Dr. Ula Al-Mousawi
Depat: Pharmacognosy

Classification of chromatography according to the type of Technique used

1. Paper Chromatography P.C.
2. Thin Layer Chromatography ,TLC.
3. Column Chromatography, CC.
4. Gas Chromatography, GC. (2 types depending on st. phase : GLC & GSC)
5. High Performance Liquid Chromatography, HPLC
6. Ion- Exchange Chromatography
7. Gel Chromatography
8. Electrophoresis

Classification of Chromatography according to mobile phase:

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

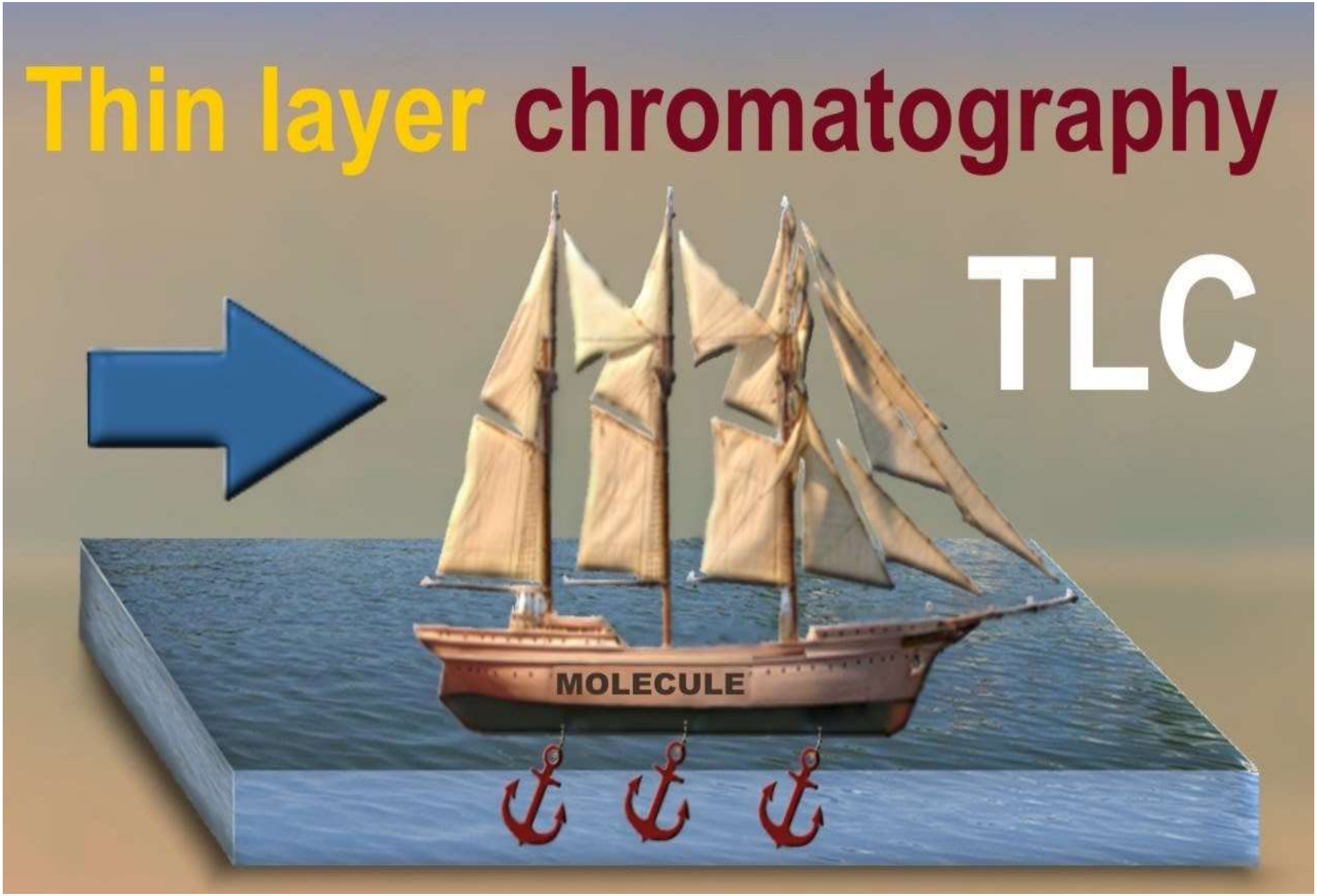
2- Gas chromatography : mobile phase is a gas. (GSC, GLC).

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 aluminium 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.


Thin layer chromatography
TLC
MOLECULE

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.

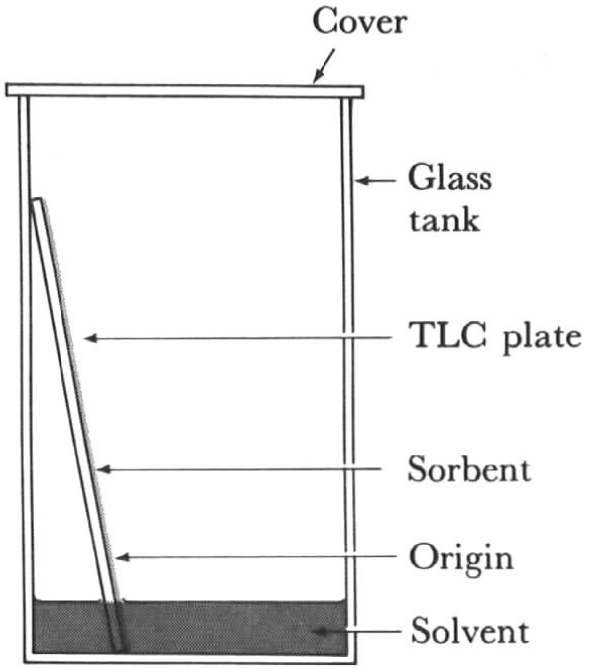
The mobile phase:

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.

Thin layer Chromatography (TLC)



Preparing the Chamber

To a jar with a tight-fitting lid add enough of the appropriate developing liquid so that it is 0.5 to 1 cm deep in the bottom of the jar.

Close the jar tightly, and let it stand for about 30 minutes so that the atmosphere in the jar becomes saturated with solvent.

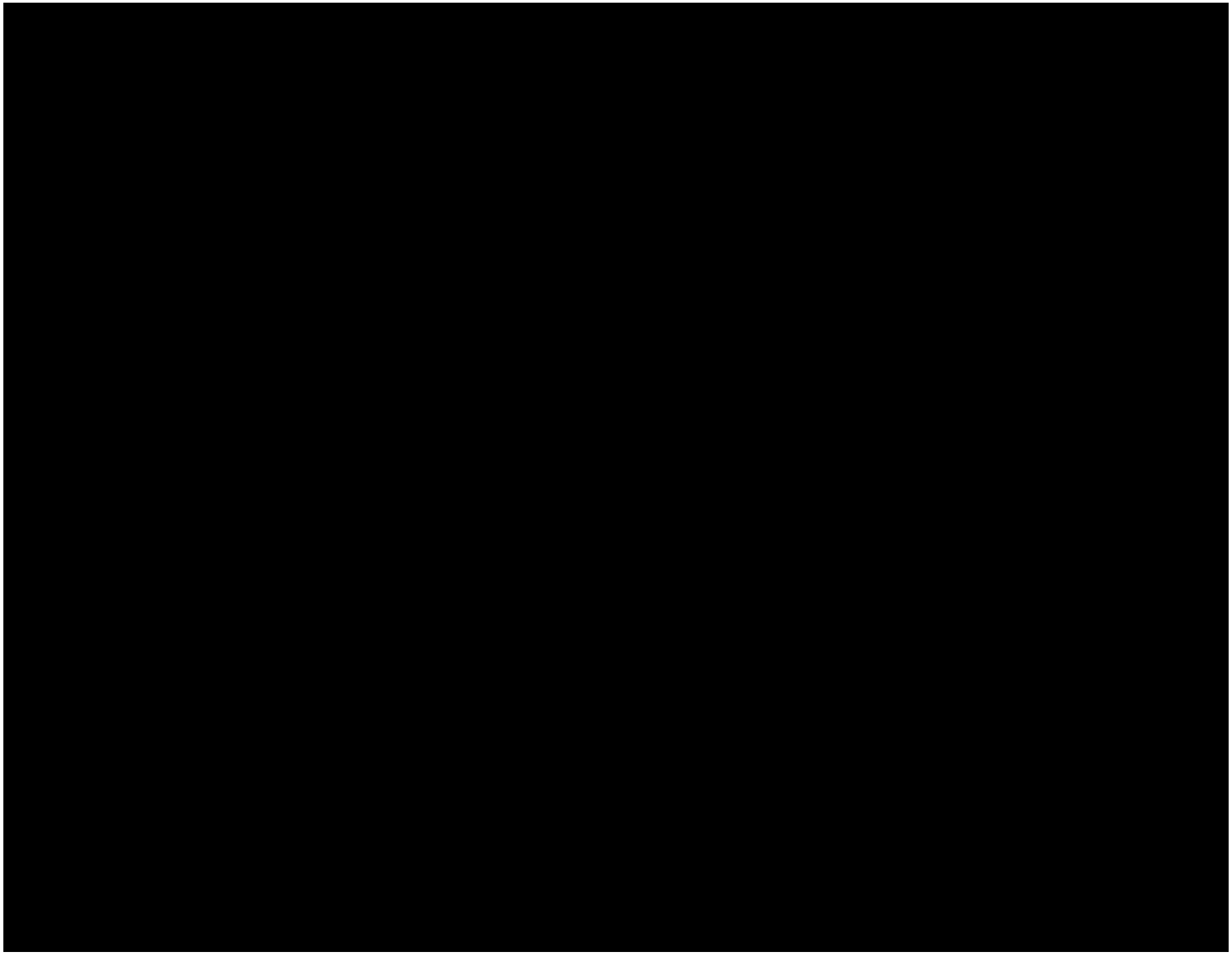
Preparing the Plates for Development

With a pencil, etch two small notches into the adsorbent about 2 cm from the bottom of the plate.

The notches should be on the edges of the plate, and each notch should be the same distance up from the bottom of the plate.

The notches must be farther from the bottom of the plate than the depth of the solvent in the jar.

Using a drawn-out capillary tube, spot the samples on the plate so that they line up with the notches you etched.



Developing the Plates

After preparing the development chamber and spotting the samples, the plates are ready for development.

Be careful to handle the plates only by their edges, and try to leave the development chamber uncovered for as little time as possible.

When the plates are removed from the chamber, quickly trace the solvent front (the highest solvent level on the plate) with a pencil.

Visualizing Agents

Alkaloids: Dragendorff's reagent

Cardiac glycosides: Antimony trichloride

Sugar: Aniline phthalate

Amino acids: Ninhydrin

Identifying the Spots (visualization)

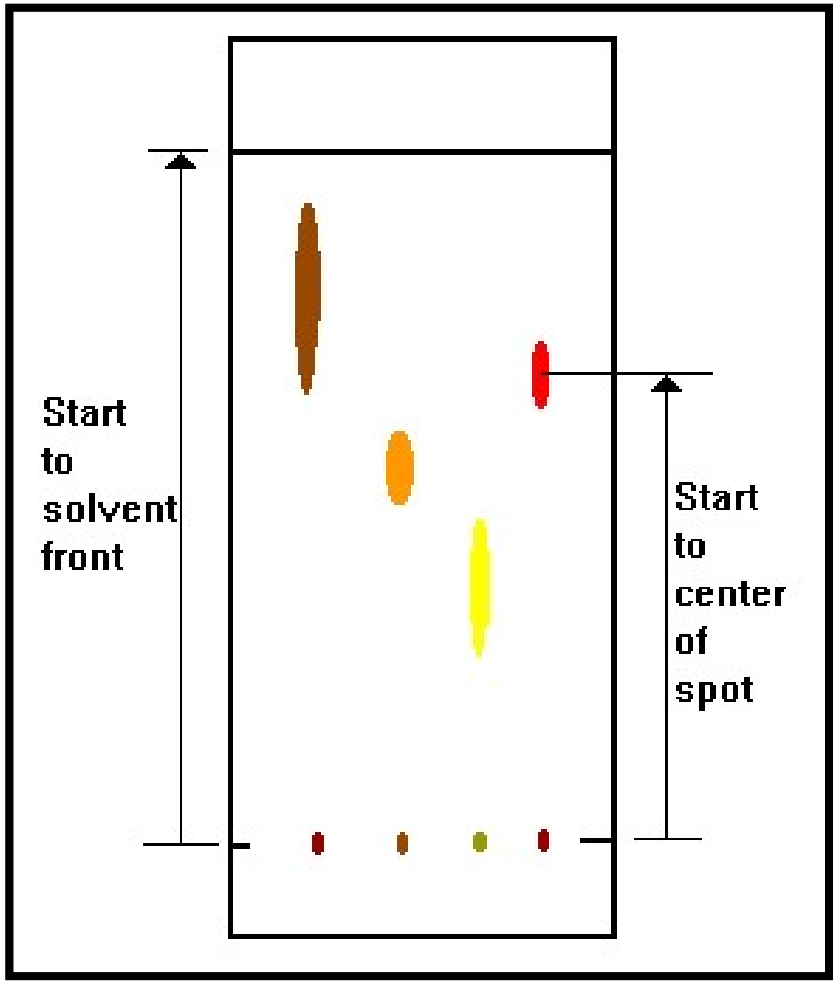
If the spots can be seen, outline them with a pencil.

If no spots are obvious, the most common visualization technique is to hold the plate under a UV lamp. Many organic compounds can be seen using this technique, and many commercially made plates often contain a substance which aids in the visualization of compounds

Explaining the Data

- 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.

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



R_f values often depend on the temperature and the solvent used in the TLC experiment.

The most effective way to identify a compound is to spot known substances – authentic - next to unknown substances on the same plate.)

In addition, the purity of a sample may be estimated from the chromatogram.

An impure sample will often develop as two or more spots, while a pure sample will show only one spot.

Larger Rf value ⟶ more soluble

Smaller Rf value ⟶ less soluble

Paper Chromatography

Paper Chromatography

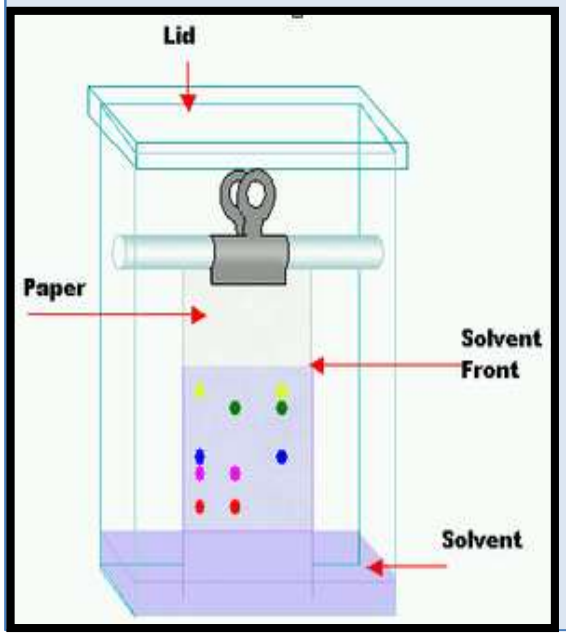
A method of partition chromatography using filter paper strips as carrier or inert support.

- **Cellulose support is in the form of sheet of paper which has large amount of water bound to it.**
- **Partitioning occurs between the bound water and developing solvent.**
- **The solvent used is water.**
- **Uses: To identify unknown samples**
- **Isolation of components of mixtures**

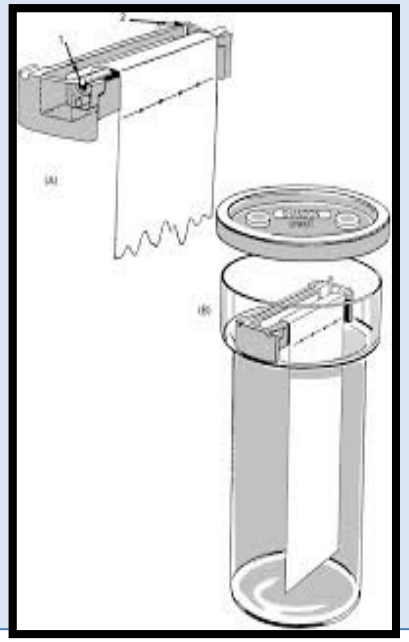
Types of Paper Chromatography

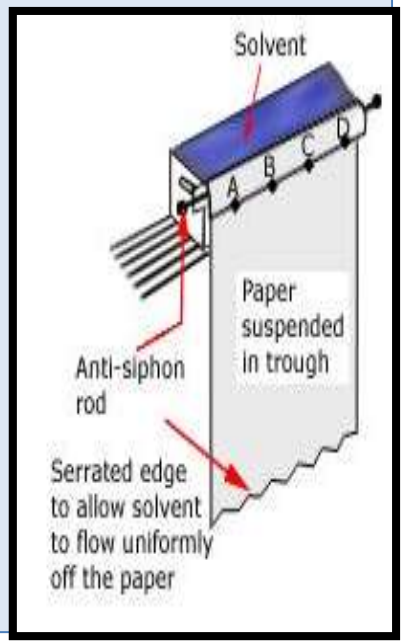
Paper chromatograms can be developed various flow directions by either ascending or descending solvent flow.

ascending



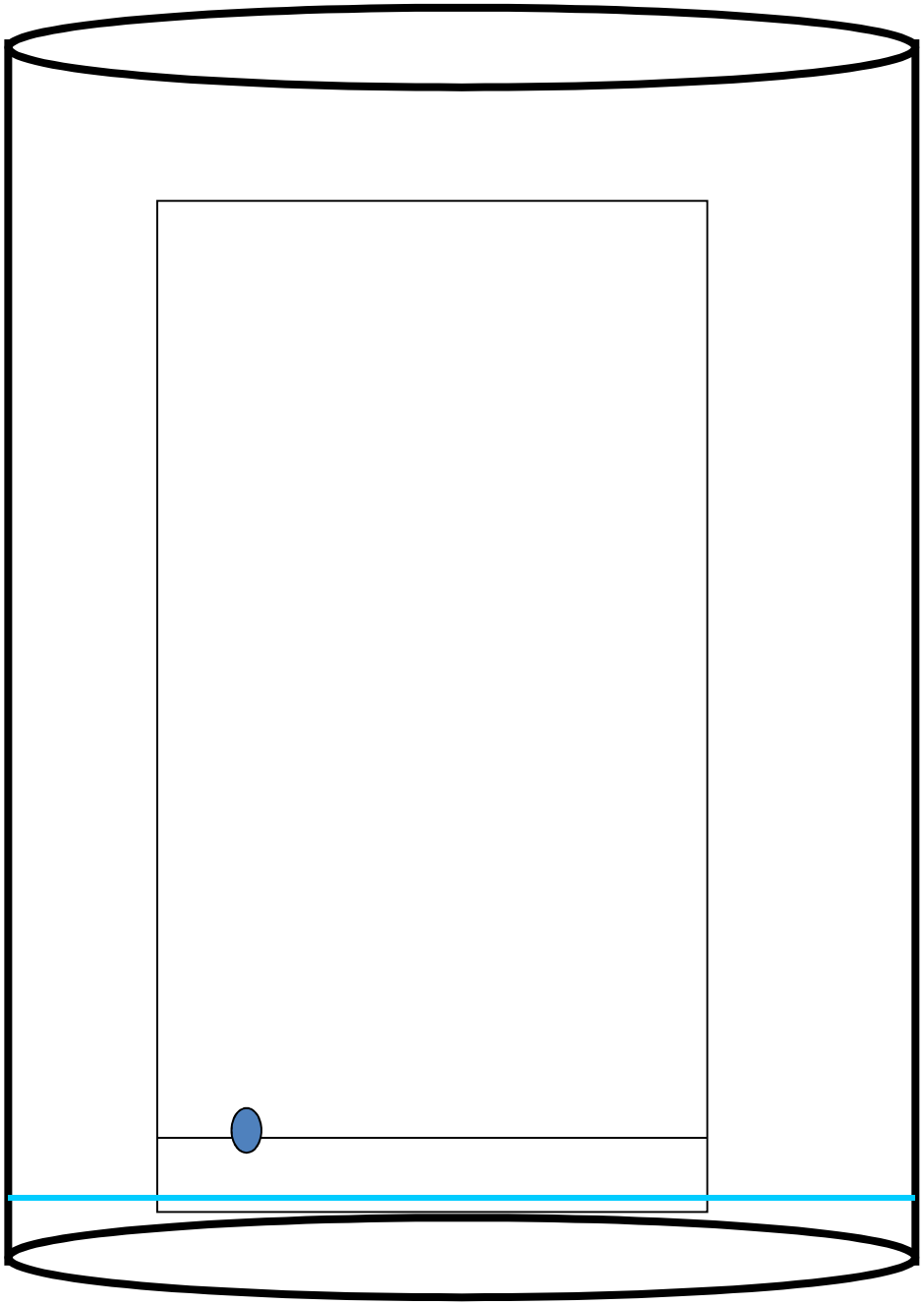
descending

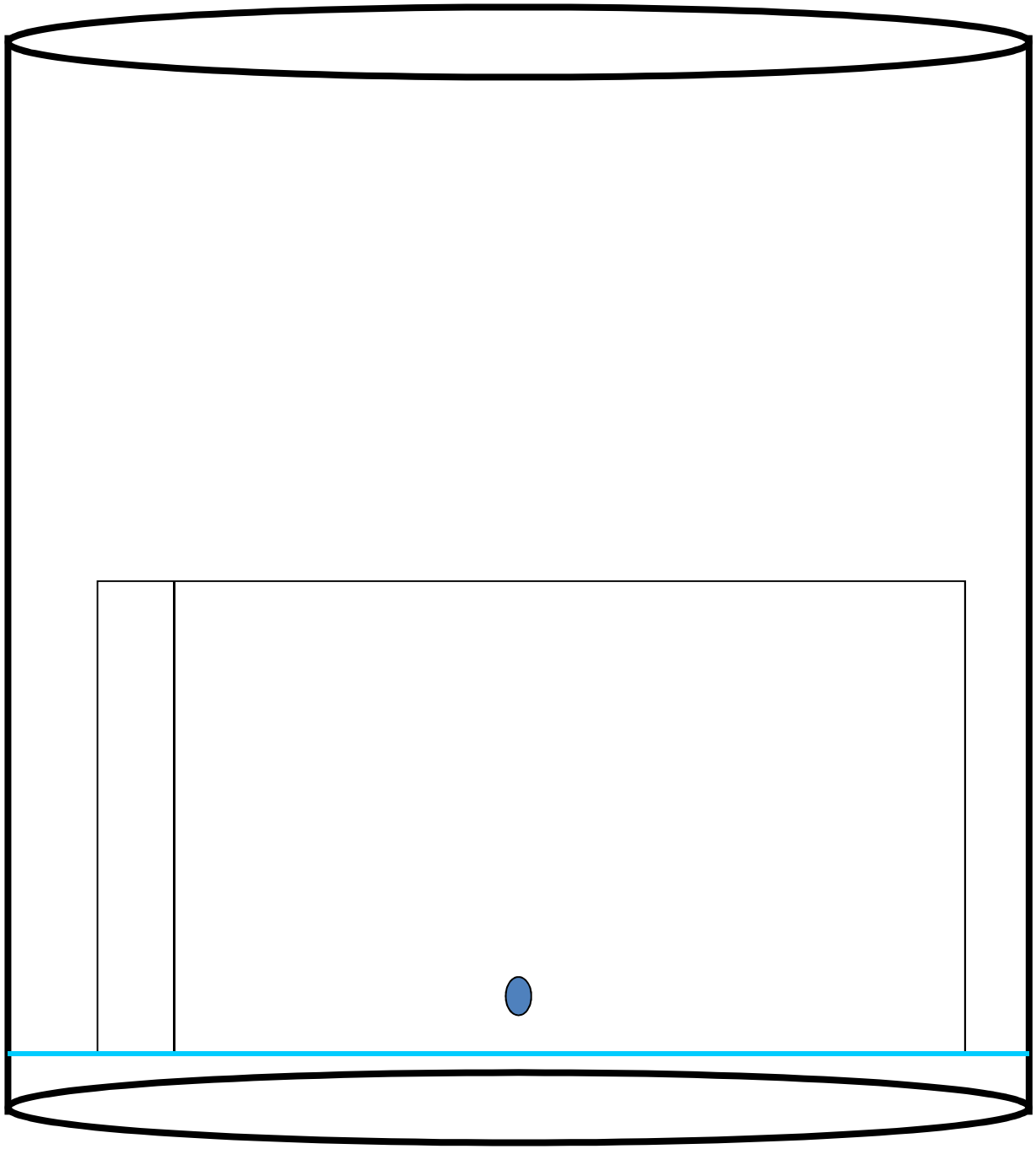




Two Dimensional Paper Chromatography

- When large numbers of substances are to be separated on a single chromatogram.
- 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^{o} 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.
- Two dimensional chromatography helps resolve substances having similar R_f values.





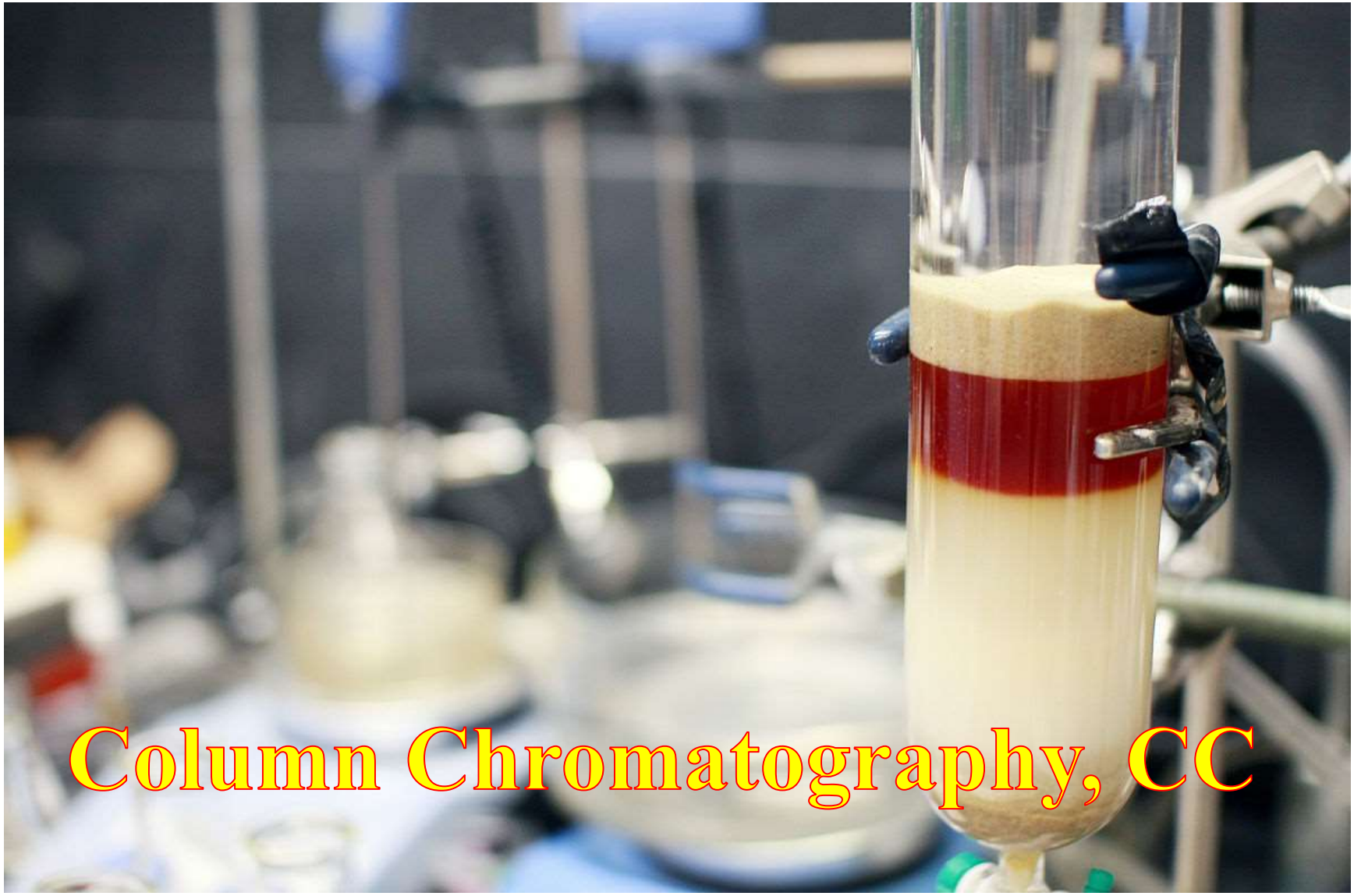
Detection of spots in the paper

By color

Fluorescence

Chemical reaction after the paper is sprayed with various reagents

Identification is based on comparison with standards of known Rf or by elution.


Column Chromatography, CC

Column Chromatography

- It is a Chromatographic technique in which the sample is allowed to pass through column filled or (packed) with stationary phase.
- When the mobile phase move through the column, it carries with it the sample compounds & during its migration through the column separation of the sample compounds takes place.
- The stationary phase used could be solid or liquid, while the mobile phase could be liquid or gas

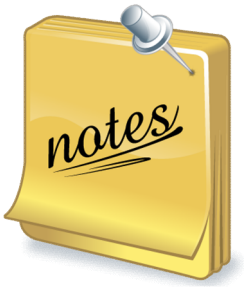
Therefore, we have the following types of column Chromatography:--

1) Liquid- Solid Column Chroma., LSC.

2) Liquid- Liquid ColumnChroma., LLC.

3) Gas Column Chrom., 2 types:

- a- GSC
- b-GLC



The column should be

1) dry
2) clean

TECHNIQUES

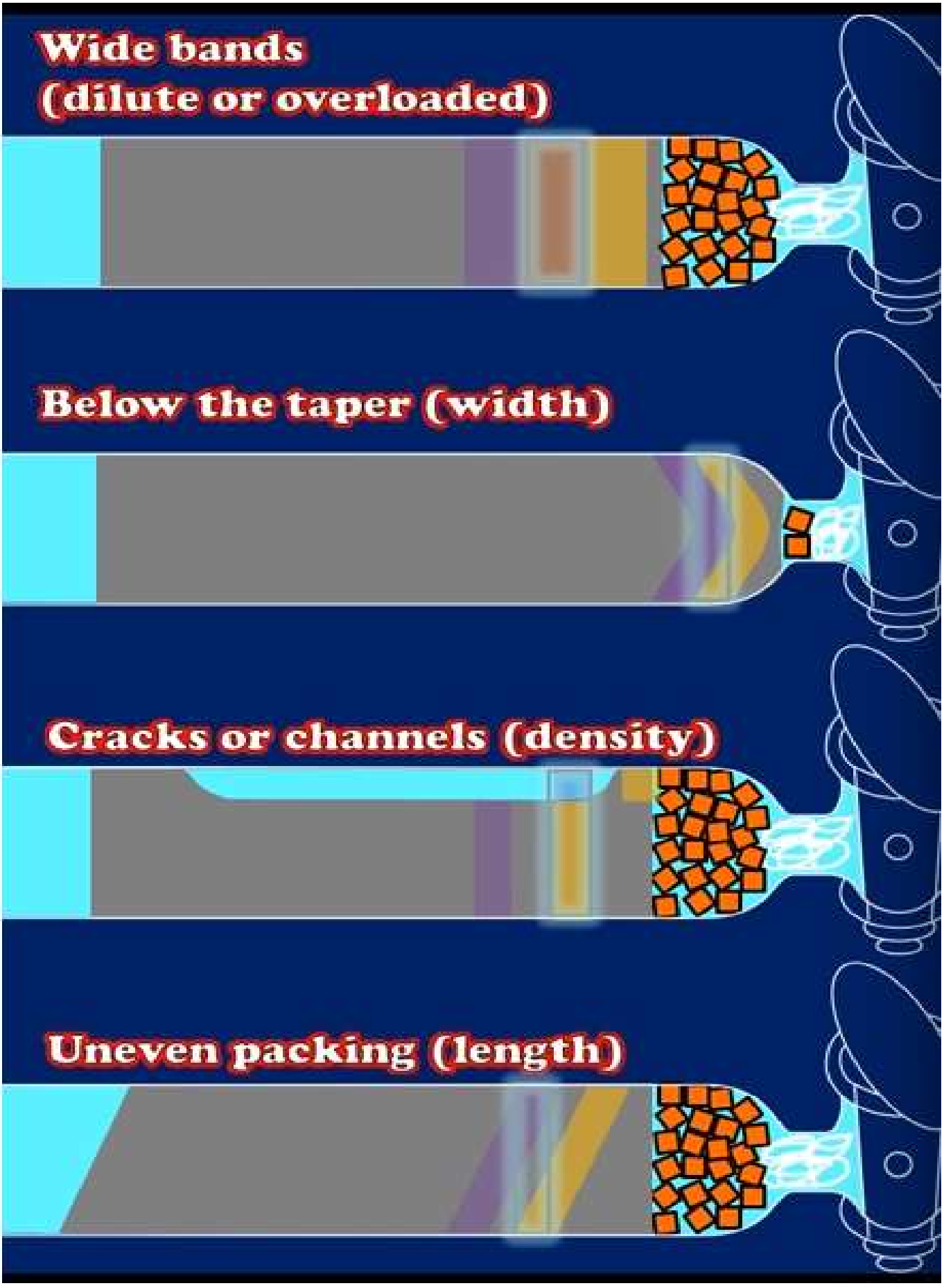
- **Elution: (development in CC)**
  It is the process by which the solvent is allowed to pass through the column so that separation of the sample compound occur.
- **The mobile phase passed from the top of the column (by separatory funnel) which is called the eluent & when the solvent comes from the bottom of the column called eluate.**

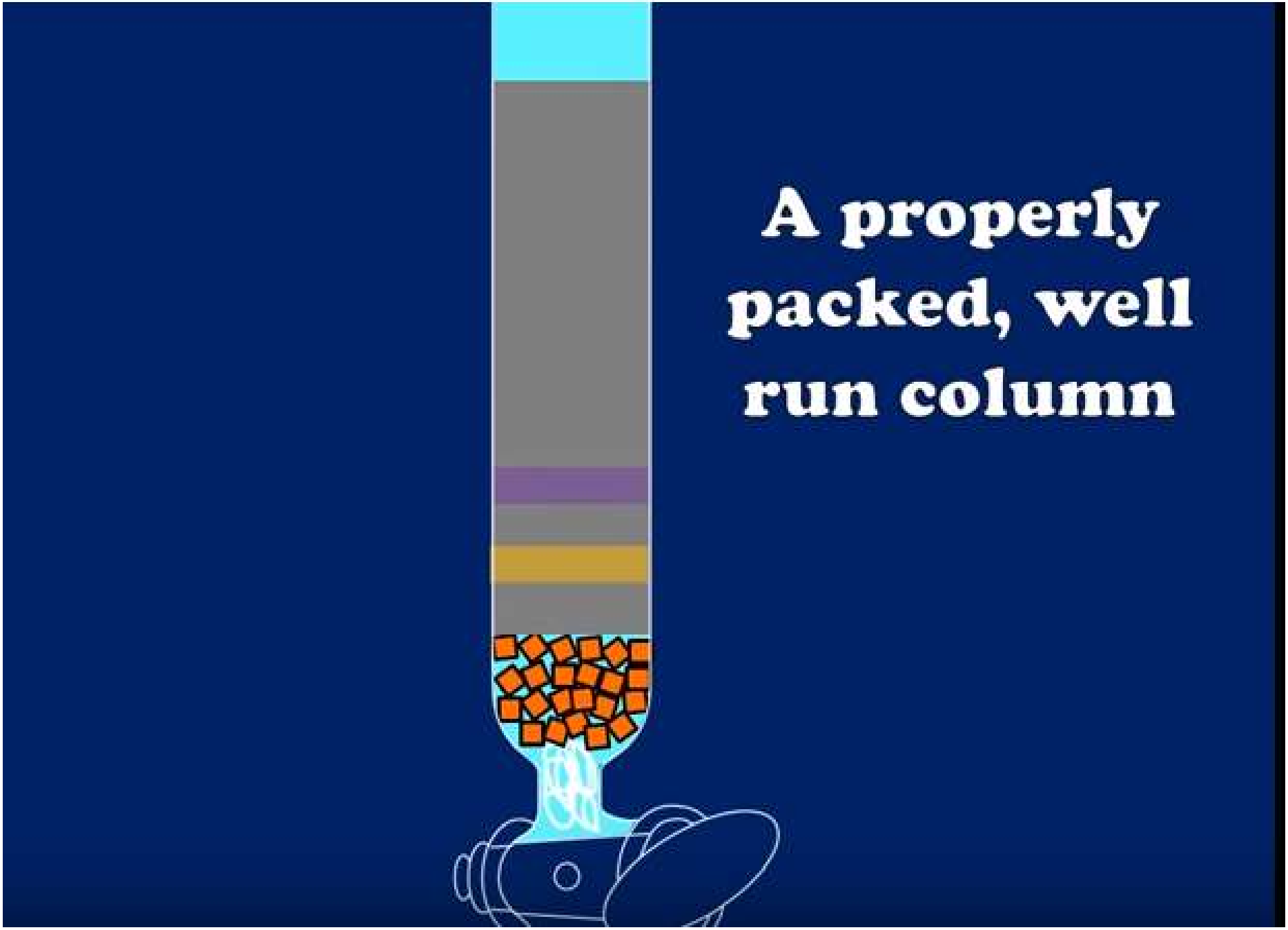
Column packing can be divided into:

1) Dry packing: solid stationary phase (silica gel or alumina) is added to the dry column with tapering to get uniform packing of the stationary phase because any bubble will affect the process of separation.

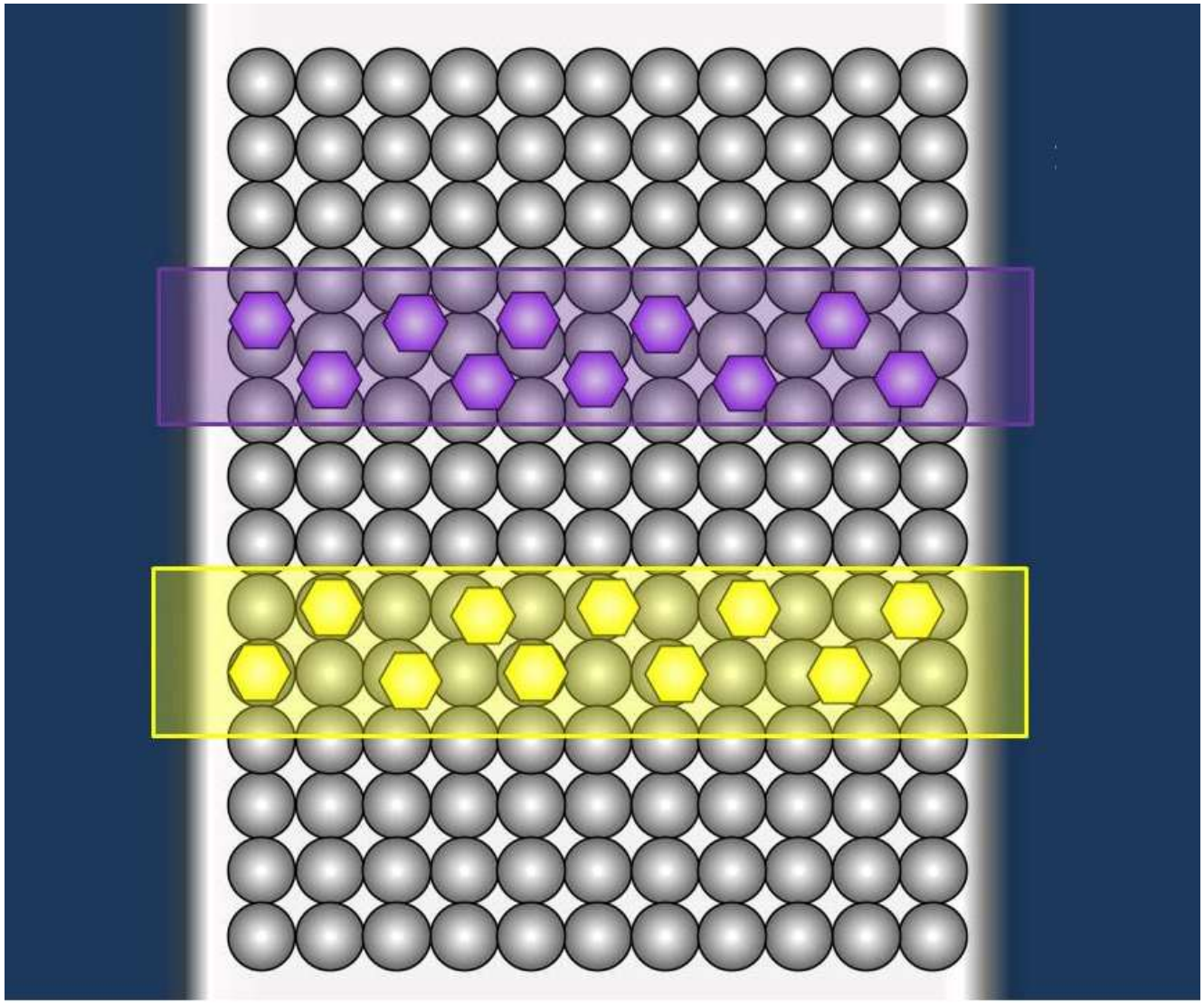
2) Wet packing:

solid stationary phase is mixed with suitable quantity of water in a beaker with stirring to form slurry, then poured it through the column with settling until we reach 2/3 of the column. We must leave a space for a layer of the solvent above it.


Wide bands
(dilute or overloaded)
Below the taper (width)
Cracks or channels (density)
Uneven packing (length)


A properly
packed, well
run column

Column packing can be divided into:



1)Dry packing:

solid stationary phase (silica gel or alumina) is added to the dry column with tapering to get uniform packing of the stationary phase because any bubble will affect the process of separation.

2) Wet packing:

solid stationary phase is mixed with suitable quantity of water in a beaker with stirring to form slurry, then poured it through the column with settling until we reach 2/3 of the column. We must leave a space for a layer of the solvent above it.

There are several methods of elution:

1) Simple elution or called isocratic elution

2) Fractional or stepwise elution

3) Gradient elution method

1) Simple elution (isocratic elution):

- **The column is eluted by simple solvent until separation of the sample compound is completed.**
- **Sometimes mixture of solvents can be used to elute the column from the beginning of the chromatography process until we complete it without changing the composition of the mixture.**

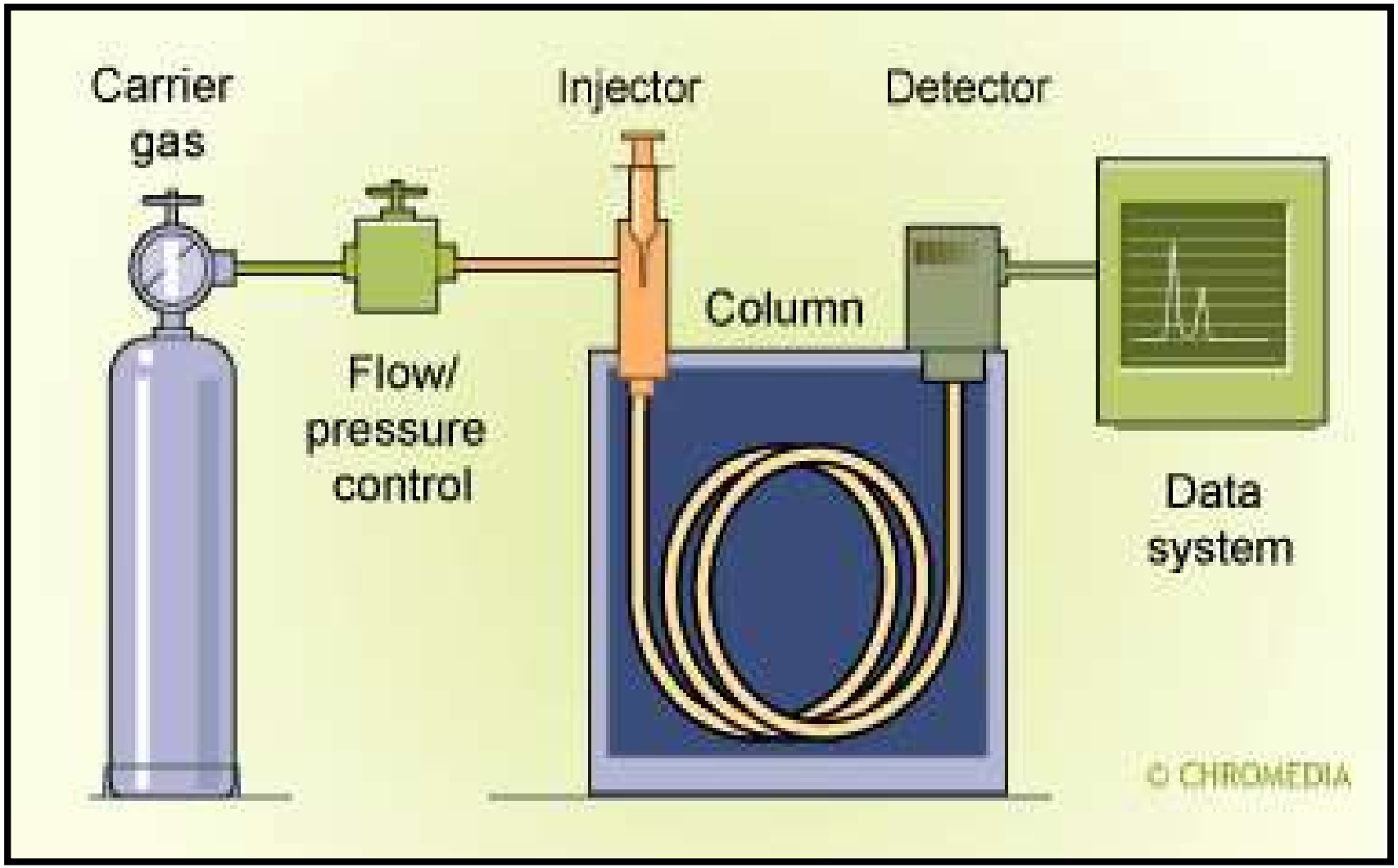
2) Fractional or stepwise elution:

- If simple elution can't give a complete separation so it is necessary to use strong solvent (as a mobile phase) to elute the remaining compounds from the column for example: when the first solvent is Acetone elute some of the sample compounds, we have to use stronger solvent as ethanol to elute the other compounds.

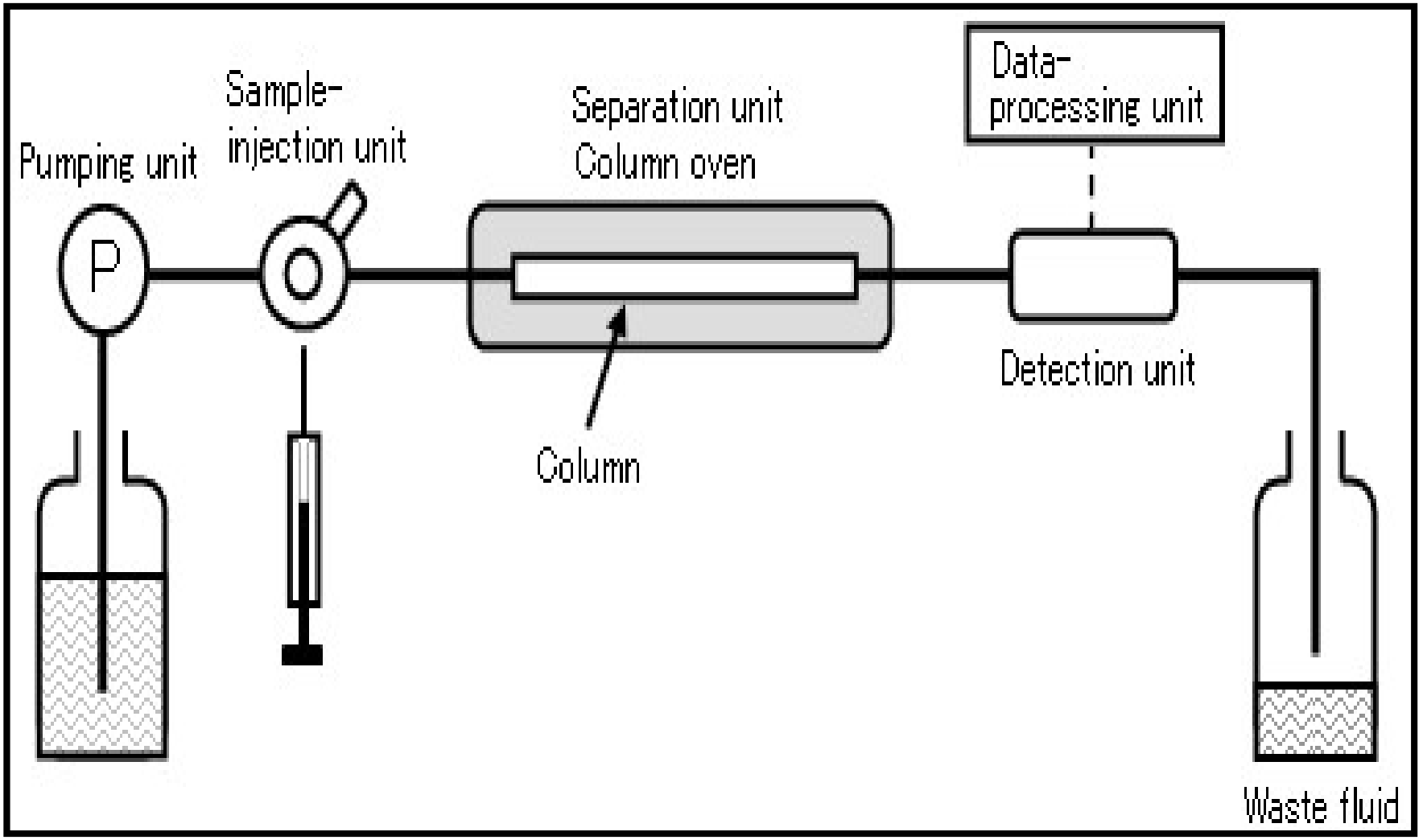
3) Gradient elution method:

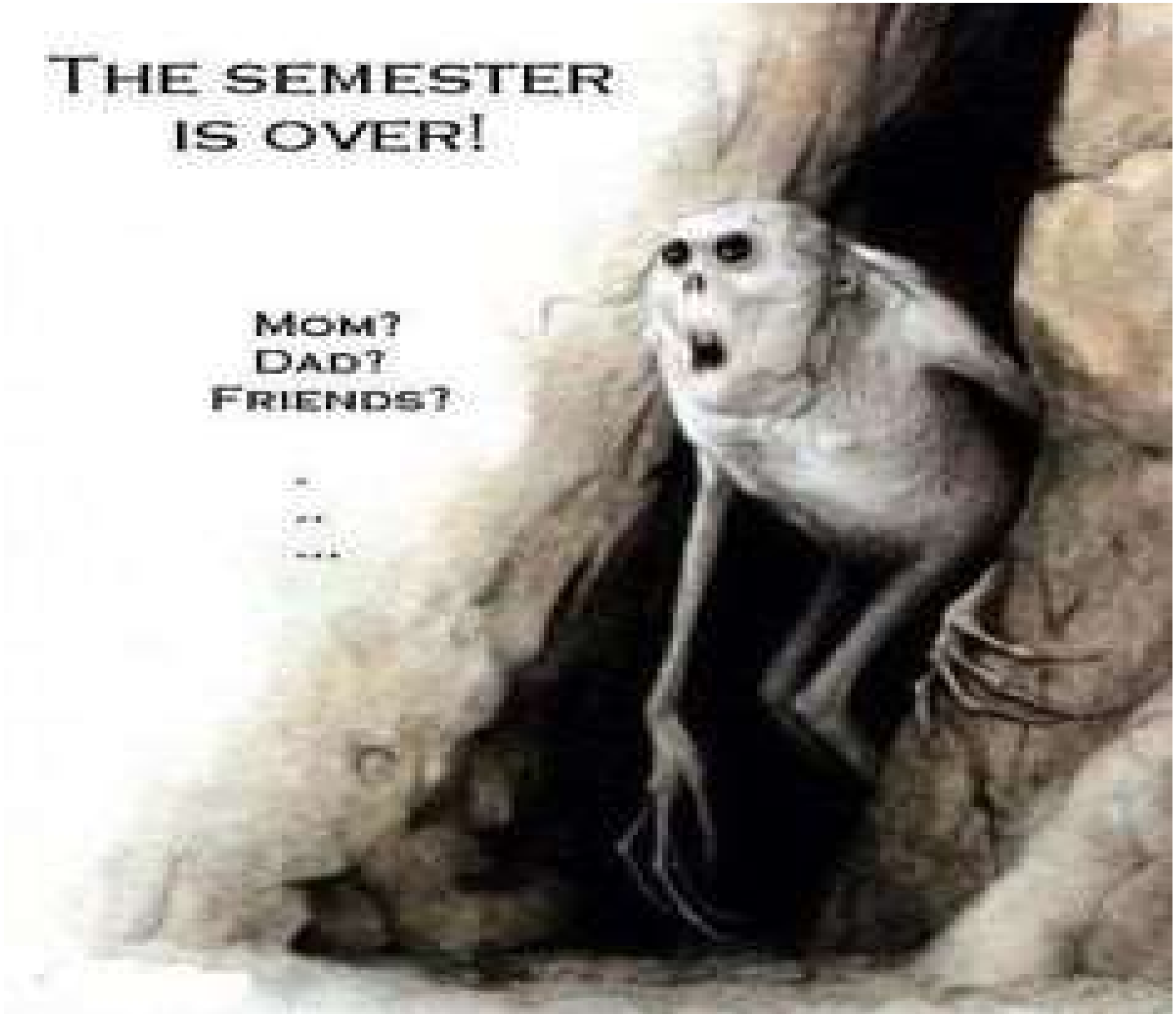
- The solvent is not changed completely but we can increase the eluting capacity by gradual increase in its polarity

Gas Chromatography



High Performance Liquid Chromatography (HPLC)




THE SEMESTER IS OVER!
MOM?
DAD?
FRIENDS?
.
..
...