- As a general visualization procedure, TLC plates normally contain **a fluorescent indicator** which makes the TLC plate **glow green under UV light of Wavelength 254 nm**.





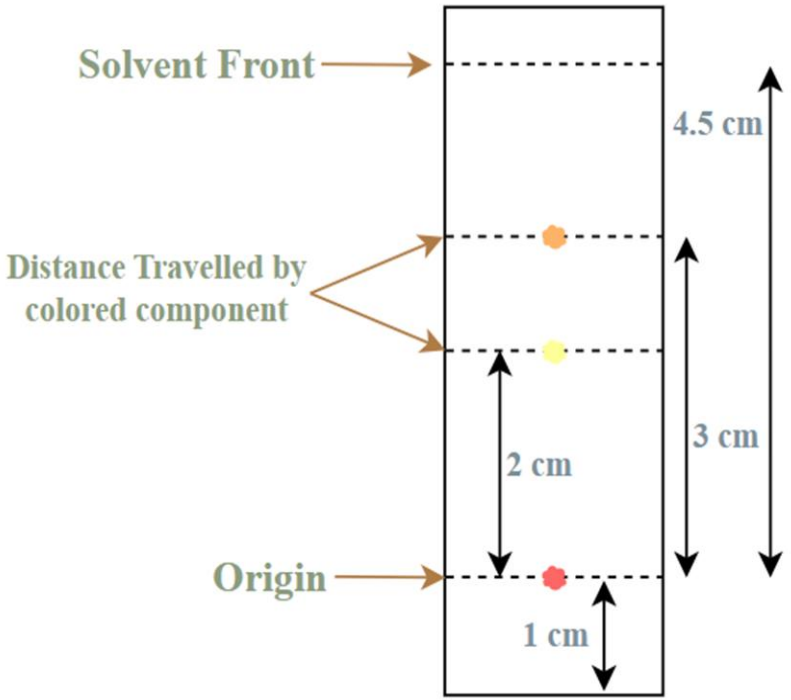
- **Compounds that absorb UV light** will quench the green fluorescence yielding **dark purple or bluish spots on the plate**. Simply put the plate under a UV lamp, and the compounds become visible to the naked eye.
- Another useful visualizing technique is an **iodine chamber**. Iodine sublimes and will **absorb to organic molecules in the vapor phase**. The organic spots on the **plate will turn brown and can be easily identified**. Also circle these observed spots, since the color stain will eventually fade from the plate.
- **Destructive methods:**
- **Staining** For compounds that are **not UV-active,** there are several varieties of stains that can be used depending on the nature of the compound of interest. To use a stain, simply dip the TLC plate into the staining solution (**sulfuric acid/ vanillin**) as quickly as possible, and then immediately absorb the excess stain with paper and heat carefully with a **heat gun or on a hot plate at 110°C until spots are revealed**.

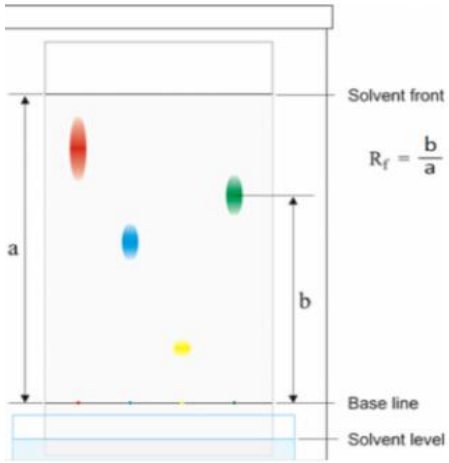


Calculation of retardation factor (Rf Values)

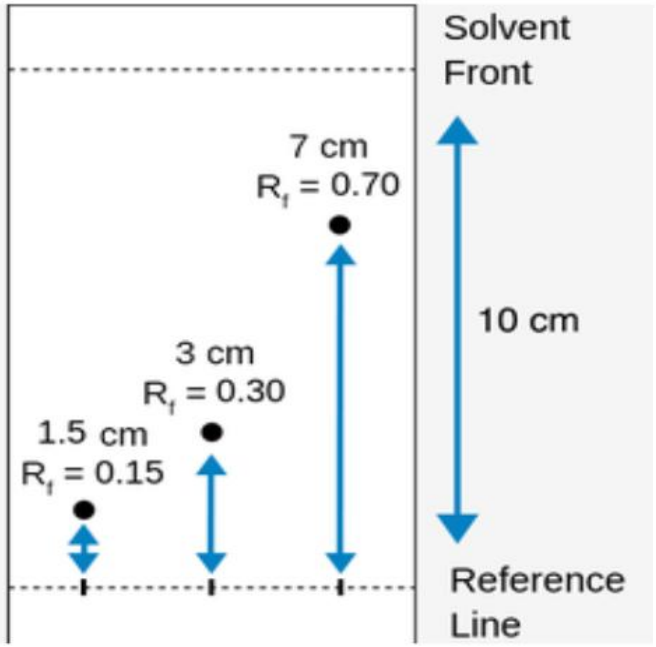
- Thin layer chromatography use for both **quantitative and qualitative analysis** of compounds mixture.
- **Qualitative analysis** is based on **measuring Rf values**, a retardation factor (Rt) (also known as retention factor) which describes the **ratio of time spent in the stationary phase relative to time spent in the mobile phase** basically is **the distance traveled by the compound divided by the distance traveled by the solvent.**

$$R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent front}}$$

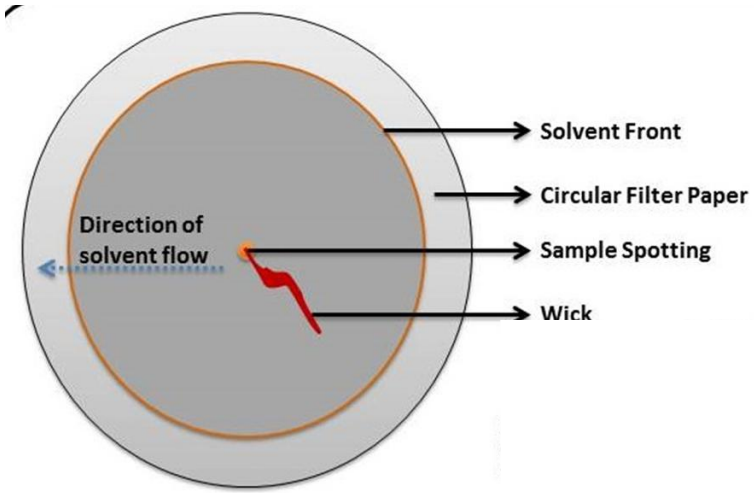


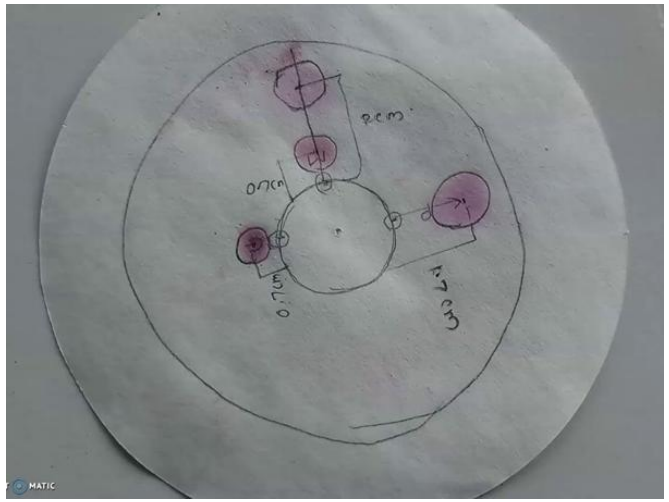


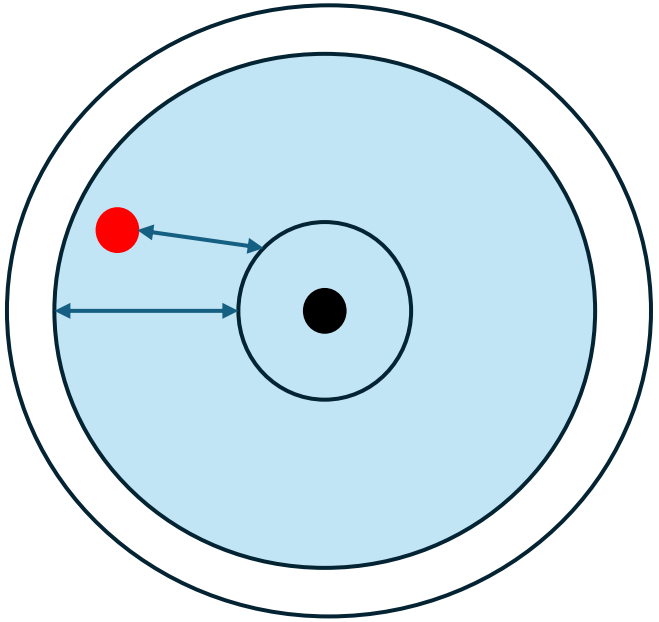
$$R_f = \frac{\text{Distance travelled by the substance}}{\text{Distance travelled by the solvent front}}$$



Q2:A round filter paper having a diameter of 12 Cm was used for chromatography, the solvent moved until it reached the circumference of the paper and the sample was spotted 1.5 Cm from the center of the paper. Calculate the Rf value for compound that move 3Cm from the place of spotting? (3 Marks).







The end

