

Title: Chromatographic analysis of samples. Thin Layer Chromatography.

Introduction:

Thin Layer Chromatography (TLC) is a widely used technique in analytical chemistry for the separation, identification, and quantification of organic compounds. It is a simple and cost-effective method that utilizes a stationary phase (thin layer of adsorbent material) and a mobile phase (solvent) to separate the components of a mixture based on their different affinities towards the stationary phase. This protocol outlines the steps involved in performing a TLC experiment.

Materials:

1. TLC plates (silica gel or alumina-coated)
2. Sample(s) to be analyzed
3. Solvent system (appropriate for the sample)
4. Capillary tubes or micro-pipettes
5. Developing chamber (glass or plastic)
6. Glass cover or watch glass
7. UV lamp or iodine chamber
8. Pencil or marker
9. Ruler or measuring tape
10. Gloves and safety goggles

Procedure:

1. Preparing the TLC Plate:

- a. Take a TLC plate and measure the desired distance (usually 1-2 cm) from the bottom using a ruler or measuring tape.
- b. Mark a horizontal line across the plate at the measured distance using a pencil or marker.
- c. Repeat the process for multiple plates if required.

2. Applying the Sample:

- a. Prepare the sample by dissolving it in a suitable solvent to obtain a concentrated solution.

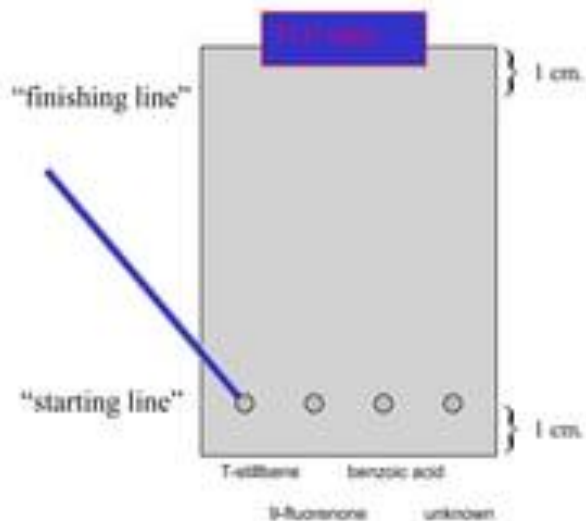
b. Using a capillary tube or micro-pipette, spot the sample solution as a small dot on the marked line of the TLC plate.

c. Allow the spotted sample to dry completely before proceeding.

Sample Application (spotting)

Process

- A. Draw "guide lines" lightly with pencil
- B. Dissolve solid sample in CH_2Cl_2
- C. Use TLC capillary to transfer and spot dissolved sample



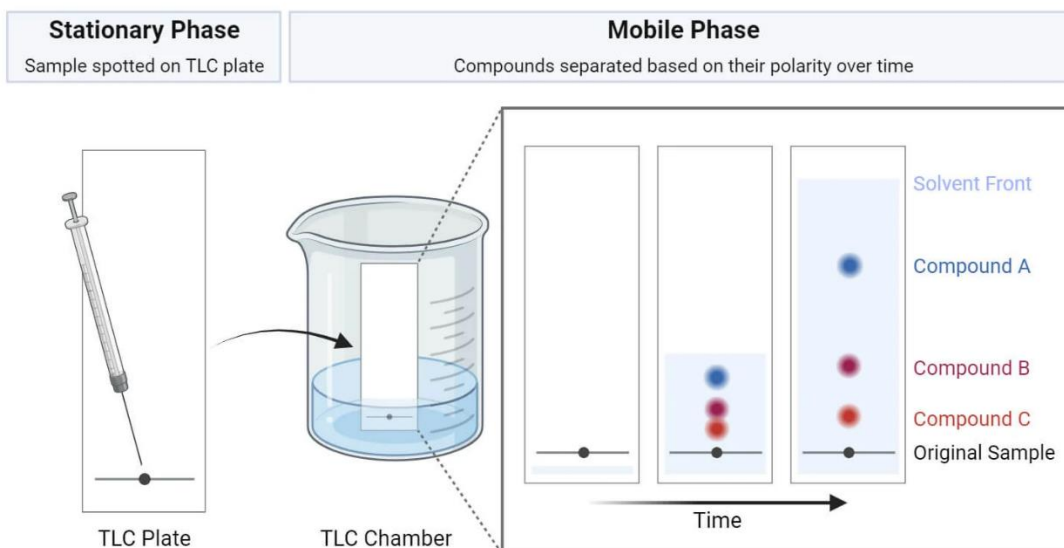
3. Preparing the Developing Chamber:

- a. Take a glass or plastic developing chamber and add the solvent system to a depth of about 1 cm.
- b. Ensure that the solvent level is below the marked line on the TLC plate.

4. Developing the TLC Plate:

- a. Place the TLC plate vertically into the developing chamber, ensuring that the spotted line is above the solvent level.
- b. Cover the chamber with a glass cover or watch glass to prevent evaporation.
- c. Allow the solvent to ascend the plate until it reaches near the top.
- d. Remove the TLC plate from the chamber and mark the solvent front with a pencil or marker.
- e. Allow the plate to air dry completely.

Thin Layer Chromatography



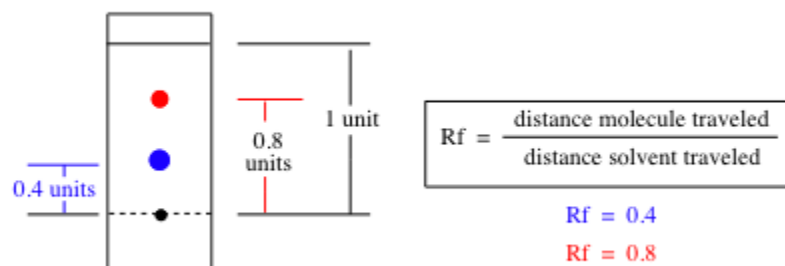
5. Visualizing the Separated Components:

- Expose the dried TLC plate to an iodine chamber or visualize under a UV lamp to observe the separated components.
- If using an iodine chamber, place the TLC plate inside and allow it to develop color spots.
- If using a UV lamp, turn on the lamp and observe the plate under UV light. The separated components may appear as fluorescent spots.

6. Calculating the R_f Values:

- Measure the distance traveled by each component from the origin (spotted line) to the solvent front using a ruler or measuring tape.
- Calculate the Retention Factor (R_f) value for each component using the formula: $R_f = \text{Distance traveled by the component} / \text{Distance traveled by the solvent front}$.

Determining R_f Values:



7. Recording and Analyzing the Results:

- Record the observations, including the number and appearance of spots, R_f values, and any additional relevant information.
- Compare the R_f values obtained from the sample with known standards or literature values to identify the components.

Note: Ensure proper safety precautions are followed throughout the experiment, including the use of gloves and safety goggles. Dispose of the used TLC plates and solvents according to appropriate laboratory waste disposal procedures.