Chromatography (404 C)

Fourth year Chemistry / Biochemistry students

by

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Thin Layer Chromatography (TLC)

What Is Thin Layer Chromatography?

Thin Layer Chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminium foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminium oxide, cellulose, or silica gel.

On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (R_f) expressed as:

R_f = dist. travelled by sample / dist. travelled by solvent

The factors affecting retardation factor are the solvent system, amount of material spotted, absorbent and temperature. TLC is one of the fastest, least expensive, simplest and easiest chromatography technique.

Thin Layer Chromatography (TLC)

TLC: is a solid - liquid technique used to separate chemical compounds.

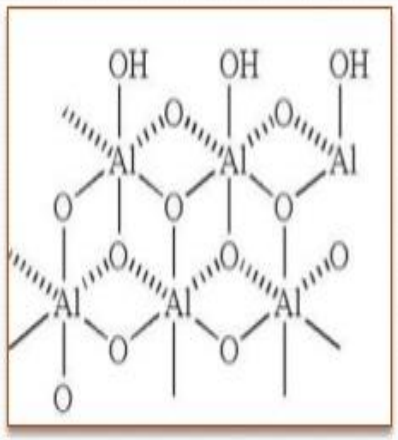
TLC plate : is a sheet of glass, metal or plastic which is coated by a uniform thin layer of a solid adsorbent.

The solid adsorbents that most commonly used:

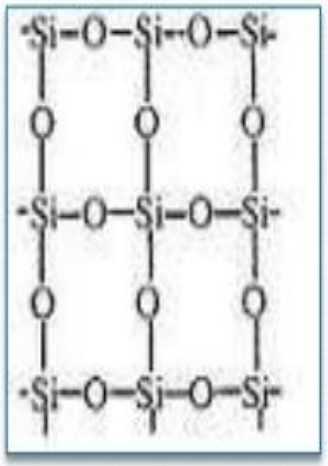
1) Silica gel or silicon dioxide (SiO2.XH2O).
2) Alumina (Aluminum oxide) (Al2O3.XH2O).
3) Cellulose powder.

Both of them are polar but alumina is more polar than silica gel.

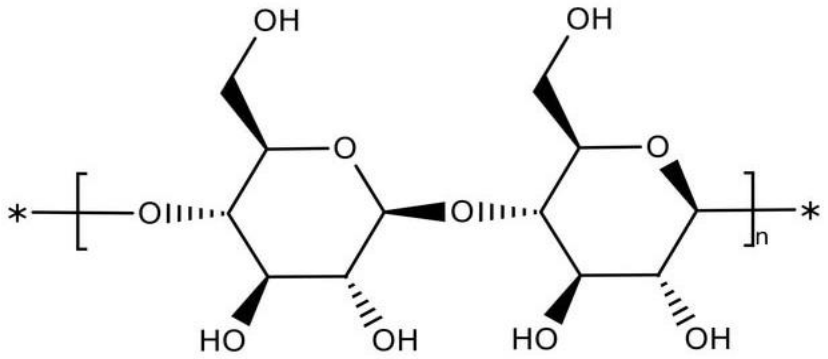
Adsorbents in TLC may contain a binding agents as CaSO4 (gypsum) which is facilitate the adsorbent sticking on glass plate.



Structure of Alumina



Structure of Silica



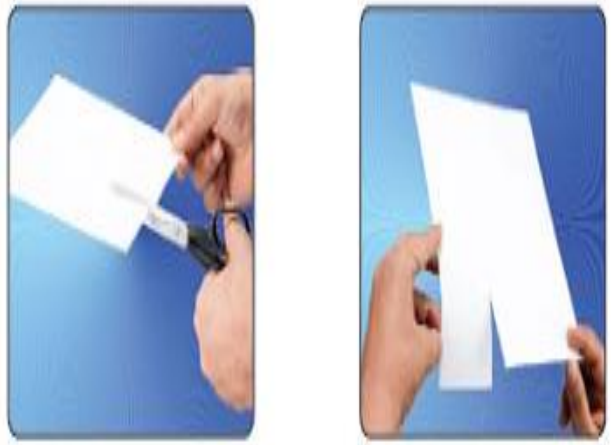
Structure of Cellulose powder


1


2


3


4



Activation Of adsorbent

Silica gel adsorbs some vapors and becomes inactive, so it must be activated by:

1) Air-drying the TLC plates for a duration of 30 minutes.

2) Then put in oven maintained at 105 °C for 30 minutes.

3) Then cooling them in a desiccator.

This drying process helps a great extent in rendering the adsorbent layer active.

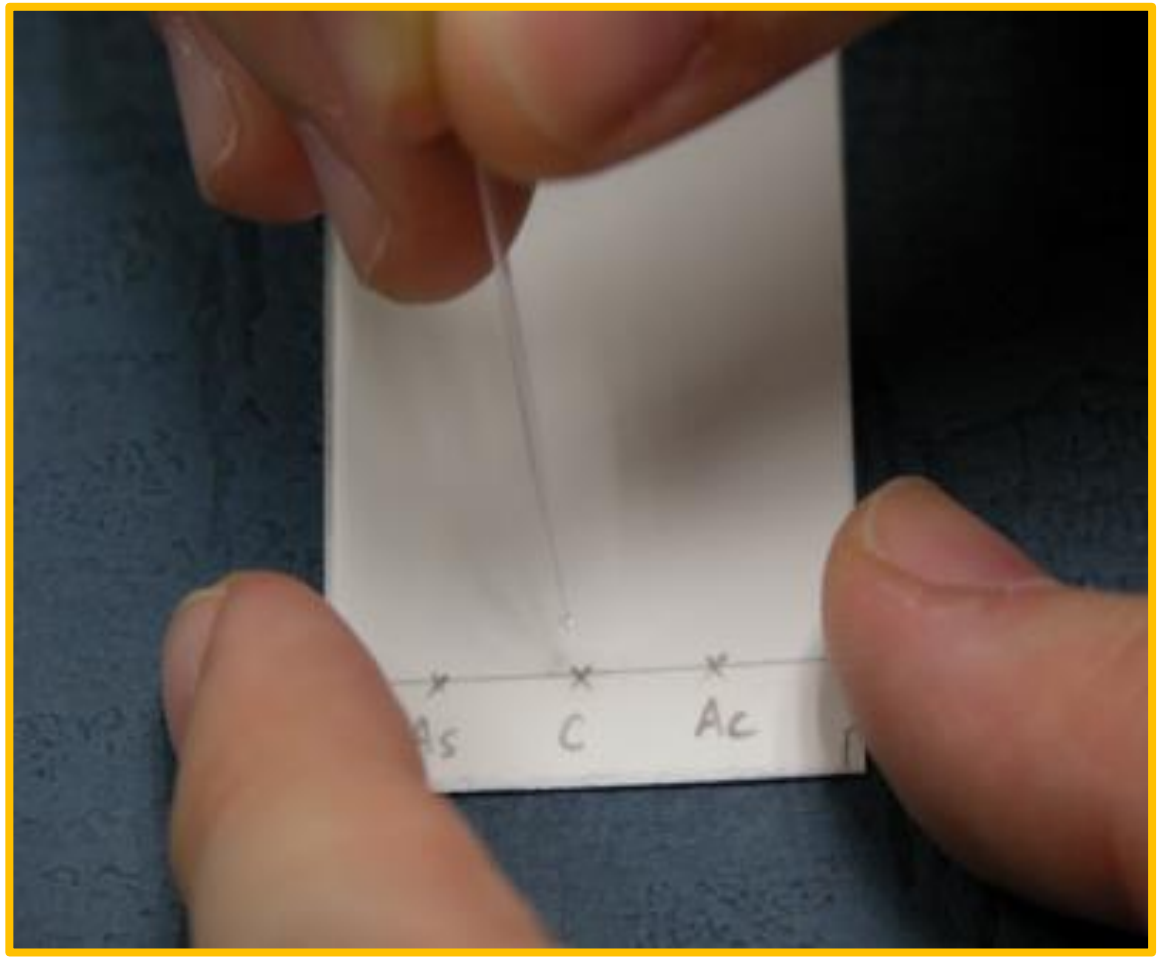
- To achieve very active layers, silica gel and alumina coated plates may be heated up to 150 °C for a duration of 4 hours and cooling them in a desiccator.

Steps of thin layer chromatography

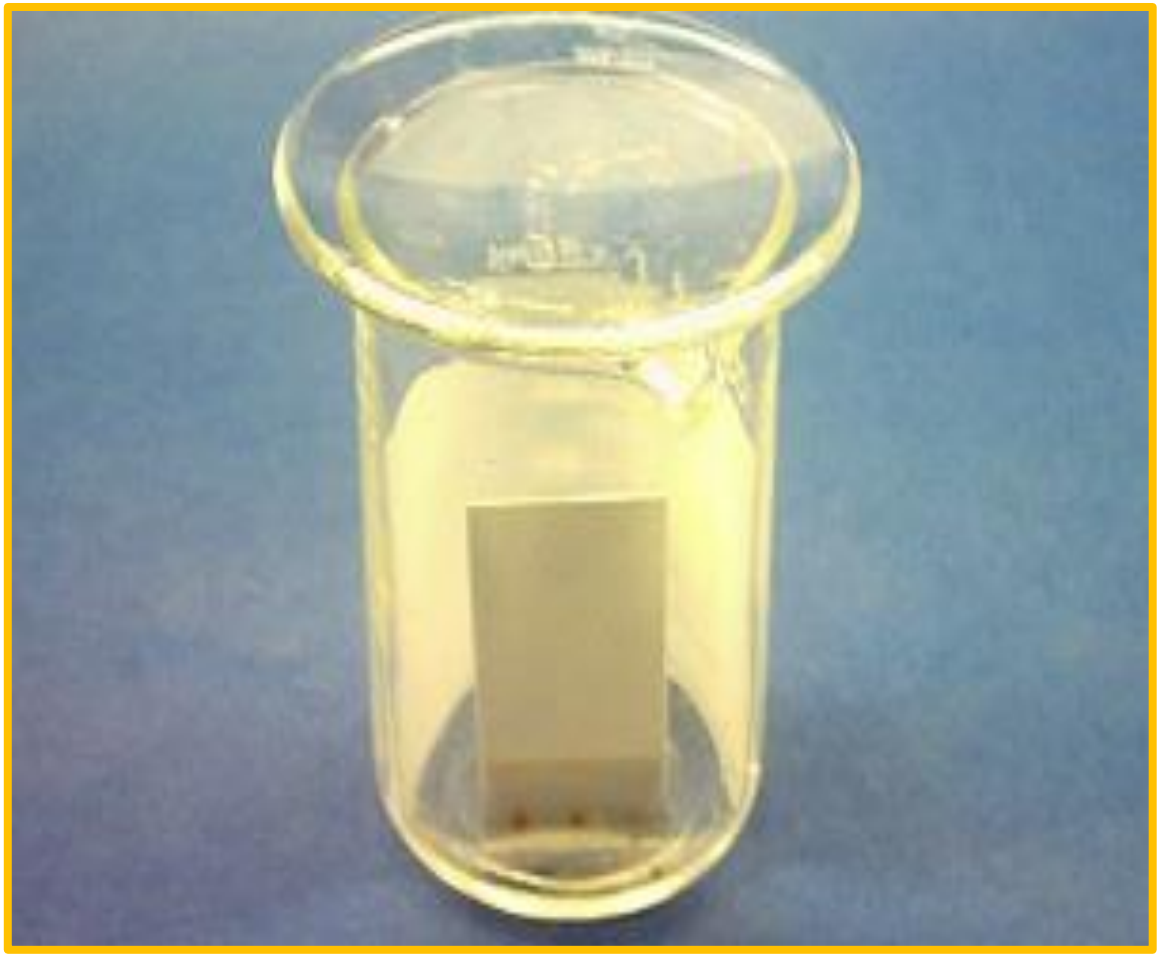
1) Prepare the jar by pouring a small amount of mobile phase (solvent).

2) Prepare the TLC plate

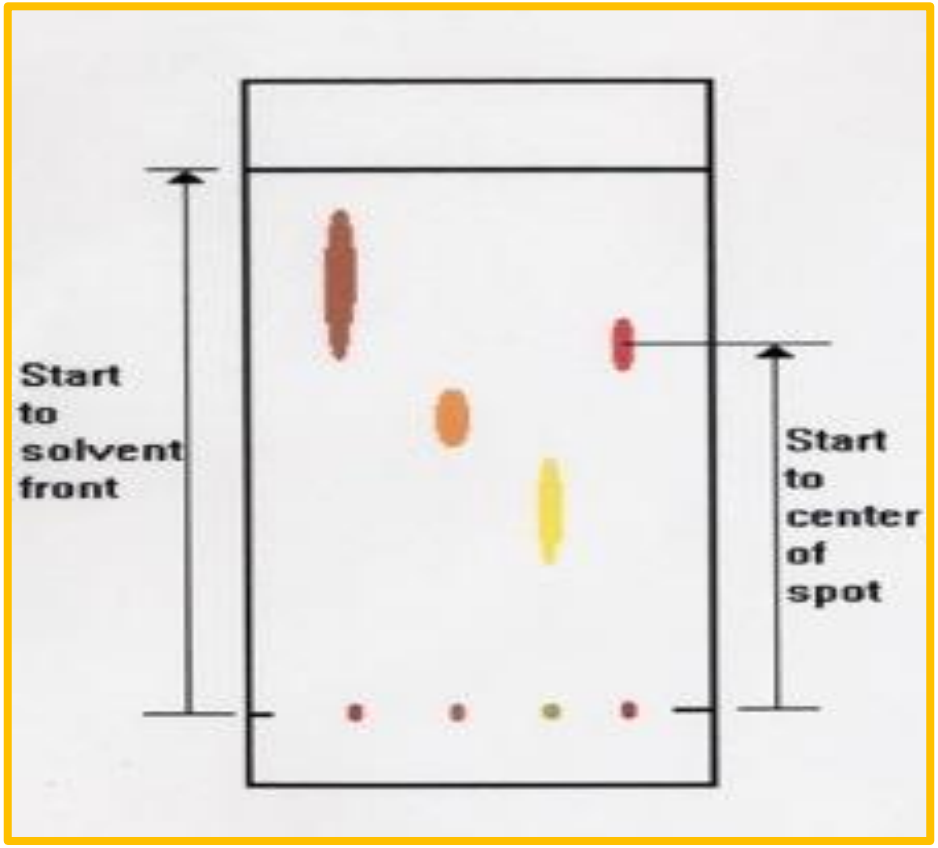
3) Spot the TLC plate

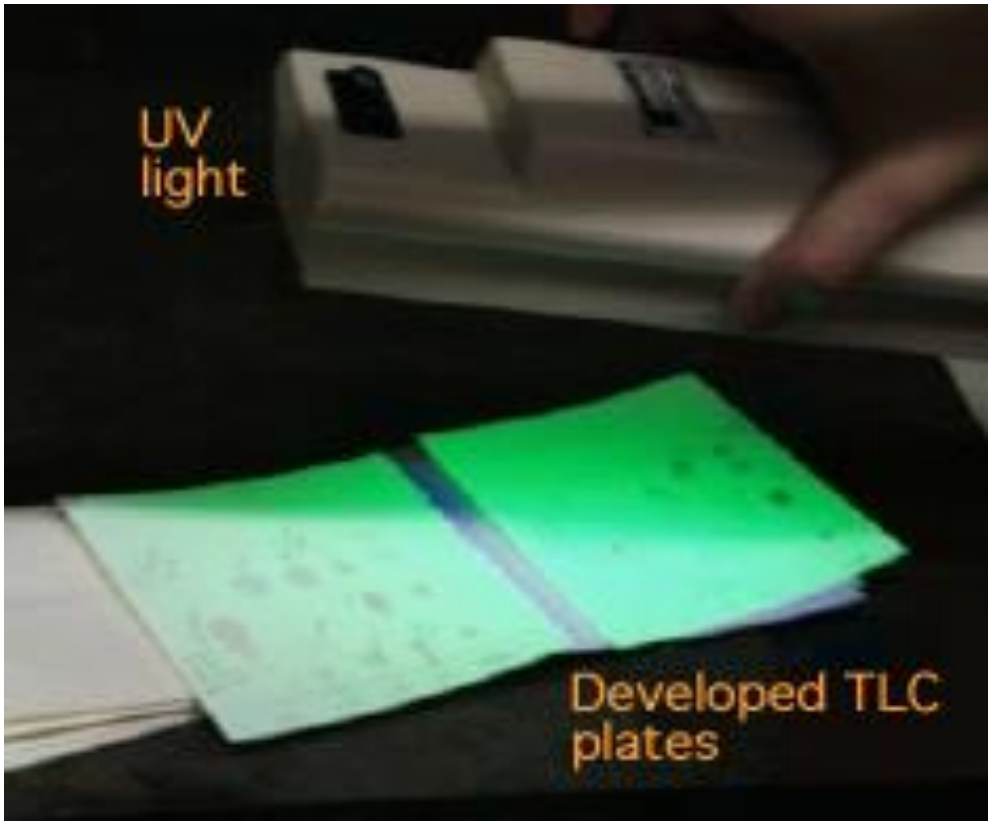


4) Development of the plate



5) Visualize the spots and calculate Rf




UV
light
Developed TLC
plates

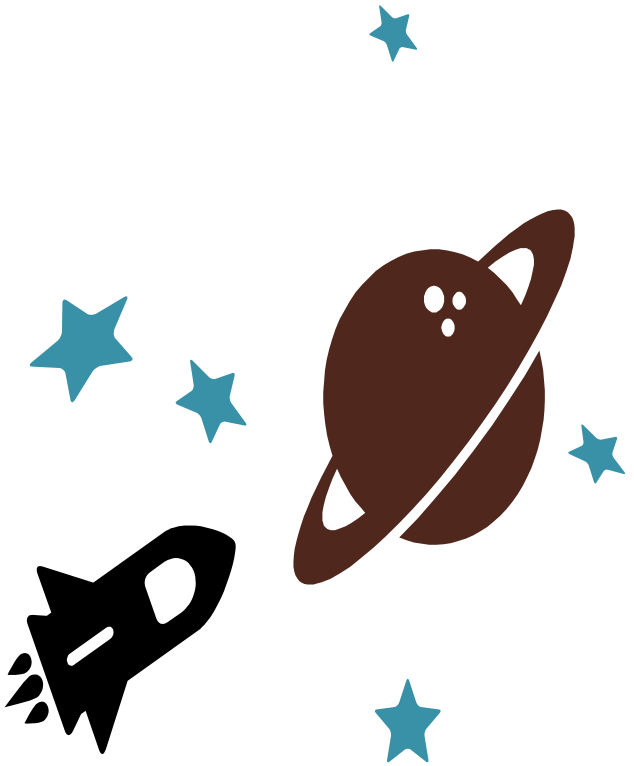
The advantages of TLC

- 1) Sensitive
- 2) Requires only a small amount of sample
- 3) Faster in time
- 4) Simple
- 5) Inexpensive
- 6) Requires only a small amount of solvent
- 7) Wide choice of the adsorbent (cellulose, celite, silica gel, alumina)
- 8) Capillary diffusion is eliminated (i.e. fibrous nature of cellulose in paper produce capillary diffusion which increase spot size.

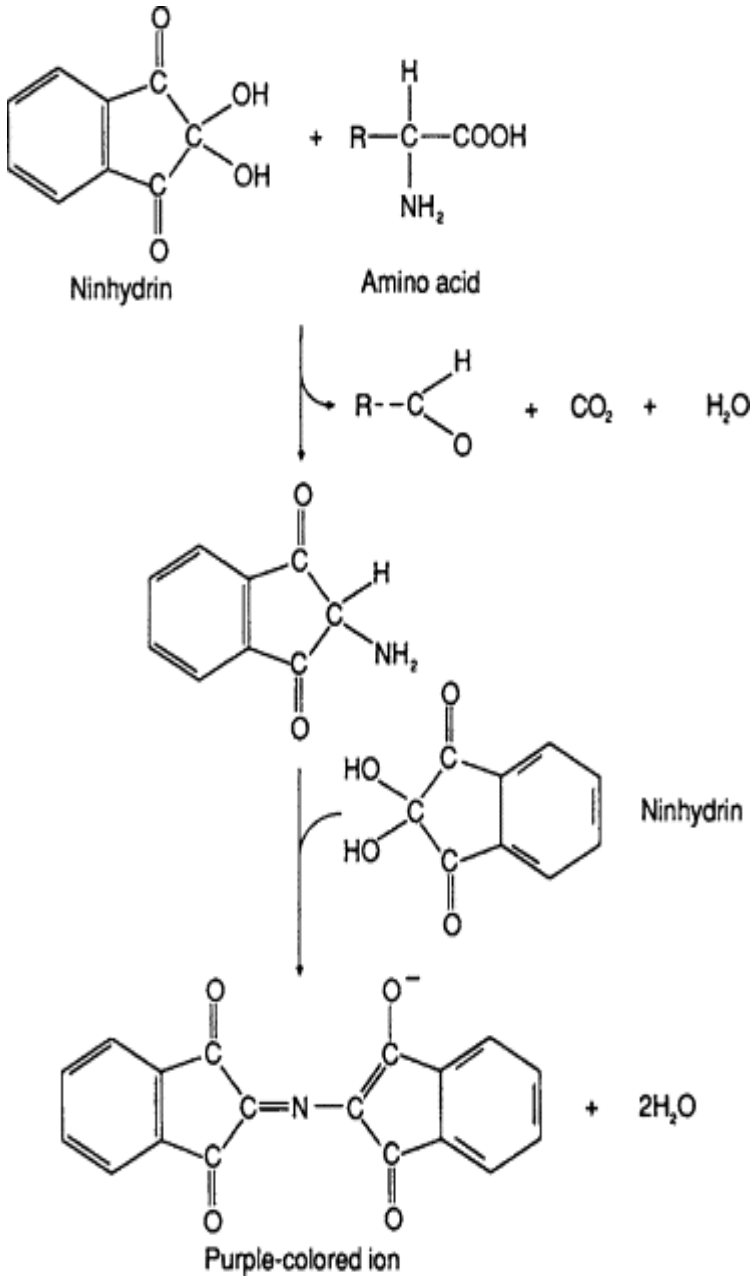
The disadvantages of TLC

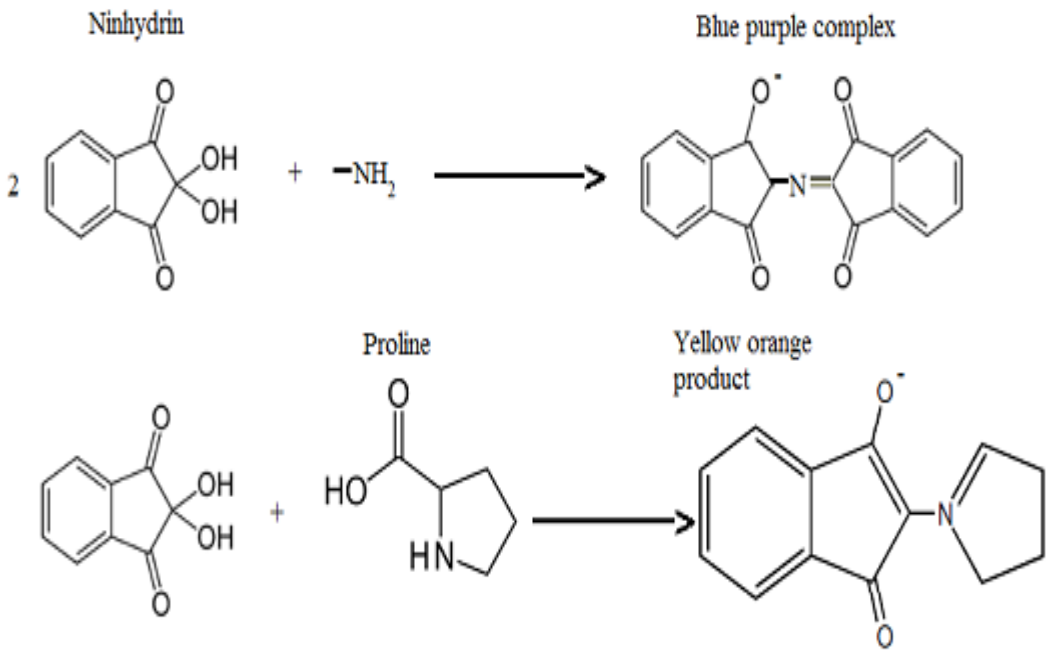
- TLC plates do not have long stationary phases. Therefore, the length of separation is limited compared to other chromatographic techniques. Also, the detection limit is a lot higher. If you would need a lower detection limit, one would have to use other chromatographic techniques.
- TLC operates as an open system, so factors such as humidity and temperature can be consequences to the results of your chromatogram.

Separation of amino acids by TLC

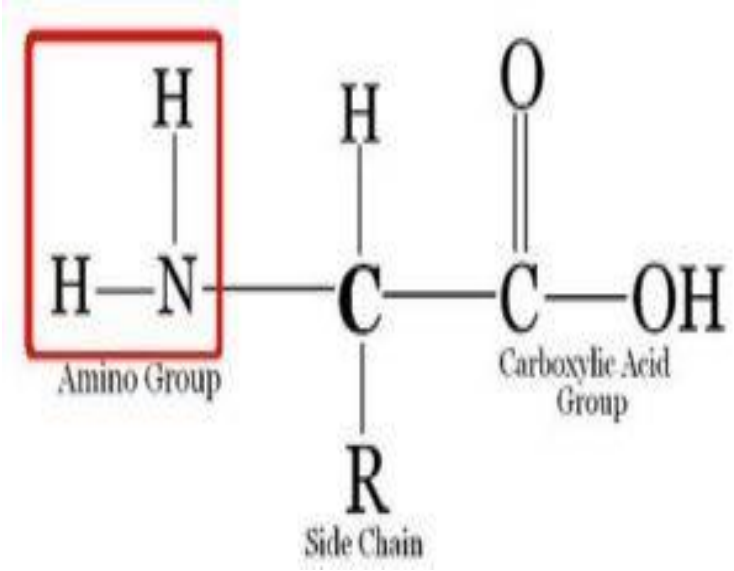


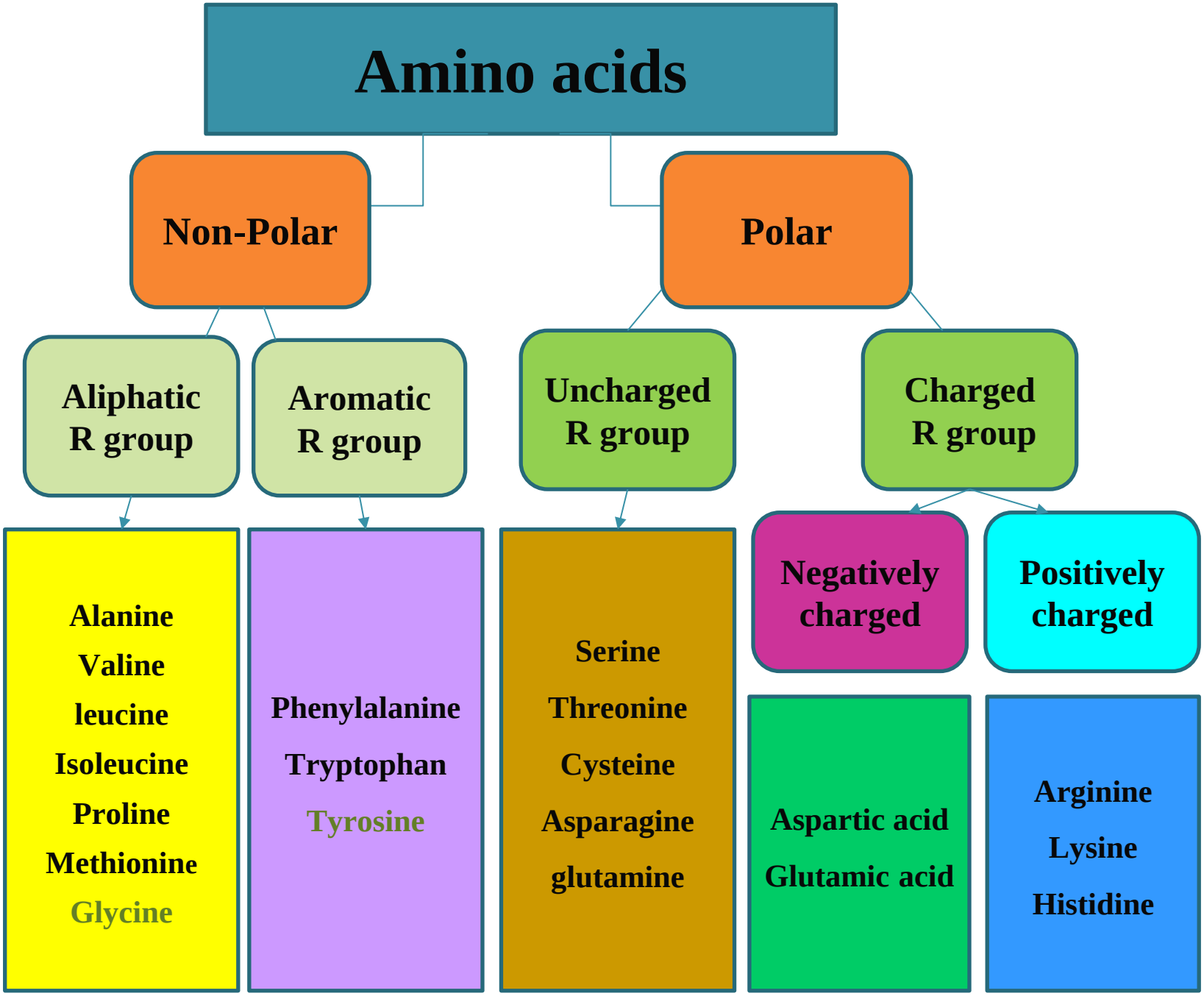
- **Since amino acids are colorless compounds, ninhydrin is used for detecting them.**
- **To identify this, after development, the TLC plate is sprayed with ninhydrin reagent and dried in an oven, at 105°C for about 5 minutes.**
- **Ninhydrin reacts with α-amino acids that results in purple colored spots for all amino acids except proline and hydroxyproline which gives yellow color.**


Ninhydrin
Amino acid
+ CO2 + H2O
Ninhydrin
+ 2H2O
Purple-colored ion


Ninhydrin
Blue purple complex
2
OH
OH
O
O
+
−NH2
O-
N
Proline
Yellow orange
product
HO
HN
O-
N

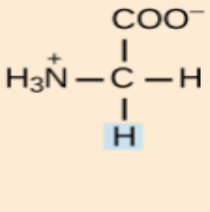
Classification of amino acids based on the properties of their side chain

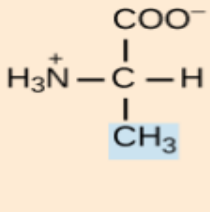


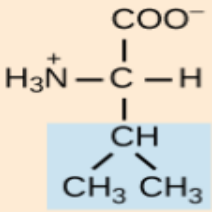

Amino acids
Non-Polar
Polar
Aliphatic R group
Aromatic R group
Uncharged R group
Charged R group
Negatively charged
Positively charged
Alanine
Valine
leucine
Isoleucine
Proline
Methionine
Glycine
Phenylalanine
Tryptophan
Tyrosine
Serine
Threonine
Cysteine
Asparagine
glutamine
Aspartic acid
Glutamic acid
Arginine
Lysine
Histidine

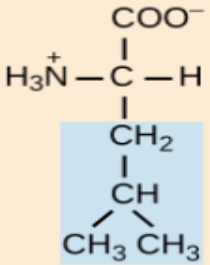
AMINO ACID

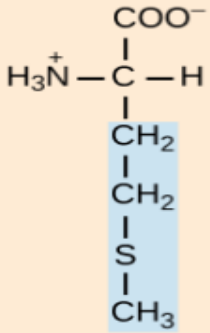
Nonpolar, aliphatic R groups

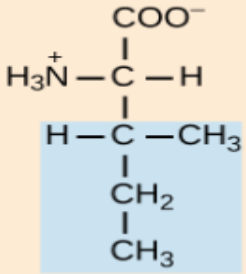

Glycine


Alanine

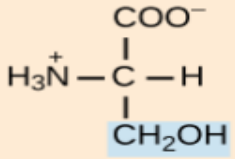

Valine

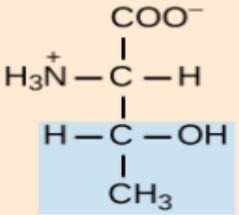

Leucine

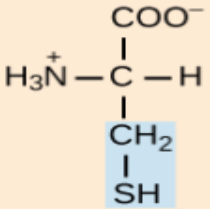

Methionine

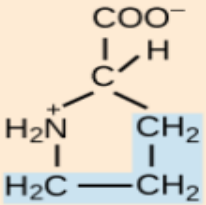

Isoleucine

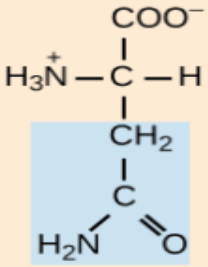
Polar, uncharged R groups

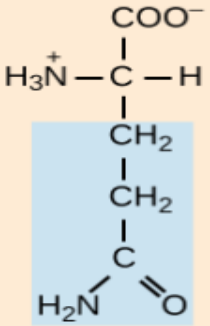

Serine


Threonine


Cysteine

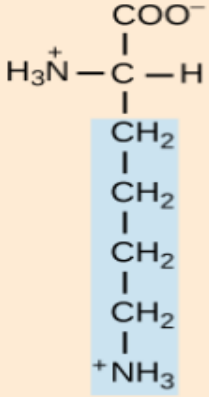

Proline

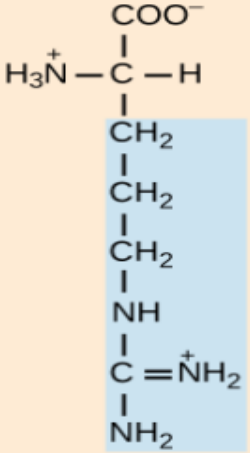

Asparagine

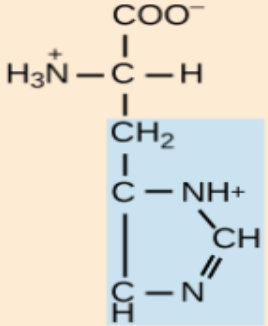

Glutamine

AMINO ACID

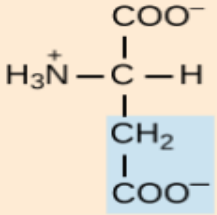
Positively charged R groups

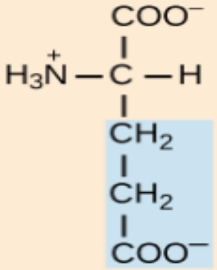

Lysine


Arginine

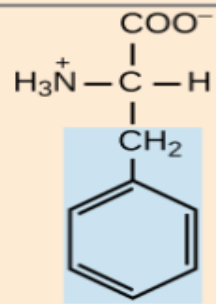

Histidine

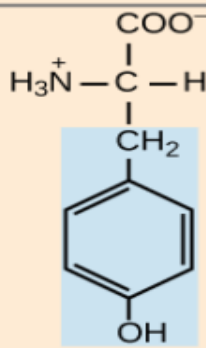
Negatively charged R groups

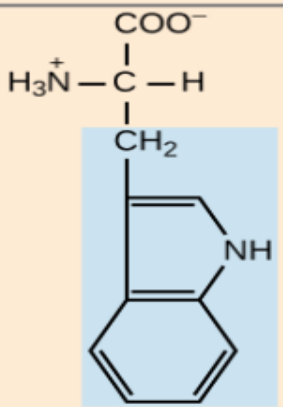

Aspartate


Glutamate

Nonpolar, aromatic R groups


Phenylalanine


Tyrosine


Tryptophan

Chromatographic terms

- The **analyte** is the substance to be separated during chromatography.
- A **chromatogram** is the visual output of the chromatograph.
- The **eluate** is the mobile phase leaving the column.
- The **eluent** is the solvent that carries the analyte
- The **detector** refers to the instrument used for qualitative and quantitative detection of analytes after separation.