

**Practical work n°5****Thin Layer Chromatography (TLC)****1- Introduction**

Thin Layer Chromatography (TLC) is a simple, rapid, and effective analytical technique used to separate and identify components of a mixture. It is based on the differential migration of compounds over a stationary phase under the influence of a mobile phase.

-The stationary phase consists of alumina or silica spread as a thin layer on an aluminum plate. These solids have adsorptive properties: they interact with the eluted molecules through Van der Waals forces and, in some cases, hydrogen bonding.

-The mobile phase is a solvent or a mixture of solvents, known as the eluent.

After the sample is deposited onto the stationary phase, the substances migrate by capillary action at different rates. This migration depends on the strength of their interactions with both the mobile phase and the stationary phase. Each component travels a characteristic distance specific to the substance, known as the retention factor or Rf value.

$$R_f = \text{Distance traveled by solvent front} / \text{Distance traveled by pigment.}$$

**2-Objective of the practical work**

-Separate the different constituents of a mixture (chlorophyll pigments) using thin layer chromatography.

**3- Materials and chemicals**

-Fresh spinach leaves

-Mortar and pestle

-TLC plates (silica gel on aluminum or glass)

- Capillary tubes ou micropipettes

-Chromatography developing chamber

-Filter paper

-Beakers

-Funnel

-Ruler

-Pencil

-Ethanol

-Petroleum ether

-Diethyl ether

-Distilled water

-Sand

#### **4- Experimental procedure**

##### **4-1-Extraction of pigments**

- Wash the spinach leaves with distilled water.
- Cut the leaves into small pieces.
- Place the leaf pieces into a mortar.
- Add a small amount of sand to facilitate grinding.
- Add 100 mL of ethanol.
- Grind thoroughly until a dark green solution is obtained.
- Filter the extract using filter paper to remove solid debris.
- Evaporate the solvent using a rotary evaporator to obtain a concentrated pigment extract.
- Collect the concentrated pigment extract in a clean and dry test tube.

##### **4-2- Preparation of the Solvent**

-The solvent system is prepared by mixing 40 mL of petroleum ether with 60 mL of diethyl ether.

##### **4-3- Chromatography chamber preparation**

- Pour the solvent into the chamber to a depth of about 0.5 cm.
- Close the chamber and allow it to equilibrate for a few minutes.

##### **4-4- Preparation of the TLC Plate**

- Using a pencil, draw a light horizontal line 2 cm from the bottom of the TLC plate (this is the baseline).
- Small drops of the pigment solution are applied carefully onto this line using a capillary tube (Each drop is allowed to dry before adding the next, ensuring the spot remains small (less than 3 mm)).

##### **4-5- Migration and Separation**

- Place the TLC plate vertically into the chamber (ensure the solvent level is below the baseline).
- Close the chamber immediately.
- Allow the solvent to rise up the plate by capillary action.
- When the solvent front reaches about 1–2 cm from the top, remove the plate.
- Immediately mark the solvent front with a pencil.
- Allow the plate to dry.

**4-6-Observation and identification****Table 1** : Rf values of some chlorophyll pigments

<b>Pigment</b>	<b>Rf Value</b>	<b>Color</b>
Carotene	0.89–0.98	Yellow
Pheophytin a	0.42–0.49	Gray
Pheophytin b	0.33–0.40	Brown
Chlorophyll a	0.24–0.30	Blue-green
Chlorophyll b	0.20–0.26	Green
Xanthophylls	0.04–0.28	Yellow

**Questions**

- Calculate the average retention factor (Rf) of each migration spot.
- Explain why the chamber must be closed, why different pigments migrate differently, and identifying the number, color, and nature of the pigments present in chlorophyll).