

1. What is Chromatography and what can we do with it?

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2. How does chromatography work?

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3. What are some types of chromatography?

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4. What does TLC mean?

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5. For TLC, which is nature of both phases?

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You want to do color pen analysis by TLC,

6. What equipment do you need?

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7. What are the steps to do this TLC?

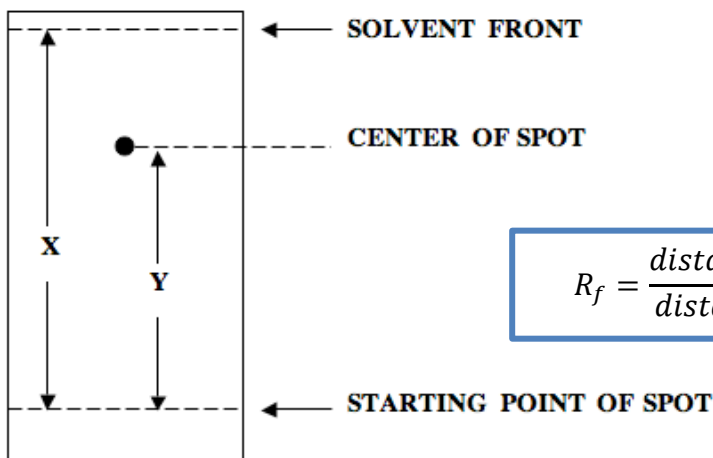
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8. What is the result of this black experiment on a TLC plate? Draw your chromatogram after migration.

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**Remarks:**

- In case of colorless substances, a specific coloring reagent can be applied to the plate. This coloring reagent will react with the analytes to produce visible spots on the chromatogram.
- It's possible to define the position of the spots by measuring the R<sub>f</sub> values. The Retention Factor (R<sub>f</sub>) is the ratio of the distance that the analyte spot moves along the plate to the distance that the solvent front travels up



$$R_f = \frac{\text{distance traveled by the center} \dots \dots \dots}{\text{distance traveled by the solvent} \dots \dots \dots}$$

## Activity 3 : Chromatography

### Analysis of pen ink by TLC (Thin Layer Chromatography)

#### 1. Step 1: Prepare the developing container

The developing container for TLC is a specially designed chamber with a lid. Pour solvent into the chamber to a depth of just less than 0.5 cm. Close the chamber with the lid, swirl it gently, and allow it to stand while you prepare your TLC plate.

#### 2. Step 2: Prepare the TLC plate

TLC plates used in this lab are purchased as 20 cm x 20 cm sheets. Each large sheet is cut horizontally into plates which are 10 cm tall by 5 cm widths; Handle the plates carefully so that you do not disturb the coating of adsorbent or get them dirty.

Measure 1 cm from the bottom of the plate. Using a pencil, draw a line across the plate at the 1 cm mark. This is the origin: the line on which you will spot the plate. Take care not to press so hard with the pencil that you disturb the adsorbent. Under the line, mark lightly the name of the samples you will spot on the plate, or mark numbers for time points. Leave enough space between the samples so that they do not run together; about 4 samples on a 5 cm wide plate is advised.

#### 3. Step 3: Spot the TLC plate

Draw a dot with your pen and a dot with the black Pentelpen® on the starting line.

#### 4. Step 4: Develop the plate

Place the prepared TLC plate in the developing chamber, close with a lid, and leave it undisturbed on your bench top. The solvent will rise up the TLC plate by capillary action. Make sure the solvent does not cover the spot.

Allow the plate to develop until the solvent is about half a centimeter below the top of the plate. Remove the plate from the chamber and immediately mark the solvent front with a pencil. Allow the plate to dry.

#### 5. Step 5: Visualize the spots

Comment your results