

Chapter 7: Thin-Layer Chromatography

The term chromatography was coined by the Russian botanist Mikhail Tswett in the late nineteenth century. Tswett studied plant pigments and found that he could separate green chlorophylls and orange carotenes from green leaf extracts using a narrow glass tube filled with calcium carbonate. In his words: “like light rays in the spectrum, the different components of a pigment mixture, obeying a law, separate on the calcium carbonate column.” Tswett called this separation process the *chromatographic method*, from the Greek *chromato* meaning color and *graphy*, which refers to writing, literally, “written in color.” The use of chromatography progressed slowly until the 1930s and the post-war era. Today, chromatography methods are the most modern and sophisticated means of separating the components of a mixture of compounds.

In chromatography, a mixture is separated by distributing the components between a stationary phase and a mobile phase. This rather broad definition covers the techniques of thin layer chromatography, gas chromatography, paper chromatography, ion-exchange chromatography, high-pressure liquid chromatography, size exclusion chromatography, and column chromatography. In each of these techniques, the mixture to be separated is placed on the stationary phase (a solid or a liquid) and the mobile phase (a gas or a liquid) is allowed to pass through the system. After a period of time, separation is achieved due to the selective interaction of chemicals with both the stationary and mobile phases.

The interactions of the chemicals with the stationary and mobile phases depend on their physical properties. Differences in absorptivity, solubility, charge, size, and boiling point cause different compounds to be more or less securely held either in the mobile phase or the solid phase.

Chromatography in general is used in organic chemistry to separate the components of mixtures. The applications of this separation fall into two categories: analytical and preparative. An analytical technique is one in which the purpose is to gather information on the amounts and mobilities of the components of the mixture. A preparative technique is one in which the purpose is to purify the compound by separating it from other components of the mixture.

The techniques of thin layer chromatography (Chapter 12), column chromatography (Chapter 13), and gas chromatography (Chapter 14) are used routinely in the organic chemistry teaching labs.

In thin layer chromatography, the stationary phase is a solid adsorbent and the mobile phase is a liquid. It is generally used as a qualitative analytical tool rather than as a means of purification, however, this technique can also be used to separate small quantities of compounds from mixtures. TLC is simple, inexpensive, and gives the user a quick answer as to how many components are in a mixture. TLC can be used to identify the compounds in a mixture by comparing data of unknown with known materials.

A TLC plate is a sheet of glass, metal, or plastic that is coated with a thin layer of a solid adsorbent (usually silica or alumina). A small amount of the mixture to be analyzed is spotted near the bottom of this plate. The TLC plate is then placed in a shallow pool of a solvent in a developing chamber so that only a small part of the bottom of the plate is in the liquid. This liquid, or the eluent, is the mobile phase, and it slowly rises up the TLC plate by capillary action (Figure 7-1).

As the solvent moves past the spot that was applied, an equilibrium is established for each component of the mixture between molecules of that component that are adsorbed on the solid and molecules that are in solution. In principle, the components will differ in solubility and in the strength of their adsorption to the adsorbent and some components will be carried farther up the plate than others. When the solvent has reached the top of the plate, the plate is removed from the developing chamber, dried, and the separated components of the mixture are visualized by one of several methods.

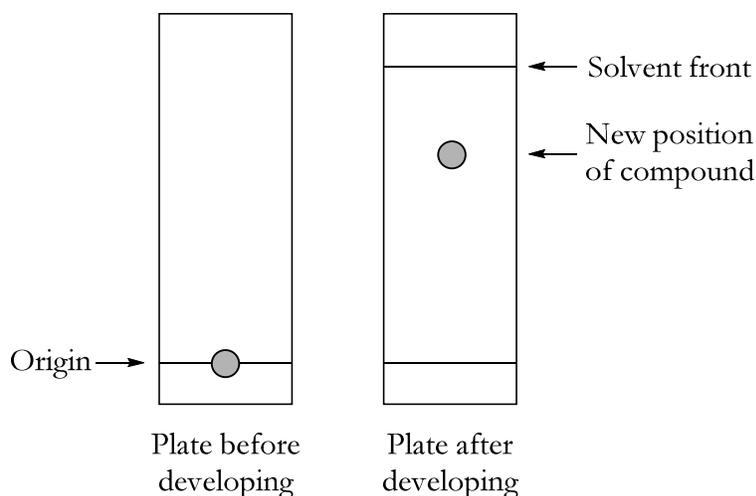


Figure 7-1: TLC plates, before and after being developed.

The rate at which a compound ascends the TLC plate depends on the properties of the adsorbent, the solvent, and the compound. Polarity is the property of each of these three factors that has the most influence on the rate of ascension. Since in a particular TLC run, the solvent and the adsorbent are the same for all components of the mixture, separation is based on differences in polarity of the compounds in the mixture. Since silica and alumina are polar adsorbents, a polar compound will interact more strongly with the adsorbent and move more slowly up the plate.

7.1 The Adsorbent

As previously mentioned, many adsorbents are available, but the most common are silica (SiO_2) or alumina (Al_2O_3). Silica is also known as silica gel or silicic acid. Both of these adsorbents are polar, with alumina being the more polar of the two.

Sometimes compounds are added to the silica gel before it is spread on the plate. For instance, gypsum (CaSO_4 , calcium sulfate, plaster of Paris) is added to help it to bind to a glass plate. Silica gel with this binder is designated silica gel G.” Compounds that fluoresce are commonly added, specifically, zinc sulfide that adsorbs at 254 nm. Silica gel products impregnate with this fluor are designated “silica gel F₂₅₄.” Silica gel comes in many size particles for use in different types of chromatography, but in general, small particle sizes are chosen for TLC applications. Silica gel can absorb moisture from the air, but this is usually not a concern on plates that are purchased already coated, as is the case in the teaching labs.

Alumina is used less often than silica in TLC applications. Although in general more polar than silica gel, the activity of this adsorbent varies greatly with the amount of water that is absorbed by the alumina, which in turn is affected by the amount of exposure to atmospheric moisture. Alumina TLC plates are generally activity III, a designation explained in the section on column chromatography.

7.2 Choice of Solvent in TLC

In many – but not all – experiments, you will be told the optimal solvent to use to develop the plate. This solvent was chosen for you by a process of trial and error as explained below.

The proper choice of a solvent is critical to separation of a given mixture by TLC. Generally, the more polar a solvent is, the more effective it is at eluting both polar and nonpolar compounds. This is because a polar solvent more effectively competes with the compounds for adsorption on the relatively polar surface of the adsorbent. The elutropic series, or the order of ability to displace solutes from adsorbents, is given for a number of solvents in Table 7-1.

Table 7-1: Eluting order of common chromatography solvents.

Hexanes	Least polar
Toluene	
Dichloromethane	
Diethyl ether	
Ethyl acetate	
Acetone	
Methanol, ethanol	
Acetic acid	Most polar

The best solvent for a particular application must be determined by a trial and error process. Generally, a mixture of solvents, or solvent system, is used in TLC. For instance, if one wants to separate two compounds and finds that they don't move up the plate in pure hexanes (a nonpolar solvent), one might try using a mixture of hexanes and a more polar solvent, such as ethyl acetate. Therefore, a solvent system consisting of a mixture of 90 parts (by volume) hexanes to 10 parts (by volume) ethyl acetate (usually written 90:10 hexanes/ethyl acetate, v:v) would be tried. If the two compounds still have similar retention factors, a solvent system of 80:20 hexanes–ethyl acetate might be tried, and so on.

A highly polar solvent will move even highly polar molecules rapidly up the TLC plate. If a solvent is too polar, movement becomes too rapid, and all the components end up at the top of the plate with little or no separation of the components of the mixture. Proper choice of an eluting solvent is thus crucial to obtaining meaningful results in thin layer chromatography.

The polarity of a solvent is not the only factor in solvent choice. Toxicity is also an issue; for instance, we discourage the use of chloroform, carbon tetrachloride, and benzene in our labs. We also avoid extremely flammable solvents such as diethyl ether. Availability and cost are also factors in solvent choice.

7.3 Interactions between the Adsorbent and the Compound

The strength with which an organic compound binds to an adsorbent depends on the strength of the following types of interactions: ion-dipole, dipole-dipole, hydrogen bonding, dipole induced dipole, and van der Waals forces. With both silica gel and alumina the dominant interactive forces between the

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adsorbent and the materials to be separated are of the dipole-dipole type. Highly polar molecules interact fairly strongly with the polar Si – O and Al – O bonds of these adsorbents and will tend to stick or adsorb onto the fine particles of the adsorbent while weakly polar molecules are held less tightly. Weakly polar molecules thus generally tend to move through the adsorbent more rapidly than the polar species. Roughly, binding of organic compounds to silica follows the order in Table 7-2.

Table 7-2: Usual eluting order of organic compounds.

Saturated hydrocarbons	Least polar
Unsaturated hydrocarbons	
Ethers	
Esters	
Halides	
Ketones	
Aldehydes	
Amines	
Alcohols	
Acids and bases	Most polar

The more polar a molecule is, the more strongly it will stick to the plate, and the less distance it will cover. Overall, then, there are two different types of polarity to consider: the polarity of the compound, and the polarity of the solvent. A more polar solvent will carry all compounds further than a less polar solvent, since it competes with the interactions between the compound and the plate. However, a less polar compound will always be carried further than a more polar compound, because it does not bind to the plate so strongly (Figure 7-2).

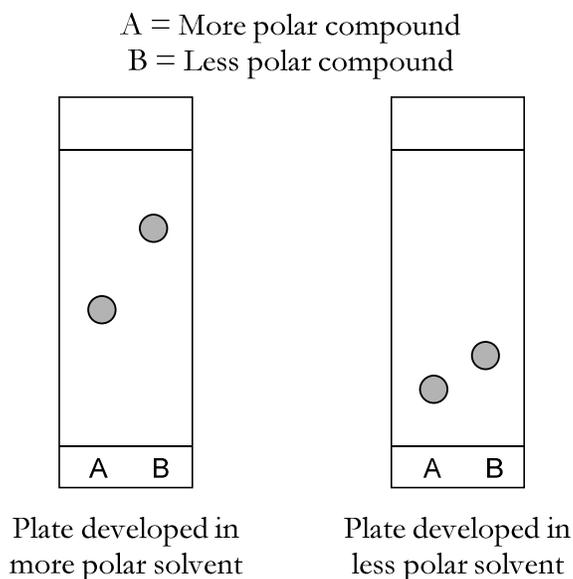


Figure 7-2: The relationship between solvent polarity, compound polarity, and spot travel distance.

7.4 Retention Factor (R_f)

The retention factor, or R_f , is defined as the distance travelled by the compound divided by the distance travelled by the solvent.

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

For example, if a compound travels 2.1 cm and the solvent front travels 2.8 cm, the R_f is 0.75 (Figure 7-3).

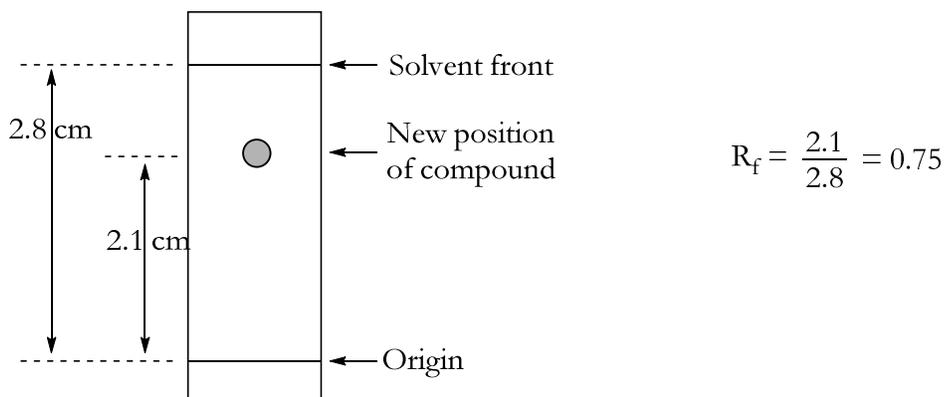


Figure 7-3: How to calculate R_f .

The R_f for a compound is a constant from one experiment to the next only if the chromatography conditions below are also constant:

- Solvent system
- Adsorbent
- Thickness of the adsorbent
- Amount of material spotted
- Temperature

Since these factors are difficult to keep constant from experiment to experiment, relative R_f values are generally considered. “Relative R_f ” means that the values are reported relative to a standard, or it means that you compare the R_f values of compounds run on the same plate at the same time.

The larger an R_f of a compound, the larger the distance it travels on the TLC plate. When comparing two different compounds run under identical chromatography conditions, the compound with the larger R_f is less polar because it interacts less strongly with the polar adsorbent on the TLC plate. Conversely, if you know the structures of the compounds in a mixture, you can predict that a compound of low polarity will have a larger R_f value than a polar compound run on the same plate.

The R_f can provide corroborative evidence as to the identity of a compound. If the identity of a compound is suspected but not yet proven, an authentic sample of the compound, or standard, is spotted and run on a TLC plate side by side (or on top of each other) with the compound in question. If two substances have the same R_f value, they are likely (but not necessarily) the same compound. If they have different R_f values, they are definitely different compounds. Note that this identity check must be performed on a single plate, because it is difficult to duplicate all the factors that influence R_f exactly from experiment to experiment.

7.5 Applications of TLC

TLC is a quick and powerful technique for checking the purity of a sample. The sample can be a product of a reaction, or perhaps it's an old bottle of a reagent that you would like to use if it is pure. The composition of fractions obtained from a column chromatography experiment is also assessed by TLC.

If a sample shows a single spot on a TLC plate, it is not *necessarily* pure. This is supportive but not conclusive evidence of purity. A sample must show a single spot in more than one solvent system to be considered pure, and even this should be upheld by other physical property data.

Another very important use of TLC is in the monitoring of the progress of a chemical reaction. The procedure is to spot a plate with a sample of the initial reaction mixture, and then compare this sample by TLC to samples taken periodically from the ongoing reaction mixture. The appearance of new spots, corresponding to products, and the disappearance of the spots corresponding to starting materials allows the observer to follow the progress of the reaction. Figure 7-4 shows an example of a reaction mixture spotted and run on a TLC plate at several points during a reaction.

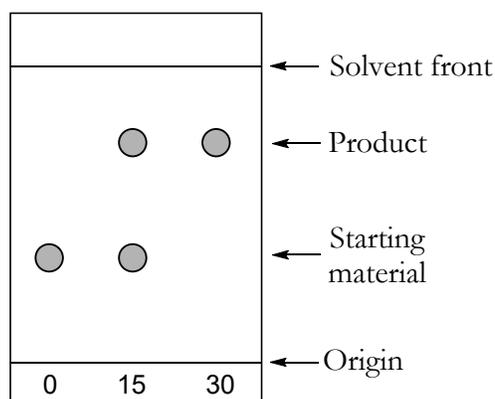


Figure 7-4: TLC of a reaction sampled at 0, 15, and 30 minutes.

7.6 Visualizing the Spots

If the compounds are colored, simple observation of the plate after the plate has been developed is all that is required. However, most organic compounds are not colored and one of many visualization methods is employed.

The most common method used in the organic chemistry teaching labs is that of observing the plates under UV light. Many aromatic compounds will show a dark blue spot under a UV lamp. The TLC plates most commonly used in the teaching labs are labeled “silica gel 60 F₂₅₄, precoated TLC aluminum sheets for TLC, layer thickness 0.2 mm.” The F₂₅₄ stands for the zinc sulfide fluorescer that is added to the silica gel adsorbent. When viewed under UV light of wavelength 254 nm, the zinc sulfide will fluoresce green, *except* where there is an organic compound that absorbs light at 254 nm. The compound serves to quench the fluorescence and a dark spot is seen on the plate.

Plates can also be visualized by staining with a chemical. Iodine will cause most organic compounds on a TLC plate to turn dark, as does treatment with sulfuric acid followed by heating. Ninhydrin causes amino acids and amines to turn blue, while 2,4-dinitrophenylhydrazine causes aldehydes and ketones to turn red or yellow. Stains using vanillin or PAA (*p*-anisaldehyde) are useful for visualizing many different functional groups. The two stains used most commonly in the undergraduate organic labs are iodine and PAA.

When staining a plate with iodine, it is possible to simply place the plate in a jar with some iodine crystals in the bottom and allow the iodine vapors to react with the plate. However, this takes some time and can lead to uneven staining. Instead, the organic labs at CU use a mixture of silica and iodine. The plate is placed in a jar of this mixture, and after a few minutes the compounds on the plate should react with the iodine and turn dark. This is a reversible reaction, so afterwards the plate will slowly return to its previous appearance while releasing iodine. For this reason, it is important to keep iodine-stained plates in the fume hood, where the iodine vapors will not cause irritation.

Staining with PAA involves placing the TLC plate on a wire rack in a spraying box (to prevent the benchtop from becoming coated with stain) and then spraying the plate with a solution of *p*-anisaldehyde, sulfuric acid, acetic acid, and ethanol. The plate is then placed on a foil-wrapped hotplate set to roughly 100 degrees. As the stain develops, it will turn a light pink, while the spots will turn different colors depending on which functional groups are present. The TLC plate is now considered solid chemical waste, so it too should be kept in the fume hood.

7.7 Procedure for TLC

1. Prepare the developing chamber.

The developing chamber can be a specially designed chamber, a jar with a lid, or a beaker with a watch glass on the top. In the CU teaching labs, a beaker with a watch glass is typically used (Figure 7-5).

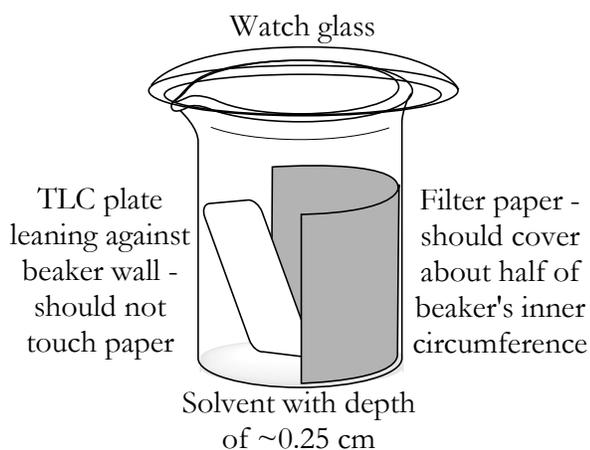


Figure 7-5: A TLC developing chamber in use.

It is important for the atmosphere in the developing container to be saturated with the vapors of the eluting solvent. This prevents the plate from drying out during the run. To aid in this saturation, line part of the inside of the beaker with filter paper, which will serve as a wick to saturate the atmosphere in the jar.

Fill the beaker with solvent to a depth of roughly 0.25 cm. Cover the beaker with a watch glass, swirl it gently, and allow it to stand while you prepare your TLC plate. If the solvent volume drops too much due to the solvent being absorbed into the paper, you may have to top it up a little before developing your plate.

2. Prepare the TLC plate.

Now you are ready to prepare your TLC plates. The plates you will use for TLC are cut from a larger plate, which is normally 5 cm wide by 20 cm long. For your TLCs to run properly, you should cut plates that are 5 cm tall, not 5 cm wide (Figure 7-6). This is because the larger plates tend to have significant issues with solvent evaporation and give inaccurate results, and because they use up the expensive TLC plates faster.

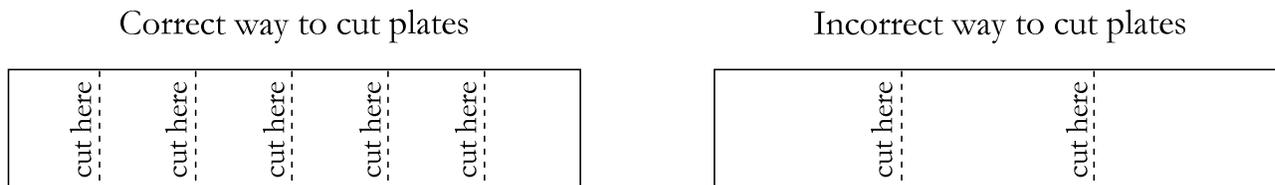


Figure 7-6: The correct way and the incorrect way to cut TLC plates.

You should ensure that the edges of your plate are as smooth as possible, so that the solvent flows evenly up the plate. If the silica along the edge is rough or ragged due to being flaked off from the backing, you might get some sideways movement of spots, making it hard to tell which of your spots came from which TLC sample (Figure 7-7).

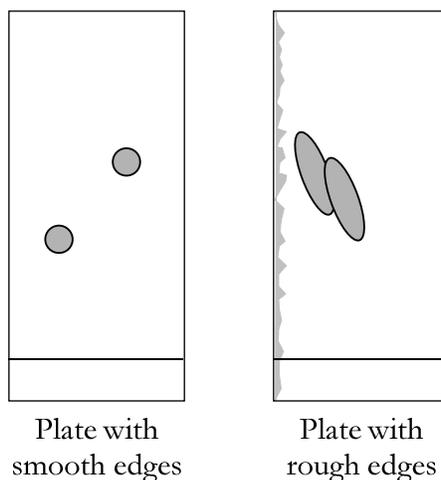


Figure 7-7: If a TLC with rough edges is used, the spots will be distorted during development.

To avoid this, trim the edges of the plate to ensure they are smooth (Figure 7-8). Always handle the plate carefully so you do not dirty the silica coating or flake off pieces along the edges.

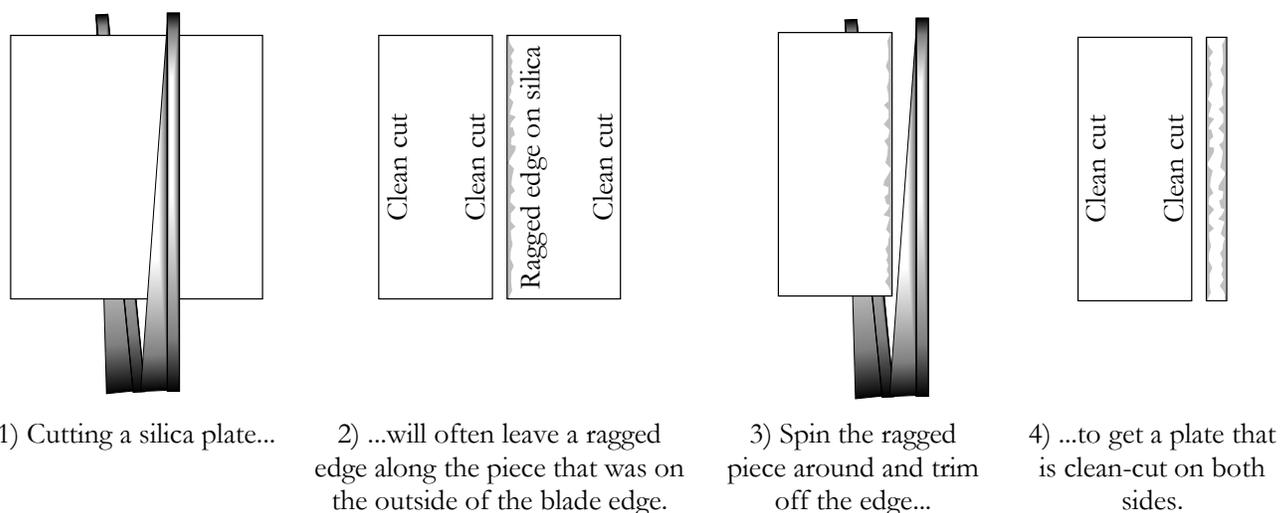


Figure 7-8: Trimming a TLC plate so that both edges are smooth.

Using a pencil, mark the origin line about 0.5 cm from the bottom of the plate (Figure 7-9). Use a ruler or the edge of a piece of paper to make sure you get a straight line; otherwise the origin will not be uniform and your calculation of R_f may be inaccurate. **Be extremely careful not to write so hard that you scratch off the silica.**

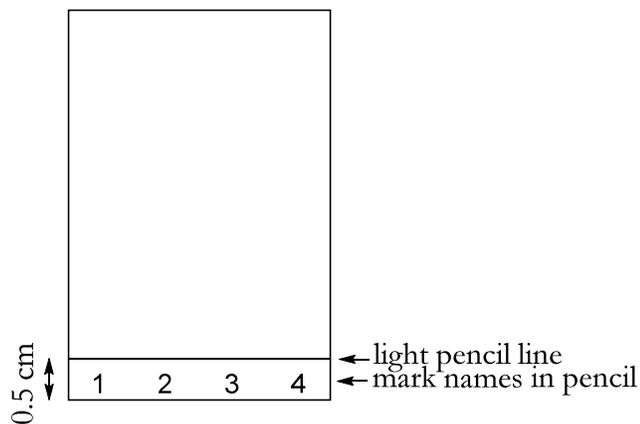


Figure 7-9: Label a TLC plate with the origin and sample names.

If you are running multiple samples on the same plate you can also write their names in pencil below the origin. Note that a pen should never be used to mark TLC plates, because the ink is pushed up the plate by the solvent. Pencil graphite, on the other hand, is made up of insoluble particles that do not move and will not overlap with your sample spots.

3. Spot the TLC plate.

Now you can make up a spotting solution of the compound you wish to develop. In a vial or other small container, add some of your compound to a volatile solvent in which your compound is soluble. As a rule of thumb, about 10 mg of a compound in a 1 mL of solvent will result in the proper size spot. It is important not to spot too much or too little sample on a TLC plate. Always use a volatile solvent such as hexanes, ethyl acetate, or dichloromethane to prepare the solutions of compounds for spotting. It is important that the solvent used to spot the plate can evaporate quickly so that it does not interfere with the TLC process. If you are spotting multiple samples on the same plate, make up a spotting vial for each one.

You are now ready to spot the TLC plate. Obtain a TLC microcap (open at both ends) and dip it into the solution of the sample to be spotted. Then, touch the microcap **briefly** to the TLC plate on the pencil line above the relevant label and allow some of the liquid to flow onto the plate. Your goal is to make a small, reasonably concentrated spot, not a large spot that overlaps with the other compounds. You can darken the spots by allowing the spotting solvent on the plate to dry (blowing on it will speed this up), and then touching the microcap to the spot a second time. If you repeat these steps, the wet area on the plate will stay small. However, don't allow the spots to become too dark – this tends to encourage “streaking”, or long, smeared-out spots with an undefined R_f . It often takes some trial and error to learn the appropriate amount of compound to spot. Be careful not to gouge a hole in the silica with the end of the microcap while you are spotting the plate.

Next, rinse the microcap so that you can use it again. Place about 1 mL of pure solvent (the same one you used to dissolve your compounds) into another vial. Place the tip of the microcap into the vial of pure solvent until it fills, then touch it to a Kimwipe until it empties. Repeat this a couple times. Now you have a clean microcap ready for reuse in spotting your next sample. Continue this process of spotting and cleaning the microcap until all of your samples are spotted onto the plate.

If your spots are UV-active, you can pre-check your plate by placing it under a UV lamp. Pre-checking your plate will not tell you what the R_f values of the compounds are, but it will allow you catch any spotting mistakes before you go to the effort of developing the plate. Your undeveloped spots should always be small, moderately dark, and centered on the origin baseline as shown by the two good spots in Figure 7-10. (This plate is turned sideways to make the text easier to read, but you should still be placing your spots in a horizontal line along the origin.) Pre-checking is not possible for the other visualization methods (iodine, staining, etc.) because they chemically alter or break down the compounds, so developing the plate afterwards would be pointless.

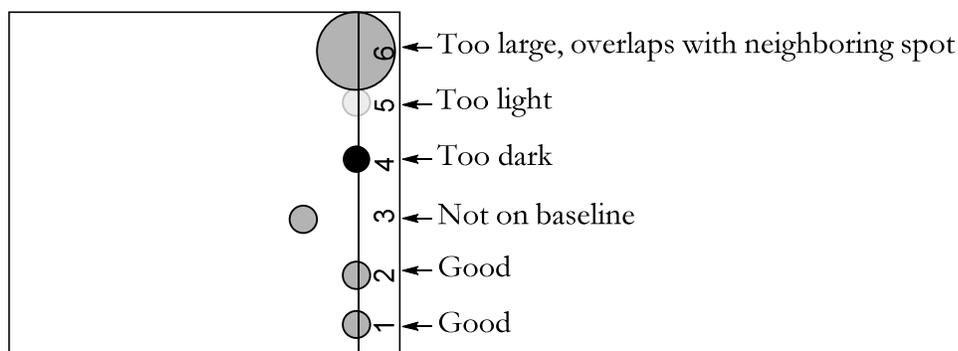


Figure 7-10: An undeveloped plate with some good spots and some bad spots.

If the microcap breaks or clogs, you may obtain a new one. The microcaps should be cleaned and reused whenever possible, both because this is an environmentally sound practice and because they are relatively expensive.

4. Develop the plate.

Using a pair of forceps, gently place the TLC plate in the developing container. Make sure that the solvent does not rise above the level of the origin, or the spots will dissolve off the plate into the solvent. Make sure the sides of the TLC plate are not touching the filter paper or the wall of the chamber, since this will cause the spots to be distorted. Cover the beaker with the watch glass and leave it undisturbed for a few minutes.

The solvent will rise up the TLC plate by capillary action. When the solvent front is almost to the top of the plate, carefully remove the plate from the beaker and quickly mark a line across the plate at the solvent front with a pencil. Allow the solvent to evaporate completely from the plate. Mark the solvent front quickly – it evaporates fast!

5. Visualize the spots.

If you are using a UV lamp for visualization (the easiest method), hold it over the plate and circle any spots you see with a pencil.

If the experiment calls for a different visualization method, you will be given more detailed instructions in the experimental procedure.

7.8 Common Problems Encountered in TLC

1. The compound runs as a long streak rather than a spot.

The sample was overloaded. Run the TLC again after diluting your sample. Or perhaps your sample might just contain many components, creating many spots that run together and appear as a streak. In this case, try running your sample in a different solvent.

2. The sample runs as a smear or an upward crescent.

Compounds that possess strongly acidic or basic groups (amines or carboxylic acids) sometimes show up on a TLC plate with this behavior. Add a few drops of ammonium hydroxide (if your compound amines) or acetic acid (carboxylic acids) to the eluting solvent to obtain clearer plates.

3. The compound runs as a downward crescent.

Likely, the adsorbent was damaged slightly by the tip of the microcap during the spotting, causing the crescent shape.

4. The compounds do not travel directly upwards but move to one side.

Something is causing the solvent to move faster along one side of the plate than the other. This might be due to a rough edge on the silica, or it might be because one edge of the plate is resting against the filter paper or the wall of the chamber.

5. No spots are seen on the plate.

You might not have spotted enough compound, perhaps because the solution of the compound is too dilute. Try concentrating the solution, or spot it several times in one place, allowing the solvent to dry between applications. Some compounds do not show up under UV light; try another method of visualizing the plate. Perhaps you do not have any compound because your experiment did not go as well as planned.

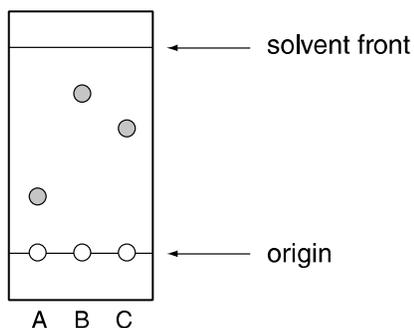
If the solvent level in the developing jar is deeper than the origin (spotting line) of the TLC plate, the solvent will dissolve the compounds into the solvent reservoir instead of allowing them to move up the plate by capillary action. Thus, you will not see spots after the plate is developed.

6. You see a blur of blue spots on the plate as it develops.

Perhaps you used an ink pen instead of a pencil to mark the origin?

7.9 Study Problems

1. Consider the following silica gel TLC plate of compounds A, B, and C developed in hexanes:

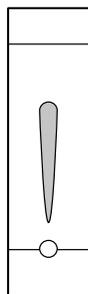


- Determine the R_f values of compounds A, B, and C.
- Which compound, A, B, or C, is the most polar?
- What would you expect to happen to the R_f values if you used acetone instead of hexanes as the eluting solvent?
- How would the R_f values change if eluted with hexanes using an alumina TLC plate?

2. You are trying to determine a TLC solvent system that will separate the compounds X, Y, and Z. You ran the compounds on a TLC plate using hexanes/ethyl acetate 95:5 as the eluting solvent and obtained the chromatogram below. How could you change the solvent system to give better separation of these three compounds?



3. After a rather lengthy organic chemistry synthesis procedure, a student ran the product of the reaction on a TLC plate and obtained the result below. What might he/she have done wrong, if anything?



4. A student spotted an unknown sample on a TLC plate. After developing in 50:50 hexanes-ethyl acetate, they saw a single spot with an R_f of 0.55. Does this indicate that the unknown material is a pure compound? What can be done to verify the purity of the sample?

5. Consider a sample that is a mixture composed of biphenyl, benzoic acid, and benzyl alcohol. The sample is spotted on a TLC plate and developed in a hexanes-ethyl acetate solvent mixture. Predict the relative R_f values for the three compounds in the sample.

6. Plate A, below, represents the TLC chromatogram of a compound run in hexanes. The same compound was then spotted on a longer TLC plate and again run in hexanes. Which TLC plate, B, C, or D, correctly represents how far the compound would run on the longer plate?

