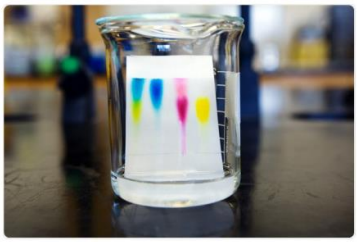
PW N° 1: Thin Layer Chromatography of ink/dyes



1. INTRODUCTION

Thin-layer chromatography is a chromatography technique used to isolate nonvolatile mixtures that separates pigments, identifying molecules. While it has many applications in a wide variety of industries, it is a particularly important technique used in forensic labs, helping scientists determine if two pieces of text were written by the same ink, which can often be an indicator of fraud.

Thin layer chromatography can be used to monitor the progress of a reaction, identify compounds present in a given mixture, and determine the purity of a substance. A number of enhancements can be made to the original method to automate the different steps, to increase the resolution achieved with TLC and to allow more accurate quantitative analysis. This method is referred to as "highperformance TLC". On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (Rf) expressed as:

$$R_f = \frac{\textbf{distance travelled by sample}}{\textbf{distance travelled by solvent}}$$

2. Aim

- Understand the principle of Thin Layer Chromatography (TLC).
- Perform TLC using food colorants as an example.

3. PRINCIPLE

Like other chromatographic techniques, thin-layer chromatography (TLC) depends on the separation principle. The separation relies on the relative affinity of compounds towards both the phases. The compounds in the mobile phase move over the surface of the stationary phase. The movement occurs in such a way that the compounds which have a higher affinity to the stationary phase move slowly while the other compounds travel fast. Therefore, the separation of the mixture is attained. On completion of the separation process, the individual components from the mixture appear as spots at respective levels on the plates. Their character and nature are identified by suitable detection techniques.

4. Materials and Products

Jar, Absorbent paper, TLC plate (silica), Acetone (for cleaning the capillary), Capillary, Colorants, Eluents: 70% saline solution (40 g/L) and 30% ethanol.

5. Procedure

Place about 10 mL of eluent (70% by volume of saline water at 40g/L, 30% by volume of ethanol) in the jar, ensuring that there is half a centimeter of eluent at the bottom. Draw a line with a pencil about 1.5 cm from the bottom of the plate. This line is called the **baseline**.

- Cut a strip of Whatman paper with dimensions slightly narrower than the diameter of the jar and slightly shorter than the total height of the jar.
- Draw a horizontal line with a pencil: this will be the baseline. Ensure that this line does not touch the solvent in the jar. Mark regularly spaced crosses along this line.
- Using capillary tubes, deposit a micro-drop of each dye (red, green, yellow).
- Clean the capillary with acetone after each deposit.
- Be careful to leave enough space between the different products deposited. Then, immerse the paper vertically in the eluent, ensuring that the baseline is above the level of the eluent. Allow the solvent to rise to near the top of the plate.
- Observe carefully!
- When the eluent has finished migrating, remove the paper from the jar and mark the level reached by the eluent (called the solvent front) with a pencil.

6. Questions

1. Write down carefully the different steps of TLC chromatography.
2. What do you observe once the paper is dipped into the eluent? Do all the dyes migrate at the same speed? If not, which dye migrates the fastest?
3. We say that the plate and the eluent are two distinct "phases." What is the liquid phase? What is the solid phase? What is the mobile phase? What is the stationary phase?
4. Does the eluent carry all the dyes in the same way? What happens to the green food dye after a few minutes? What conclusion can you draw from this?
5. What does chromatography allow us to do? Provide two functions explaining the principle of chromatography, particularly based on the results obtained from the green dye.
6. Sketch the chromatogram, labeling the colors of the different spots.
7. Do all chemical species migrate to the same level? What can we say about two species that have migrated to the same level?
9. Calculate $\mathbf{R_f}$ for yellow, $\mathbf{R_f}$ for red, $\mathbf{R_f}$ for blue, and the different ratios for the green dye.