# 7. Thin Layer Chromatography

## A. Introduction

Thin layer chromatography (TLC) is the second chromatographic method encountered in the Chem 235 laboratory. Like crystallization and distillation, TLC can be used to separate and purify organic compounds, however, TLC is based on a different set of principles, similar to those discussed in the GC experiment.

Chromatography is based on the interaction of the components in a mixture with a stationary phase and a mobile phase. In TLC, the stationary phase is a solid adsorbent that is adhered to a glass, plastic, or aluminum plate, and the mobile phase is a solvent or solvent mixture. The mixture to be separated is placed near the bottom of the plate. The solvent (eluent) is then carried up the plate, through the adsorbent by capillary action. As the components in the mixture interact with the stationary and the mobile phases, they travel up the plate at different rates, resulting in separation of the mixture.

### 1. TLC Plates and the Stationary Phase

In the past, chemists had to prepare their own TLC plates in the laboratory. Now, pre-prepared plates can be purchased and tend to provide better separation and more consistent results than those prepared individually.

A pre-prepared TLC plate consists of a glass, plastic, or aluminum plate coted with a solid adsorbent. The solid adsorbent is typically alumina ($Al_2O_3$) or silica gel ($SiO_2$), both of which are very **polar**. Additionally, a small amount of fluorescent indicator is added to the alumina or silica gel before the adsorbent is applied to the plate. This fluorescent indicator allows for the visualization of UV-active compounds by viewing the plate under a UV lamp. You should avoid touching the adsorbent with your fingers, as the oils on your fingers will contaminate the plate.

TLC plates can be purchased in a variety of sizes. Typically, large 20 cm x 20 cm plates are purchased and are subsequently cut to the desired size. In the Chem 235 laboratory, you will be provided with plastic backed alumina plates that are pre-cut to the necessary size.

### 2. The Mobile Phase

The mobile phase (also called the eluent) is a solvent or mixture of solvents that carries the components of interest up the TLC plate. The components to be separated interact with the **polar** solid stationary phase as they move up the plate. As the polarity of the eluent is increased so do the interaction of the components with the solvent. This results in the components traveling up the plate at a faster rate. As a general rule of thumb: *as the solvent polarity increases, the rate at which a compound travels up the TLC plate also increases.* A chemist often needs to find the “sweet spot” in regards to solvent polarity to achieve the desired separation. If the solvent is too non-polar, the components will stick to the bottom of the plate. If the solvent is too polar, the components will travel up the plate too quickly and separation will not be achieved.

A number of commonly used TLC solvents are listed in figure 1. This listing of solvents is referred to as the **elutropic series**, which is simply a list of solvents in order of increasing polarity.

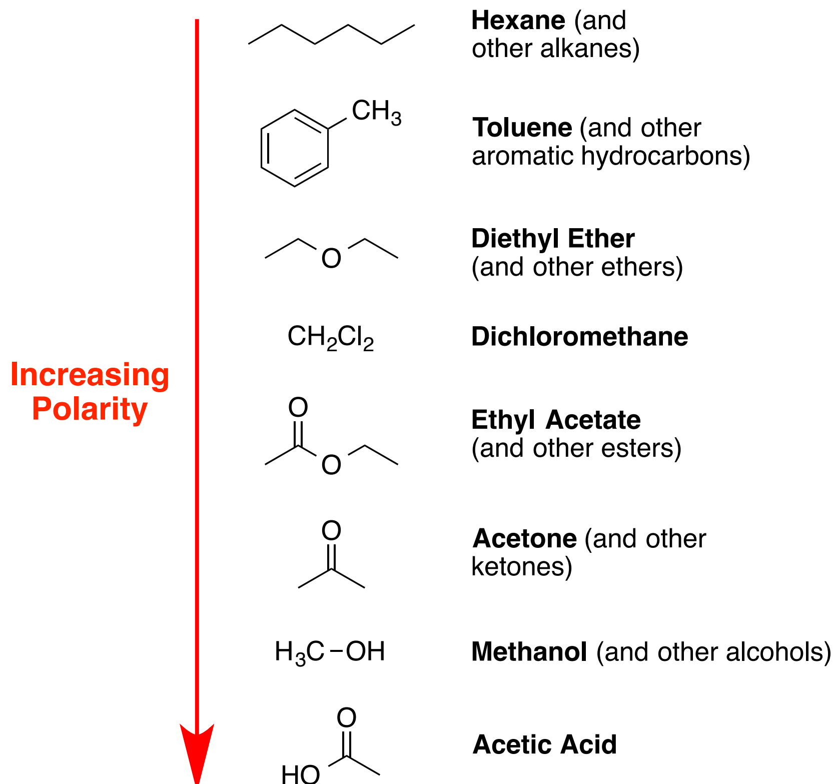



**Figure 1. Elutropic Series**

One of the most common solvent mixtures used in TLC is ethyl acetate-hexane. This provides a mixture of a relatively polar solvent (ethyl acetate) and a relatively non-polar solvent (hexane). The percentages of the two components can be varied to give a solvent mixture with the desired polarity.

## 3. Setting Up and Developing A TLC Plate

There are four basic steps to a TLC experiment. First you have to set up the developing chamber where the TLC plate will develop, next you have to put the components of interest onto the TLC plate. Third, you must develop the TLC plate in the developing chamber, and finally, you will visualize your developed TLC plate and analyzed the results.

### Preparing the Developing Chamber

A 250 mL beaker works well as a developing chamber. Cut a circle of filter paper in two and place one half into the beaker so that the flat edge of the filter paper is touching the bottom. Next, add about 5 mm of the developing solvent to the bottom of the beaker. The solvent will saturate the filter paper, which will help keep the container saturated with solvent vapors. Next, you need a means to close the top of the developing chamber. A watch glass or a piece of aluminum foil both work well for this purpose (figure 2).

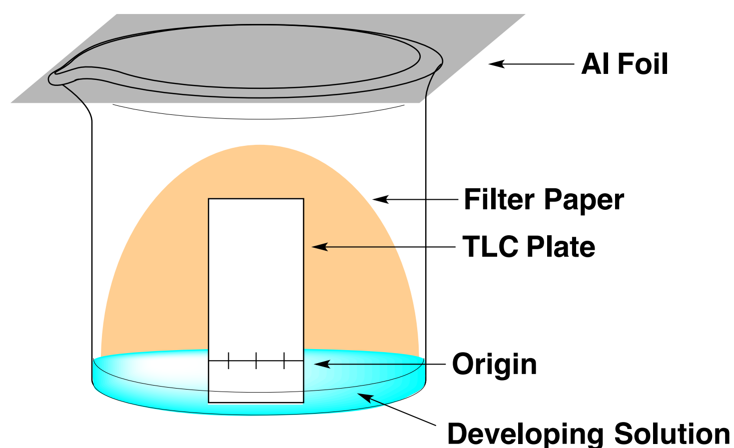



**Figure 2. TLC Developing Chamber**

**Spotting the TLC Plate**
Obtain a new TLC plate. Using a pencil (and only a pencil) draw a light line (origin line) across the plate about 7 mm from the bottom. *It is important that this line be at a greater height than the height of the solvent in the developing chamber.* Next, draw small evenly spaced perpendicular lines corresponding to the number of samples you want to put on the TLC plate. If you only have one sample to run, then you only need one perpendicular line (figure 3a). If you have three samples that you want to run simultaneously, you will need three perpendicular lines (figure 3b).

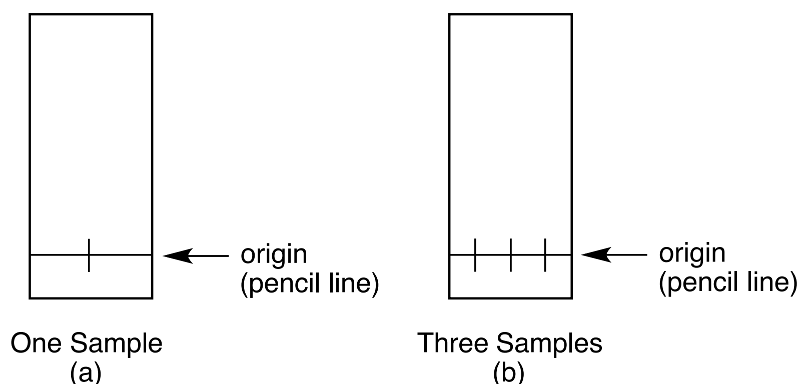



**Figure 3. TLC Plate Setup**

You are now ready to spot the compounds of interest onto the TLC plate. It is important that the sample you put on the plate is not too concentrated nor too dilute. It takes a bit of practice to get a feel as to the appropriate amount of sample to put on the plate. You do not want to put undiluted sample directly on the plate. Highly concentrated samples tend to streak and do not result in good separation.

Dilute your sample in a small volume of solvent (the developing solution often works well). Next, using a small capillary tube (aka spotter), withdraw some of the diluted sample and spot it neatly on the plate. Keep the spots a small as possible. It is best to spot once, allow the spot to dry, and then spot again. You can spot several times to build up the concentration of the sample if necessary (figure 4).

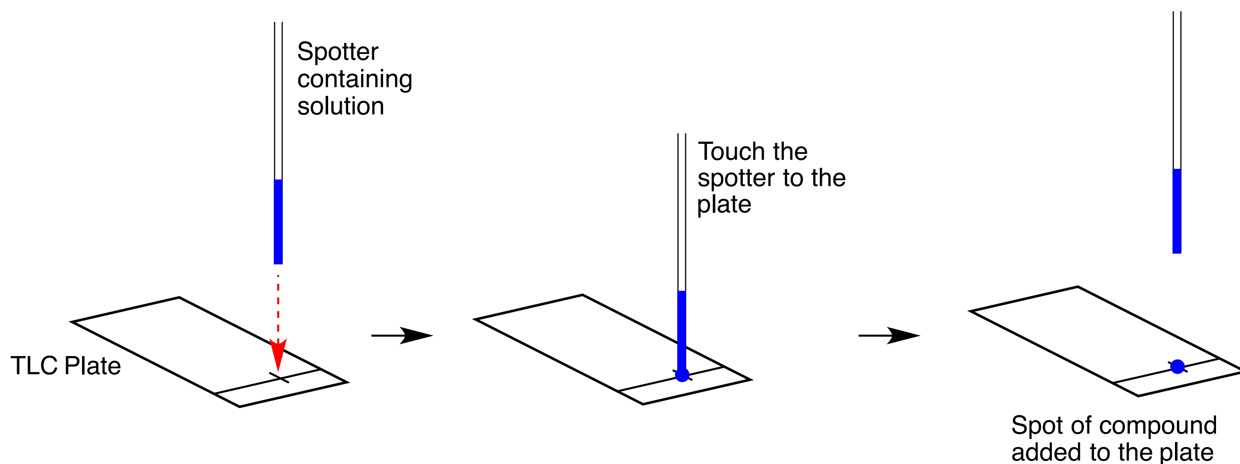



Figure 4. Spotting the TLC Plate

**Developing the TLC Plate**

Once you have spotted the TLC plate, you are ready to develop it. Carefully place the TLC plate upright into the developing chamber containing the eluent (figure 2). *Make sure the solvent level is not above the origin line containing the spot*. If the solvent covers the spot, you will need to start over and prepare a new TLC plate. Cover the chamber with a watch glass or foil. Over a few minutes, the solvent will rise up the plate and (ideally) separate the various components that are present in the mixture. Once the solvent level nears the top of the plate, remove the plate using forceps and then quickly use a pencil to mark the **solvent front** – the furthest point up the plate that the solvent was allowed to rise. Figure 5 (below) shows the separation of a two-component mixture by TLC.

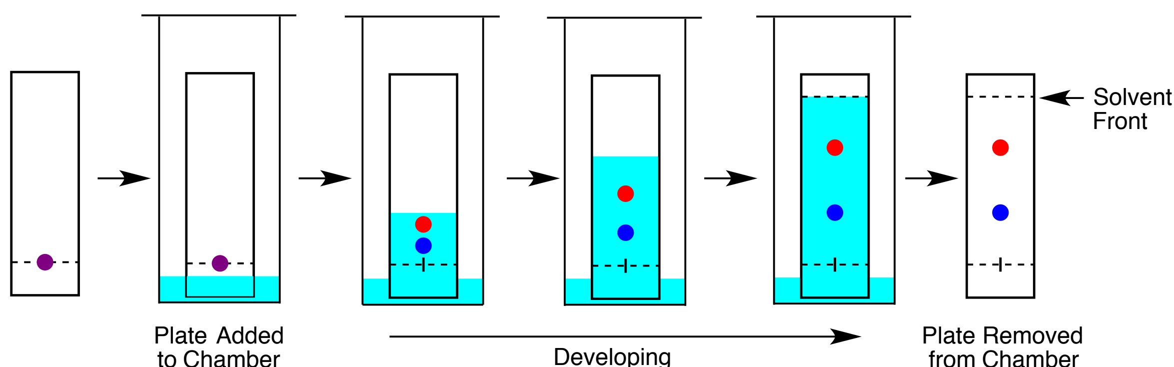



Figure 5. Developing a TLC Plate

**Visualizing the TLC Plate**

If the separated components are colored, visualization is easy because you will see the colored spots on the plate. If the components are not colored, the plate will appear blank and other visualization techniques must be utilized. Three commonly used visualization techniques are described below.

a. UV-Visualization

   Most commercial TLC plates have a small amount of a fluorescent indicator added to the silica gel or alumina. A long-wavelength UV light (254 nm) causes the indicator to fluoresce, emitting a green glow. Many compounds quench this fluorescence, and thus appear as dark spots under the UV-light. While examining the plate under UV, use a pencil, to draw a small

circle around each spot that is observed. You can also try observing your plate under short-wave UV light (180 nm).

b. Iodine Chamber
An iodine chamber is a closed container that contains a few crystal of iodine, which sublimes readily. A TLC plate can be placed in this chamber for a few minutes at which time the iodine vapors will react with the compounds present on the TLC plate resulting in the formation of dark spots. Once the plate is removed, these spots should also be circled, as they tend to fade over time.

c. Stains
One last visualization technique involved dipping the plate into a stain that reacts with the compounds present on the TLC plate. Often, the plate is heated after staining to help with rapid visualization of the spots. This technique will not be used in the Chem 235 laboratory.

## 4. Analyzing the Developed and Visualized TLC Plate

The ratio of the distance the spot travels to the distance the solvent travels is called the **$R_f$ (retardation factor) value**. The $R_f$ value has no units and is a constant for a compound analyzed by TLC as long as the conditions are held constant. In practice, $R_f$ values will vary slightly from one run to another because it is very difficult to replicate exact conditions (solvent, temperature, TLC plate manufacturer, concentration of compound spotted on the plate, etc.)

For example, in the TLC shown in figure 6, spot **A** has traveled 13 mm from the origin and the solvent front is 43 mm from the origin. The $R_f$ of compound **A** is calculated as follows:

$$R_f = \frac{\text{Distance traveled by spot A}}{\text{Distance traveled by the solvent front}} = \frac{a}{f} = \frac{13\text{ mm}}{43\text{ mm}} = 0.30$$

Spot B, on the other hand, has traveled further up the plate (30 mm) and thus has a larger $R_f$ value (0.70). It should also be noted that when silica or alumina TLC plates are used, less polar compounds tend to travel faster (have larger $R_f$ values) and more polar compounds tend to travel more slowly (have smaller $R_f$ values). Thus, spot A is likely the more polar compound.

The polarity of some common functional groups is listed below. In lecture, you should learn various ways to predict the polarity of different molecules.

**[Less Polar] Alkanes < Aromatic Hydrocarbons < Ethers < alkyl halides < Esters < Ketones < Aldehydes < Amines < Alcohols < Carboxylic Acids < Amides [More Polar]**

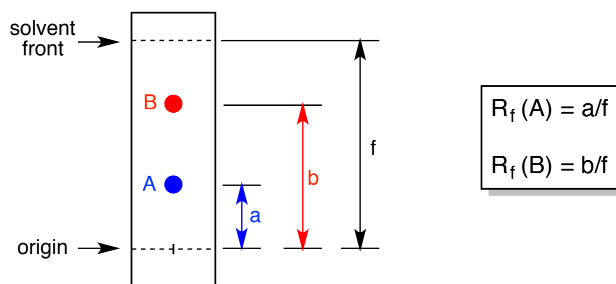
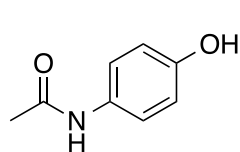



**Figure 6. $R_f$ Determination**

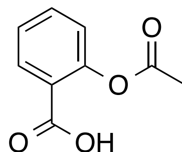
## B. Experimental Procedure

### Separation of Acetaminophen, Acetylsalicylic Acid, and Ascorbic Acid

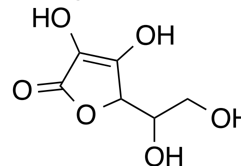
In this experiment, you will analyze an unknown mixture by thin layer chromatography. The compounds in the unknown mixture include one or more of the following:




acetaminophen
(Tylenol)




acetylsalicylic acid
(Aspirin)

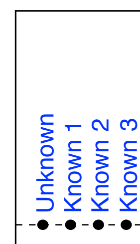



ascorbic acid
(vitamin C)

These compounds will be provided as either alcohol solutions or as solids and will serve as "knowns" to aid in the unknown identification. If the compounds are provided as solids, crush the solid into a fine powder and dissolve in a small volume of ethanol.

Obtain a capillary pipet (spotter) and a small vial of acetone. The acetone vial can be used to rinse the spotter between samples. Dip the spotter in the acetone and evacuate the liquid onto a paper towel 4-5 times to rinse the spotter.

Prepare the TLC plate and developing chamber as described in the introduction. The developing solution is a 15% ethanol-methylene chloride solution. If your unknown is a solid, dissolve it in a small amount of ethanol or reagent grade acetone (not acetone from the wash bottle). This solution can then be spotted on the TLC plate. It is recommended that you make marks to spot four things on the TLC plate (each of the three knowns and your unknown) as shown on the right.




Develop the TLC plate in the developing chamber and visualize the spots with either the UV-lamp or the iodine chamber. Once you think you know the identity of your unknown, you should run one additional TLC plate that contains the unknown, suspected known, and a co-spot. Co-spotting helps to nail down identification. In the middle, you should spot both your unknown and the suspected known. After development, if there is no separation from the co-spot than it is highly likely that the two substances that were co-spotted are identical compounds (figure 7a). If you see separation at the co-spot, then the two compounds are different (figure 7b). Without a co-spot, it can be very difficult to differentiate two compounds if the $R_f$ values are close.

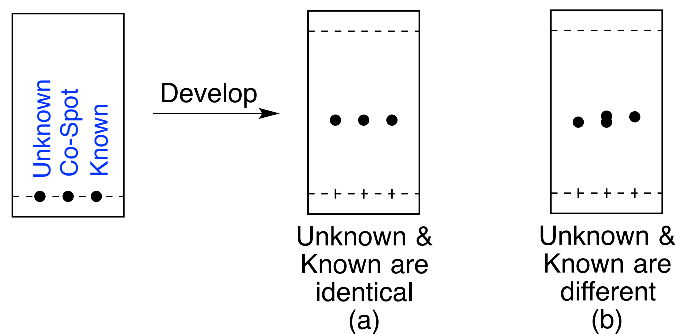



**Figure 7. Co-Spotting**

## C. Frequently Encountered Issues

### 1. You can't see any spots

If you can't see any spots then your sample was too dilute. You should prepare a new TLC plate and perform additional spotting to concentrate the sample on the plate.

*Tip: Check the plate under the UV light before developing. If you see a dark spot at the origin, then your sample is concentrated enough to see after development.*

### 2. Your spot smears up the plate

If your spot smears up the plate, the sample is much too concentrated (figure 8a). You should further dilute your sample and re-spot it on a new TLC plate.

### 3. Your spots bleed together

If you put your spots too close together at the origin, they may bleed together and be difficult to differentiate (figure 8b). If you run into this issue, prepare a new TLC plate and space your spots further apart. Additionally, bleeding can affect the $R_f$ value so it is important to avoid this complication. Sometimes the issue of bleeding can be remedied by spotting less sample on the TLC plate.

### 4. Your spot runs to the edge

When a spot runs to the edge of a TLC plate, it is likely that you spotted it too close to the edge (figure 8c). This can be fixed by preparing a new TLC plate with the spot closer to the center of the plate.

### 5. Your spot streaks

If you experiencing streaking up the plate, it is possible that your compound decomposes on the TLC medium (figure 8d). There are ways to further investigate this and to circumvent this issue (such as switching from silica to alumina), but these problems are beyond the scope of the Chem 235 course.

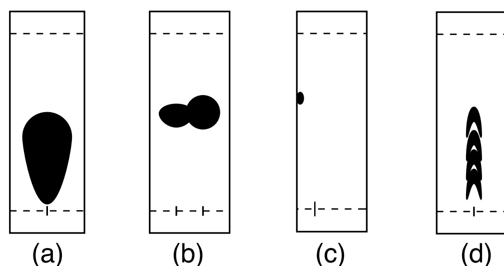


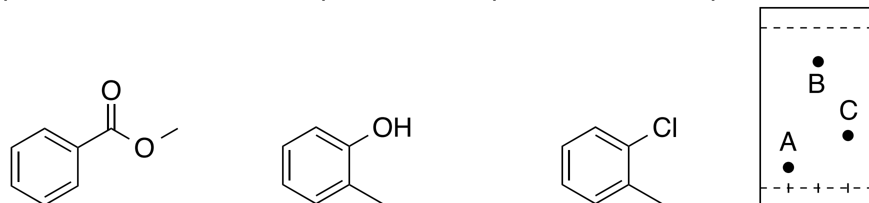

**Figure 8. Frequently Encountered TLC Problems**

## D. Pre-Lab Questions

1. Why is it important that a pencil rather than a ball-point pen or marker be used to mark the TLC plate prior to development?

2. A TLC plate is developed in a solvent system containing 10% Ethyl Acetate – Hexane. After visualization, it is found that the spot did not move from the origin. What might you do to get the spot to move up the plate?

3. The three compounds below were spotted on a TLC plate and developed to give the TLC plate shown. Which compound corresponds to which spot?




4. Calculate the $R_f$ value for spot B in the TLC plate shown above.

## E. Post-Lab Questions

1. When using polarity to determine the relative rates at which organic compounds travel up the TLC plate, we are considering compounds of similar molecular weight. It should also be noted that $R_f$ values vary with chain length when comparing molecules containing the same functional group. Would you expect 1-hexanol or 1-decanol to have a higher $R_f$ value? Explain.

2. Did you encounter any issues such as streaking or bleeding in any of your TCL runs? If so, explain what you observed.

3. List the compounds used in this lab (acetaminophen, acetylsalicylic acid, and ascorbic acid) from least polar to most polar. What structural features in each do you think accounts for the polarity?

4. An unknown mixture was found to be insoluble in a particular developing solvent. Do you think separation of this mixture will be observed on the TLC plate after development in this solvent?

5. TLC is a common method used to monitor reactions for completion. Consider the reaction below and draw the expected TLC plate prior to the reaction, during the reaction, and after the reaction has completed. *Don't worry about drawing spots for the reagents ($O_3$, DMS) or the formaldehyde product on the TLC plate.*