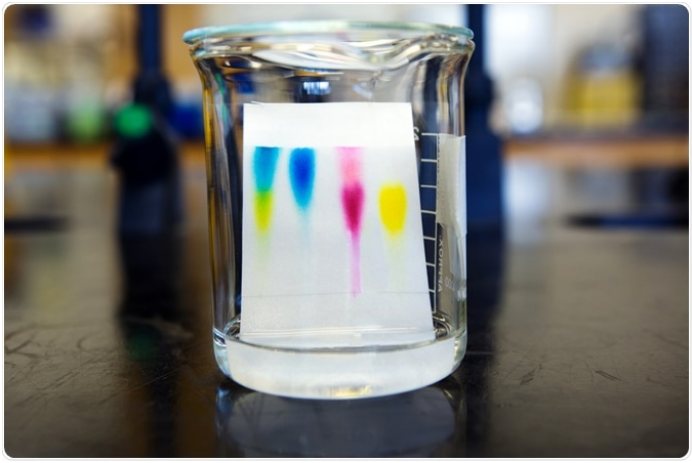
To perform Thin Layer Chromatography of ink/dyes

INTRODUCTION: Thin Layer Chromatography (TLC)

Thin-layer chromatography is a chromatography technique used to isolate non-volatile 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 "high-performance TLC". On completion of the separation, each component appears as spots separated vertically. Each spot has a retention factor (R_f) expressed as:

R_f = dist. travelled by sample / dist. travelled by solvent

AIM:

Our goal is to examine and to compare the quality of the results obtained by thin-layer chromatography, high-performance thin layer chromatography test in the analysis of samples of ink/dyes.

Thin layer chromatography as a qualitative method has shown good quality of results that can be used for a good life prediction of the ammunition.To study how to perform Thin Layer Chromatography of ink/dyes.

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.

REQUIREMENT:

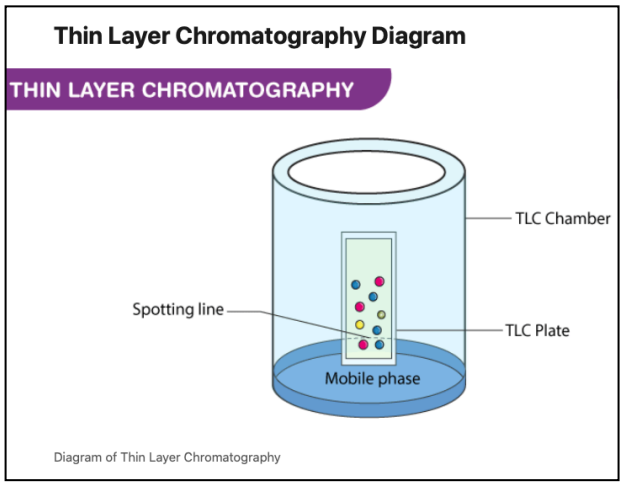
Chromatography jar	Forceps (tweezers)
Micro-capillary tubes	Methanol in dropper bottle
Ink sample Stirrer Beaker	Mobile phase (solvent): 7.0 ml Ethyl actetate + 3.5 ml ethanol + 3ml distilled water
Glass slides or TLC plates Silica Gel G and Distilled water Hot air oven	Ruler Pencil Scissors

PROCEDURE:

Thin Layer Chromatography Procedure

Before starting with the Thin Layer Chromatography Experiment, the different components required to conduct the procedure along with the phases involved.

1. Obtain a silica gel plate (TLC paper), handle it by the edges to avoid contaminating.
2. Draw a pencil line all the way across the bottom of paper 2-3 mm up from the bottom.
3. If working with an ink source: mark TLC paper at the starting line. Note using a pencil the identity of the sample being tested. More than one sample can be tested on the TLC paper.



If working with an ink on paper. First extract the ink sample from the paper. Using a capillary tube, wick up an ink sample. Apply capillary tube to starting line on the TLC paper to create a spot. Duly note on the TLC paper which sample is applied. If working with dilute samples we can achieve better results by drying the application and re-applying. Repeat until applied 3-5 times.

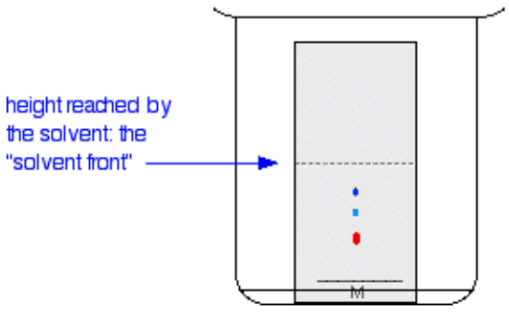
4. The TLC plate is now ready to place into a jar already adequately filled with solvent (ethanol). The TLC plate will be positioned so that the bottom is in the solvent but no solvent will be above the starting line we have marked with our pencil. The solvent jar will be covered while the TLC plate is being developed in order for the solvent vapors to equilibrate.

5. Allow the solvent to continue until all possible separations have occurred. Typically the solvent is 80% of the way up the paper.

6. Remove the plate from the jar. Immediately mark the solvent front and the dye spots with a pencil. Note all observations in our lab notebook including a complete drawing in our notebook of the finished TLC plate. Measure and record the distance each spot (from its center) moved from the starting line. Calculate the Rf values for each. Some ink will have different components, and thus will have multiple Rf values. Put Rf values in a table format to report.
7. Sometimes a critical component of the ink has fluorescence. Using an ultraviolet light source we can observe our samples for this unique property.

RESULT:

As the solvent slowly travels up the plate, the different components of the dye mixture travel at different rates and the mixture is separated into different coloured spots.

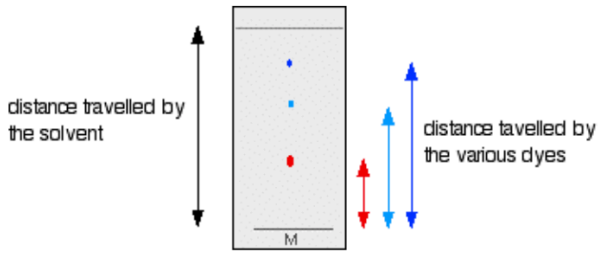


Measuring R_f values

Measurements are often taken from the plate in order to help identify the compounds present. These measurements are the distance travelled by the solvent, and the distance travelled by individual spots.

When the solvent front gets close to the top of the plate, the plate is removed from the beaker and the position of the solvent is marked with another line before it has a chance to evaporate.

These measurements are then taken:



The R_f value for each dye is then worked out using the formula:

$$R_f = \frac{\text{distance travelled by component}}{\text{distance travelled by solvent}}$$

For example, if the red component travelled 1.7 cm from the base line while the solvent had travelled 5.0 cm, then the R_f value for the red dye is:

$$R_f = \frac{1.7}{5.0} = 0.34$$

If we could repeat this experiment under exactly the same conditions, then the R_f values for each dye would always be the same. For example, the R_f value for the red dye would always be 0.34. However, if anything changes (the temperature, the exact composition of the solvent, and so on), that is no longer true. We have to bear this in mind if we want to use this technique to identify a particular dye. We'll look at how we can use thin layer chromatography for analysis further down the page.

CONCLUSION:

TLC is one of the fastest, least expensive, simplest and easiest chromatography technique. The technique of chromatography was first used in 1900 by scientist Mikhail Tsvet to separate the pigments of plants. Since its invention, TLC has evolved over the decades for use in numerous applications. One of the most prominent applications of the technology is the separation of multicomponent pharmaceutical formulations.

It is also heavily relied on for separating and identifying various colors, preservatives, cosmetic products, and sweetening agents in the food and cosmetic

sectors. In addition, forensic science has also adopted TLC that it is used in ink analysis, usually helping to determine if a document has been forged.

Ink analysis is an incredibly important analytical technique in forensic crime labs. Most commonly, it is used to determine if more than one ink was used on a document, which can help to detect fraud and forgery which can provide invaluable clues in criminal cases.