

Paper chromatography for amino acids

Asst. Prof. Dr. Noor S Jaafar
Second stage/ Second semester

Development and its types

Development refers to the modes in which the mobile phase moves through the stationary phase during separation.

**Ascending
development**

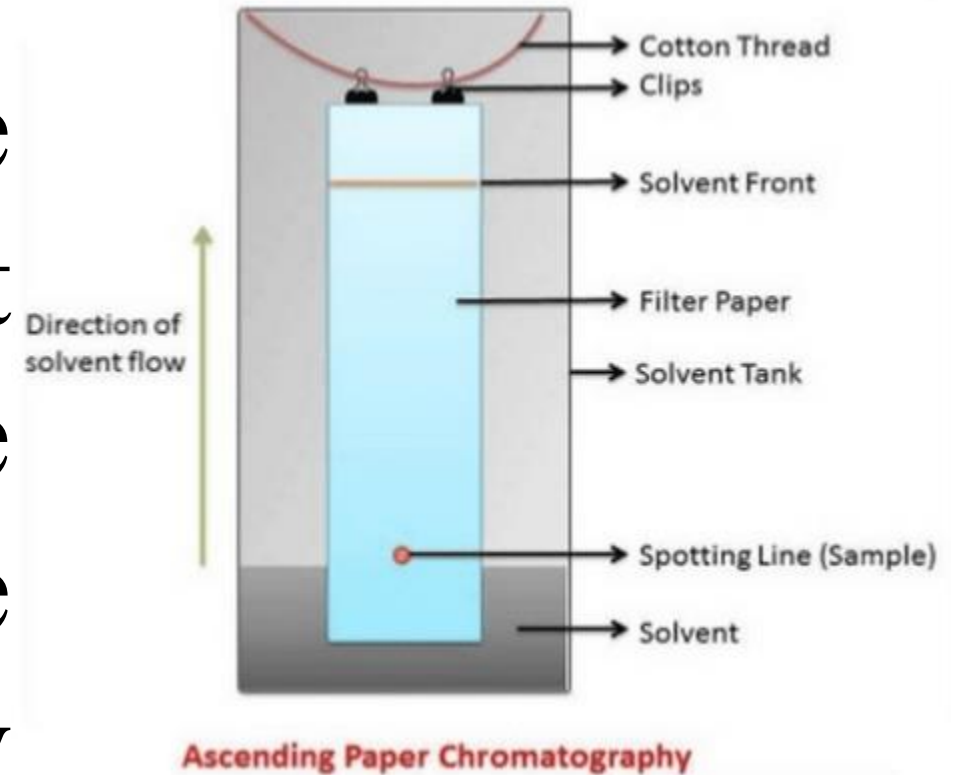
**Descending
development**

**Radial
development**

**Multiple
developments**

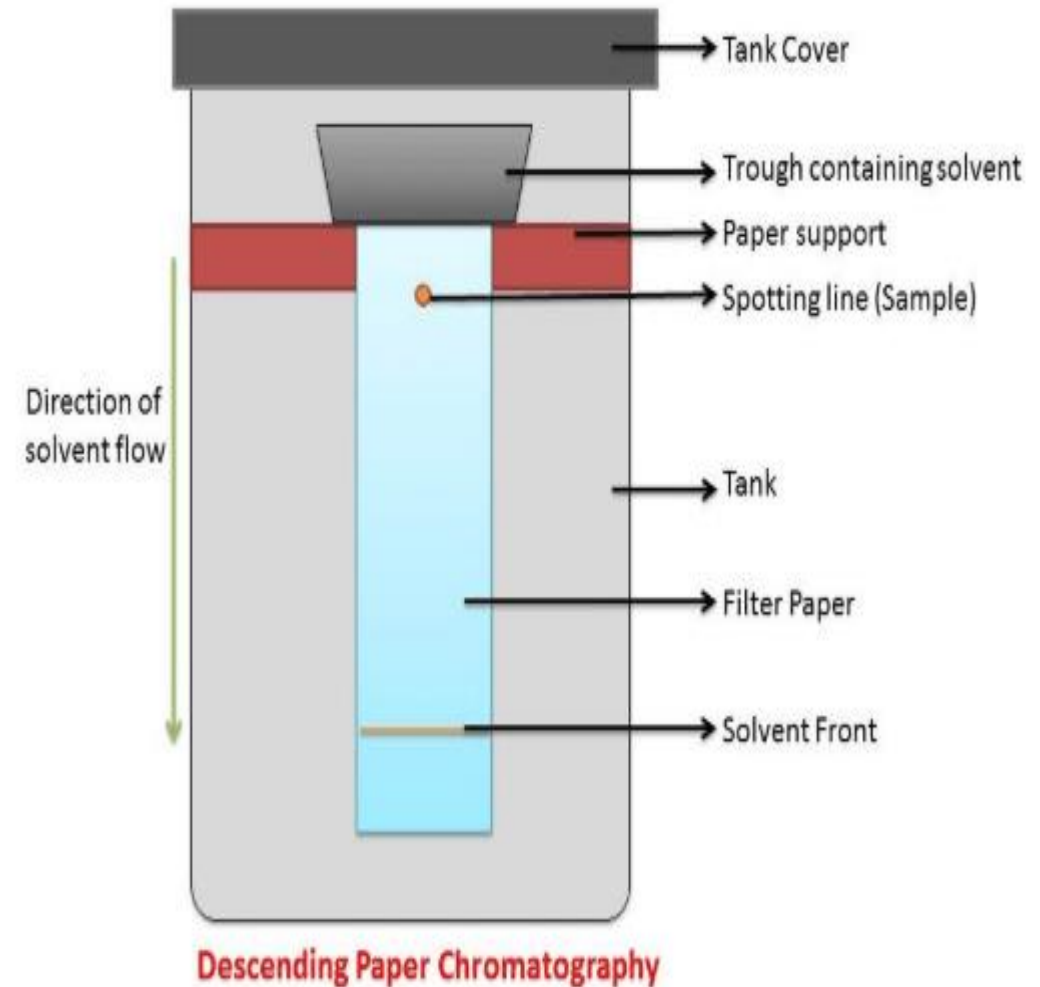
Ascending development

The paper will be dipped in the solvent mixture so that the solvent travels up the paper through capillary action.



Descending develop

Descending development in chromatography is a technique, primarily in paper chromatography, where the solvent moves **downwards** on the paper by gravity and capillary action



Multiple developments

1. Multiple developments

2. Two- dimensional chromatography

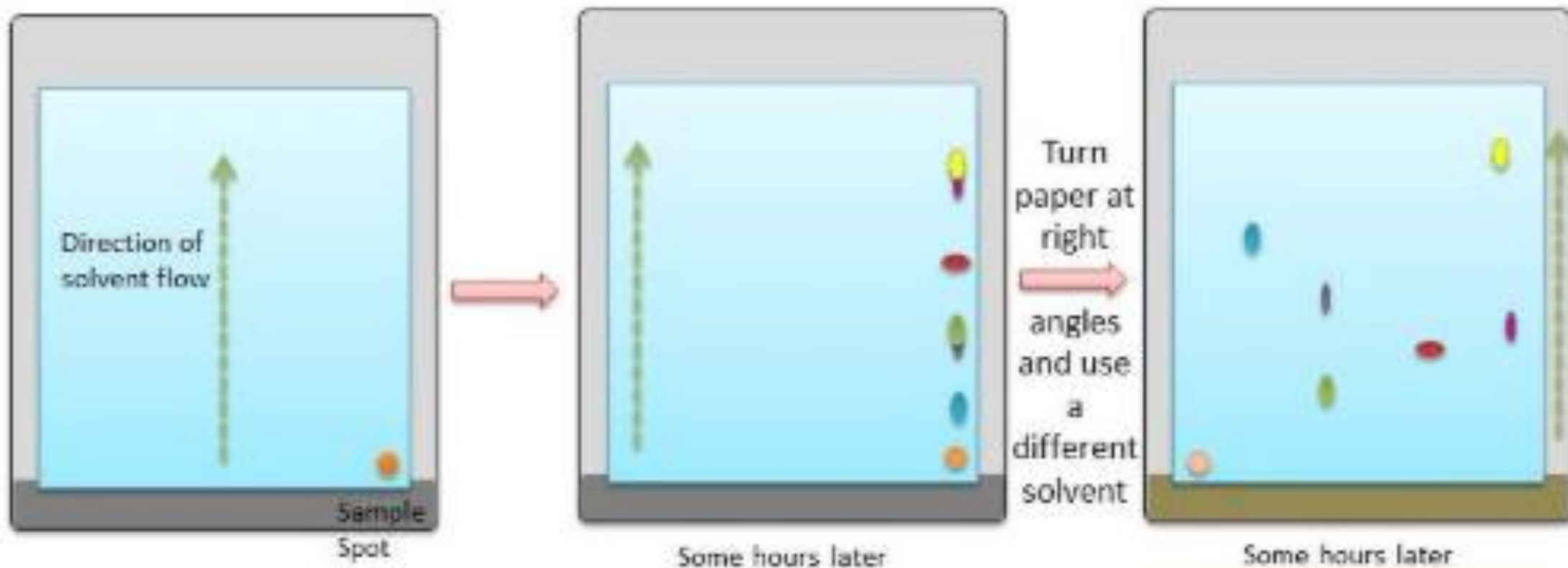
Multiple developments

The chromatogram is repeatedly developed in the same direction, and thus, the complete resolution of two or more substances that have close R_f values

The mobile phase used can be either the same solvent system or different solvent systems.

Two- dimensional chromatography

- Used 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 °, and developed in the second direction.
- Usually, different types of solvent systems are used in each direction. The first solvent must be completely volatile.

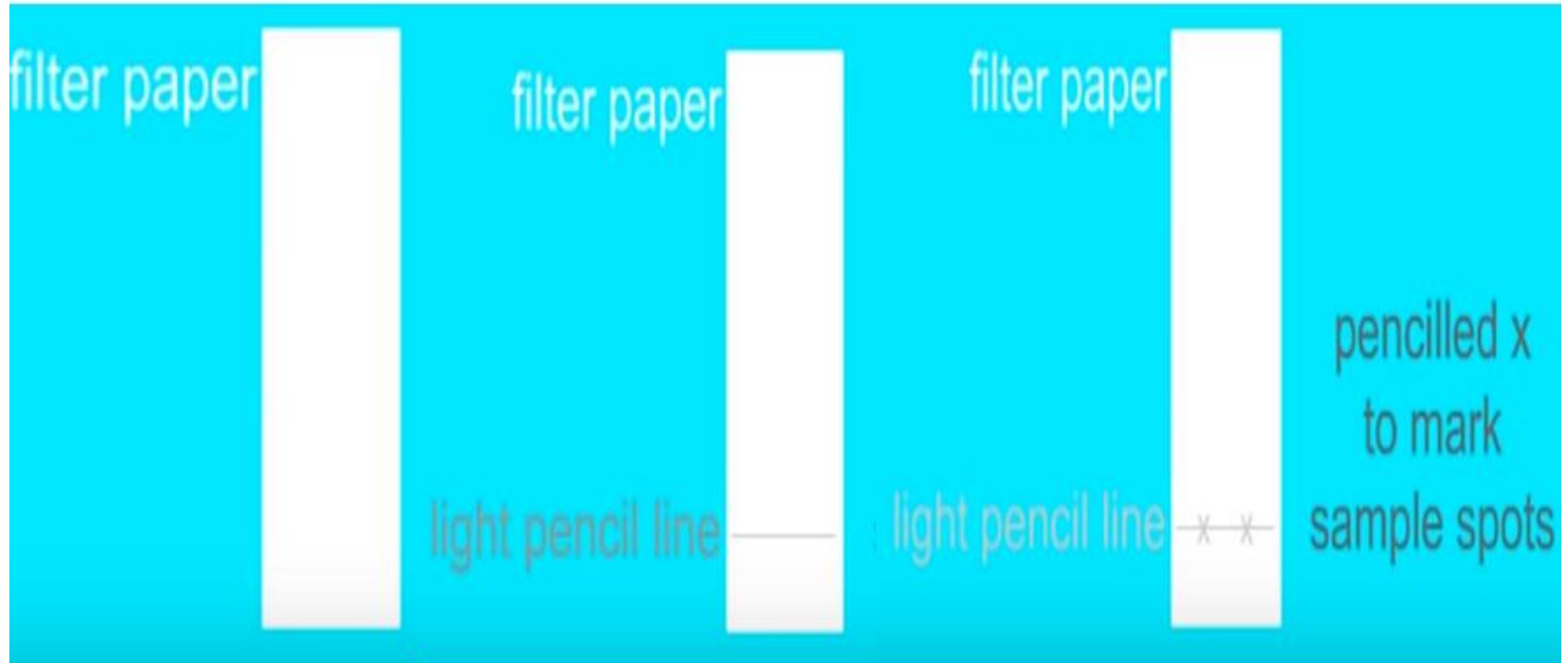


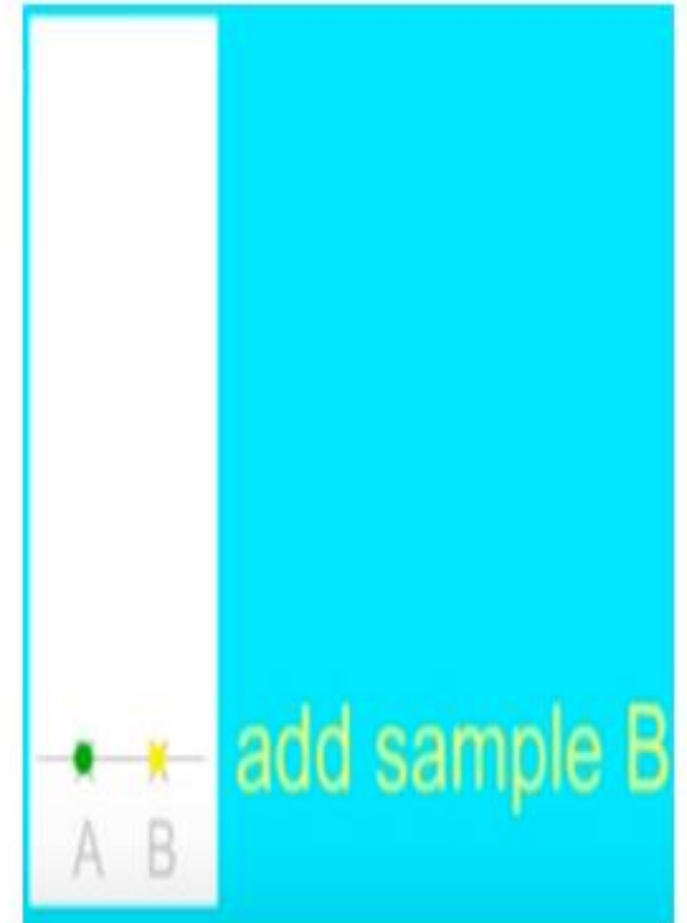
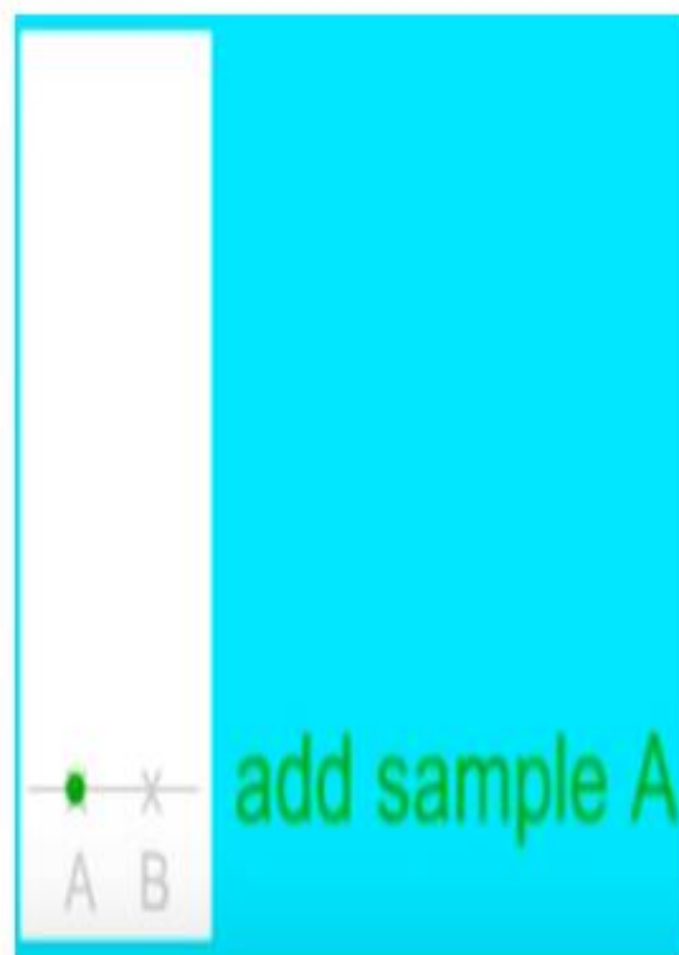
2-Dimensional Paper Chromatography

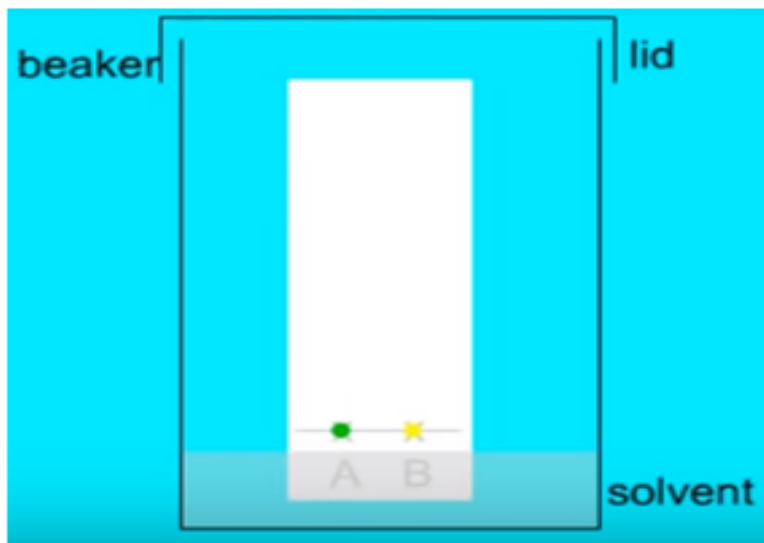
Retardation factor R_f

- Can be defined as the distance moved or traveled by the compound to the distance moved by the solvent.

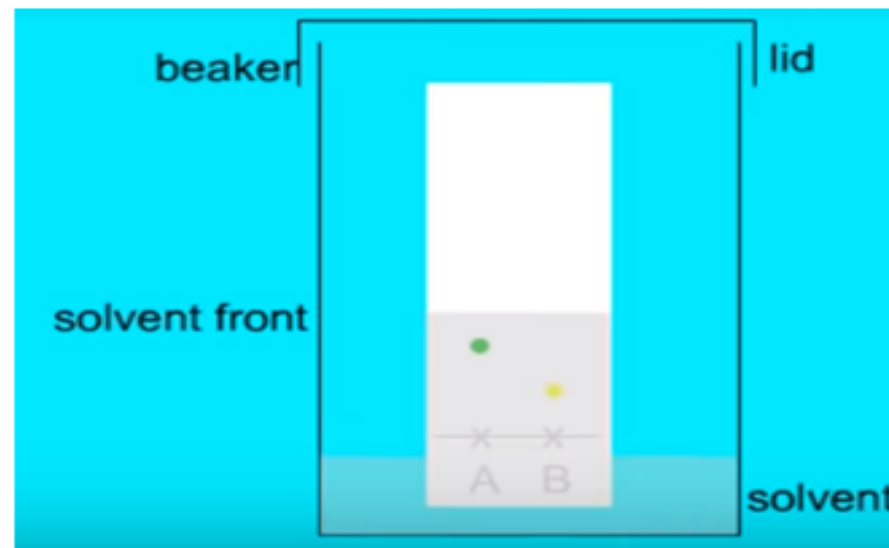
$$R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$$



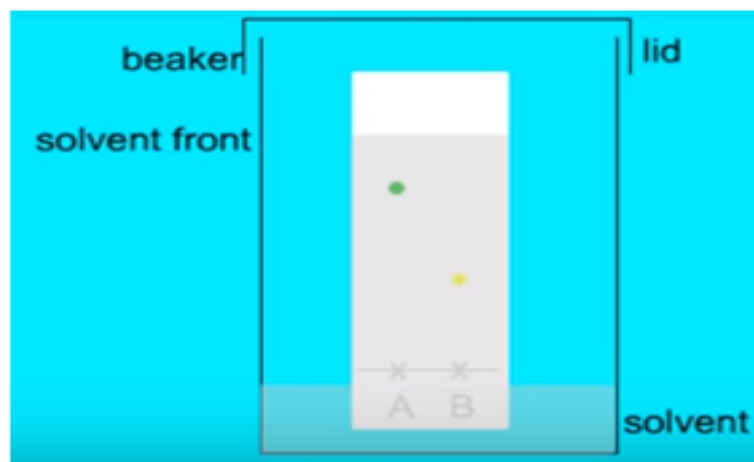




7



8

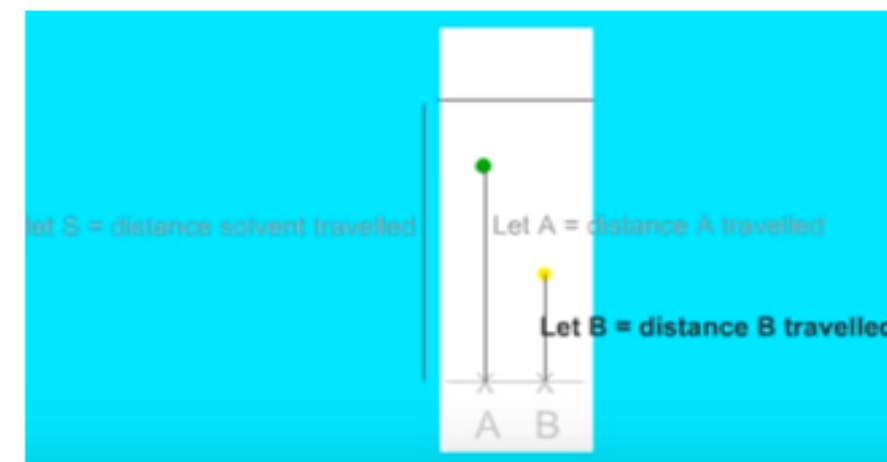


9



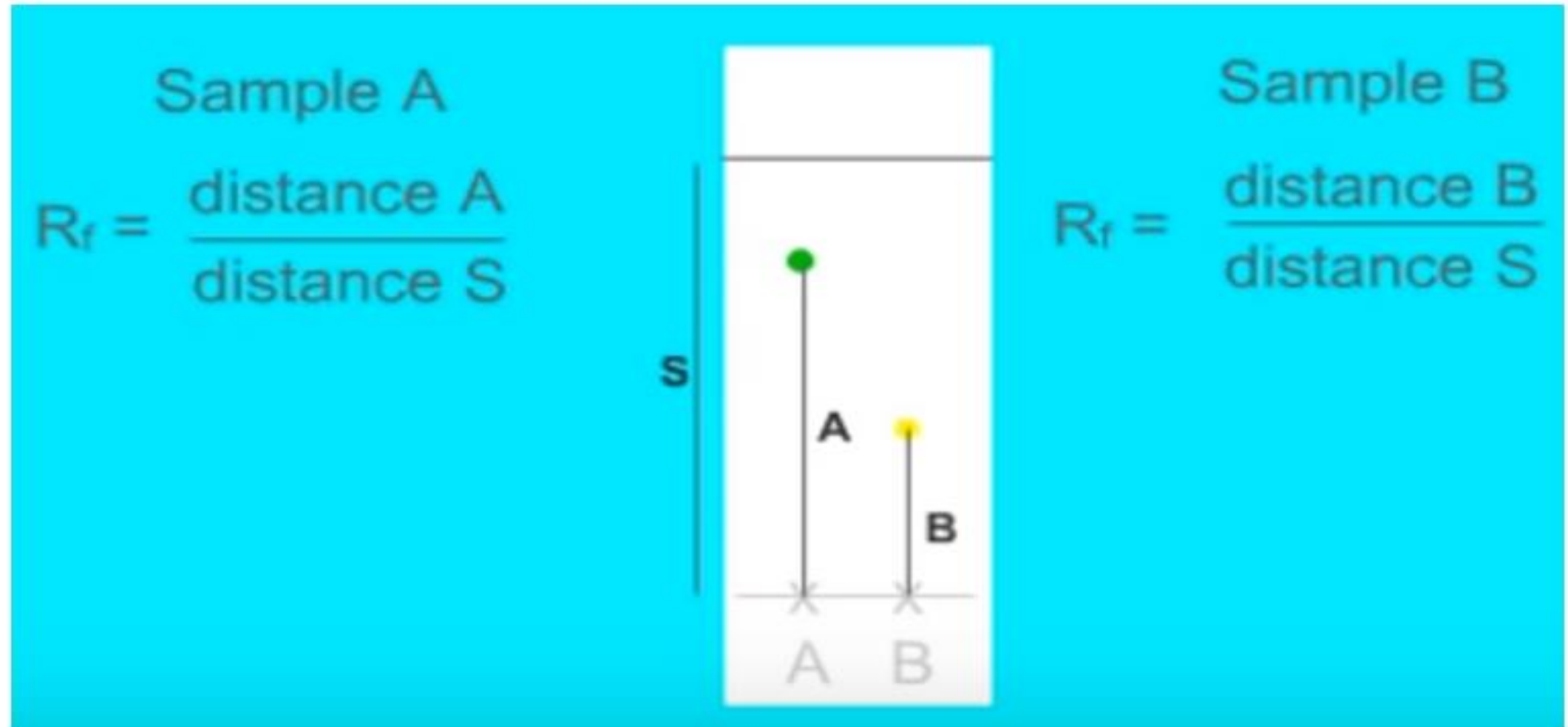
Asst.Prof. Dr. Noor S Jaafar

10

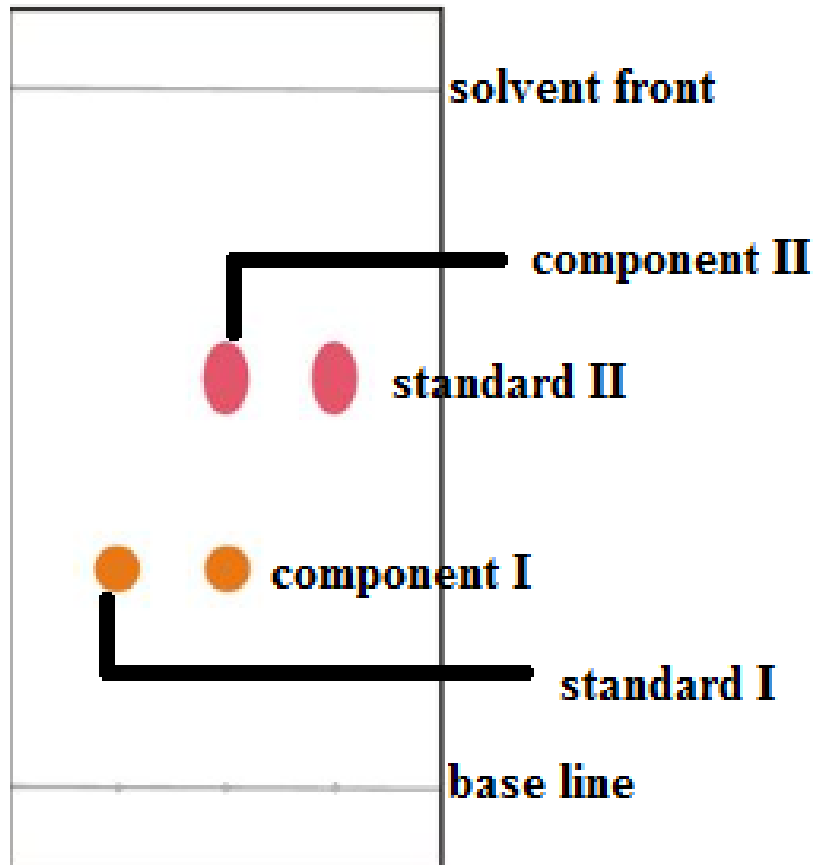


26

11



R_f value determination



R_f value = Distance traveled by the sample/ Distance traveled by the mobile phase

For standard I= 2cm / 8cm = 0.25

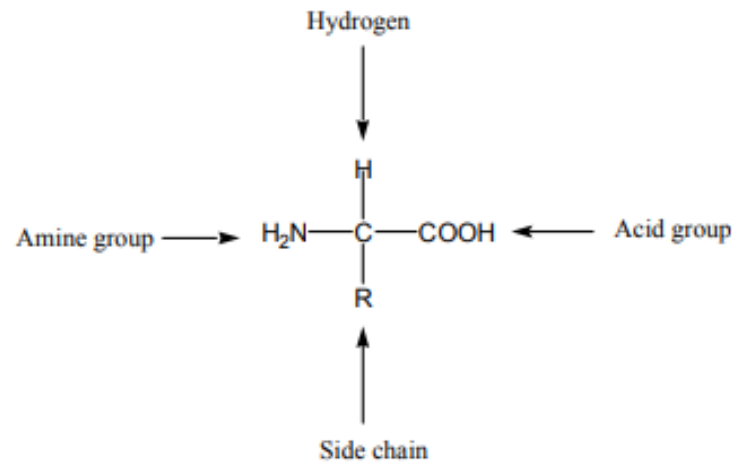
For standard II= 4cm / 8cm = 0.5

For component I= 2cm / 8cm = 0.25

For component II= 4cm / 8cm = 0.5

Paper chromatography for amino acids

- Amino acids are the building blocks for proteins and alkaloids.
- Paper chromatography is an effective method for separating and studying the amino acids.



In paper chromatography for amino acids

St.

phase

Water
adsorbed
on filter
paper

M.

phase

Organic
phase of
BAW 4:1:5

Sample

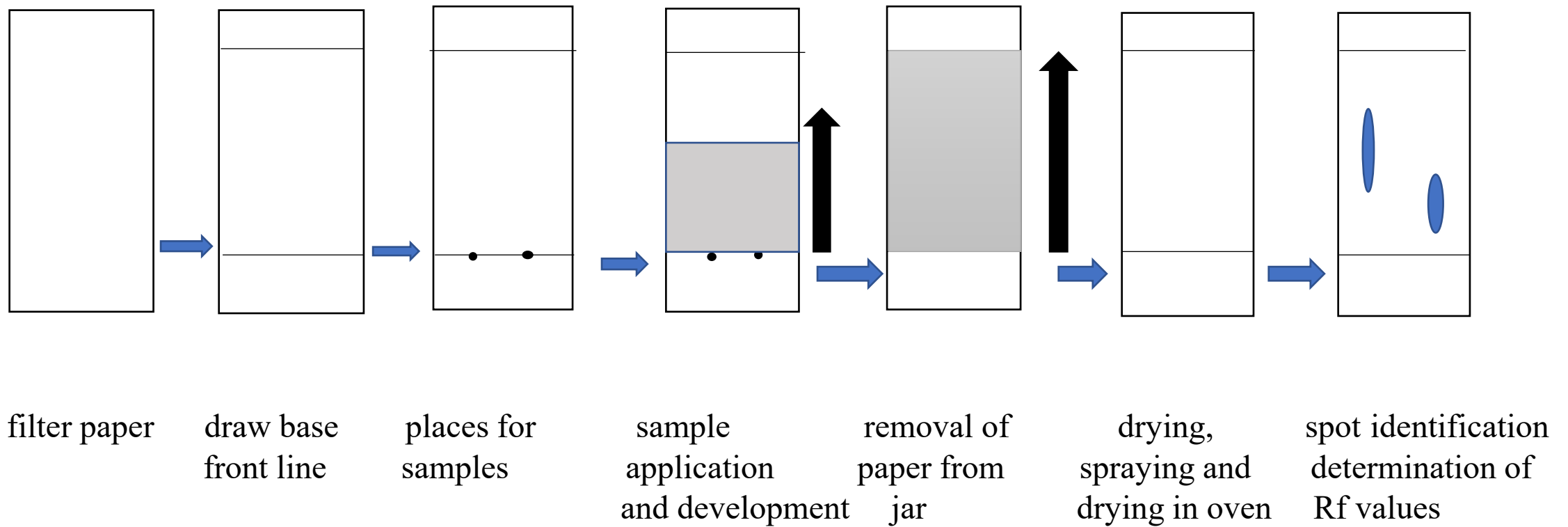
Leucine
Cysteine

Spray reagent: 0.1% Ninhydrine in n-butanol

Procedure

- Obtain a sheet of filter paper, and draw a baseline and front line.
- Mark off two equally spaced points along the baseline. (They should be separated by about 1 cm). The samples will be applied to these spots.
- The samples can be applied to the paper by using a narrow capillary tube. The spot should be about 2-3 mm in diameter.
- After spotting the paper, allow it to dry by waving it in the air or using a heat lamp or hair dryer.
- Place the paper in a jar previously saturated with the mobile phase.
- When the solvent reaches the solvent front, remove the paper and let the chromatography paper dry in the hood. When the paper is dry, spray it with ninhydrin reagent. Allow the paper to dry in an oven at about 100 °C.
- When the chromatographic paper has fully dried, outline the spots, mark the centers of each of the spots, and determine their R_f values

Procedure



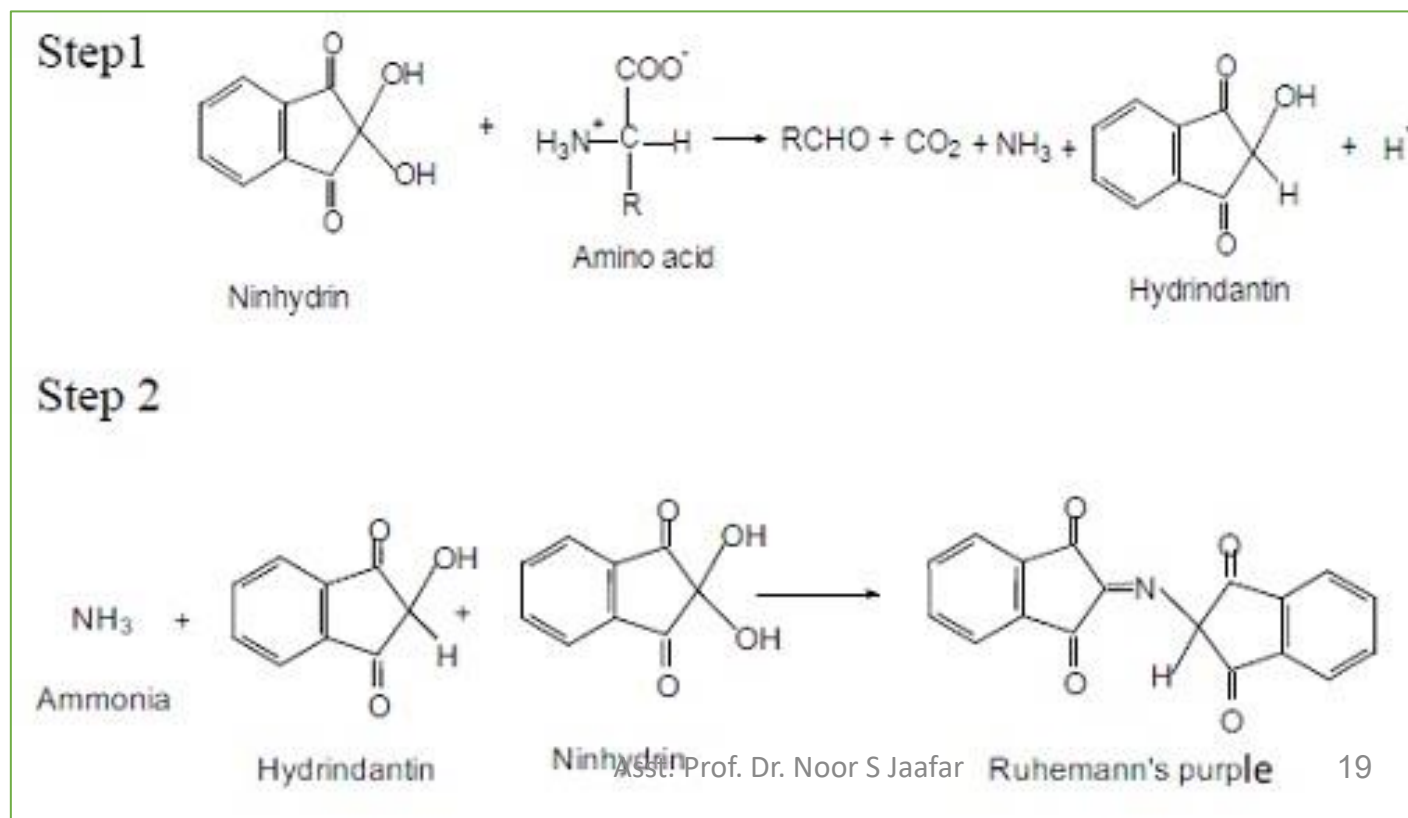


CAUTION:

Ninhydrin is poisonous: Use it only in a fume cupboard, and wear protective gloves, do not inhale its fumes, and wash it off immediately if it gets onto your skin.

Amino acid reaction with ninhydrine

- Amino acid + Ninhydrin $\longrightarrow$ RCHO + NH₃ + CO₂ + Hydrindantin
- Hydrindantin + Ninhydrin + NH₃ $\longrightarrow$ Purple color complex



- Ninhydrin oxidatively decarboxylates amino acids to CO_2 and NH_3 and an aldehyde with fewer carbon atoms than the parent amino acids.
- The reacted ninhydrin reacted with liberated ammonia and another molecule of ninhydrin, forming a purple color complex.