Thin layer chromatography

Thin layer chromatography (tlc) is an analytical technique for determining the composition of a mixture. It may be used to determine the extent of a reaction, the purity of a compound, or to ascertain the presence or absence of materials in fractions from column chromatography. Tlc analysis may indicate the best conditions for running a column. When done properly, tlc provides rapid, reliable results.

The key to obtaining a good chromatogram is having enough material on the plate to be seen after visualization, but not too much material such that the resolution is lost. The following points should aid you in obtaining good chromatograms without an excessive time investment.

The plate:
The plates used for tlc are commercially available as either silica gel or alumina adsorbents on either a plastic or aluminum foil support. The plates are supplied in a 20 x 20 cm size. For organic chemistry, the plates are usually silica gel supported on a plastic support.

The size of the plate does not have much to do with the resolution of the compounds, but does affect the time required for analysis. For rapid analysis, a plate cut to 6.5 cm high by 2.5 - 5 cm wide will provide good results in a minimum amount of time. A plate higher than 6.5 cm requires a more elaborate set-up than the one described below for comparable results. It is possible to get 4 - 6 separate spots on a 2.5 cm wide plate, so don't cut more plate than is required.

Using a pencil, carefully draw your spotting points on the plate about 1 cm from the bottom. Carefully draw a line 50 mm from your spotting points. This will be the solvent front when you remove the plate from the development tank.

The development tank:
The development tank consists of three pieces; a 250 mL beaker, a piece of folded filter paper and a watchglass. The beaker serves to hold the solvent, and should be filled to a depth of 3 - 7 mm (. 10 mL). Fold the edges of the filter paper over to form an oval, and place it in the beaker such that it is soaked with the solvent. The filter paper should be as tall as the beaker. Lastly, cover the beaker with the watchglass to prevent the solvent vapours from entering the laboratory.

The purpose of the paper is to saturate the air in the beaker with the solvent vapours. If the air is not saturated with solvent, the solvent that is rising up the plate will evaporate in an attempt to saturate the air. This will lead to high R_f values and poor resolution. When placing the paper in the beaker, ensure that the plate will sit below the top of the paper.

Sample preparation:
For neat compounds, place one drop (liquids) or ~ 15 mg (solids) into a clean vial. Add about one mL of a volatile solvent (ether or CH_2Cl_2). Cap the vial, and mix to dissolve the material. If you are analyzing column fractions, these may be analyzed directly.

Plate spotters:

Possibly the most important piece of equipment used in running a tlc. A very fine capillary is required to put only a small amount of material onto the plate. The end of a pasteur pipette is not fine enough.

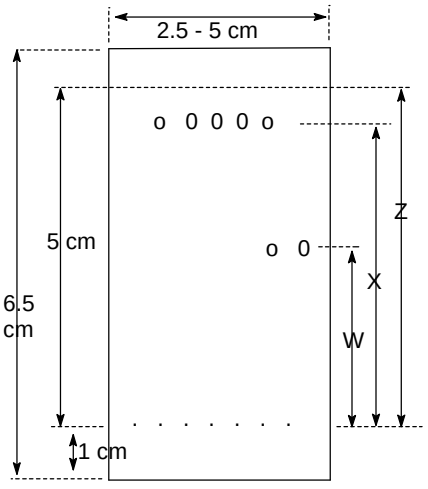
To prepare tlc spotters, perform the following in a fumehood. Light a Bunsen burner and obtain a hot flame. The burner must not be used outside of the fumehood. Hold either a capillary tube (0.6 mm) or a pasteur pipette at both ends, just above the hottest part of the flame (the light blue cone). While heating the tube, rotate it to distribute the heat evenly. When the glass is soft and rotation becomes difficult, remove the glass from the flame and immediately pull on the ends. This will cause a very fine capillary tube to be formed.

Don't pull the glass while it is in the flame, or the ends of the capillary will be sealed. Also don't wait to pull the glass when it is out of the flame, or it will cool and harden. You can break the capillary by bending it when the glass has cooled.

Spotting the plate:
Dip the capillary into your sample solution. A small amount of sample will be drawn into the capillary. To spot the plate, *briefly* touch the end of the capillary to one of your spotting points. Be careful not to scratch the plate. The spot made by the capillary should not be more that 2 mm in diameter. If you have a dilute solution you may wish to spot the plate more than once. You may also wish to blow gently on the plate to evaporate the solvent. Continue for the remaining samples. You should use a new capillary for each sample. Alternatively, clean the capillary by repeatedly drawing solvent into the capillary, and "spotting" a tissue to remove the solvent.

Eluting the plate:
Carefully place the plate into the developing tank. Ensure that your spots are above the level of the solvent. Place the plate on an angle, so that you can see the solvent rising up the plate. Allow the solvent to rise to the line drawn near the top of the plate. When the solvent reaches this line, remove the plate and allow the solvent to evaporate. If you missed the line, or did not draw a line, mark the solvent front with a pencil line.

Visualizing the plate:
There are two common methods available for visualizing the spots on the tlc plate. If the compounds under study contain a chromophore (*ie* an aromatic compound), the plate may be placed under an ultraviolet lamp. The spots will show as dark circles on a fluorescent background. The position of the spots is then marked with a pencil. To turn on the uv lamp in our lab, press the button on the right of the cabinet for 4 - 5 seconds, then release. If you are using uv to visualize the spots, you may wish to check your plate before elution to see if there is enough material on the spotting point.



For other organic compounds, visualization is accomplished by placing the plate in a glass jar containing a few iodine crystals.

Most (but not all) compounds will absorb some of the iodine vapours, and will be visible as dark brown spots on a tan background. The absorption of iodine requires a minute or two. The spots should be marked with a pencil upon removal of the plate since the iodine will desorb in air.

Other visualizing agents that react chemically with the compounds are available. Your instructor will describe these if necessary.

Analysis of the plate:
The diagram shows a plate after development and visualization. The results are typical of a separation by column chromatography.

Note that the dimensions of the plate are shown. The width of the plate in this example is about 4 cm, and the spotting points are 5 mm apart. The spotting points are numbered corresponding to the fractions from the column. The solvent front is shown as a dashed line at the top of the plate. The high R_f spots are due to a non-polar compound and the low R_f spots are due to a polar compound.

The R_f values, the ratio of the distance travelled by the compound to the distance travelled by the solvent, are calculated as follows. The distance travelled by the solvent is shown as Z, and is equal to 50 mm. The distance travelled by the non-polar compound is shown as X, and is about 40 mm. The R_f for the non-polar compound is therefore X/Z = 40 mm/50 mm = 0.80. For the polar compound the distance travelled is W, about 15 mm, and the R_f is therefore W/Z = 15 mm/50 mm = 0.30.

The results of the column separation may be interpreted as follows. Fraction 1 contains no material. This is expected since the compounds would not have had time to travel down the column. Fraction 2 shows that the non-polar compound has begun to elute from the column. Fractions 3 - 6 show the non-polar compound continuing to elute, with a maximum amount of material in fraction 4. Fractions 6 and 7 show that the polar compound has begun to elute from the column. Note that fraction 6 is a mixture of the two compounds, and the separation was not quite complete. We could now combine fractions 2 - 5, concentrate the solution and recover pure non-polar compound. Further tlc analysis would show us if there was more polar compound in higher fractions, and these could yield pure polar compound. Fraction 6 unfortunately requires either reseparation or disposal, depending upon the need for the compounds.

R_f values as a physical constant:
Unfortunately, it is not possible to use R_f values as an absolute physical constant. There are many variables in the use of tlc that make the R_f values unreliable. These variables include temperature, adsorbent thickness, type of adsorbent and exact solvent composition. Thus, one should never compare R_f values of compounds run on different plates, even though the conditions may appear similar.

To compare two substances suspected of being identical, they must be compared on the same tlc plate. In addition to a direct comparison of R_f, the two materials should be spotted on the same

spotting point. When mixed, two materials which have nearly identical R_f values will often appear as an elongated spot or overlapped pair of spots clearly indicating that the substances are not identical. If they are the identical, only one spot will appear. Several different solvent systems may be used to make this comparison.