
7. Thin-Layer Chromatography

You will need to bring your package of TLC plates on this day. Not all plates will be used in this experiment; some will be needed in lab the following week. Be sure to include all the analgesics and solvents used in this experiment in your Chemical Data Tables. Don't forget to include the Chromatographic Behavior Comparison section in this PreLab. An example can be found in section 3.2.

PreLab exercise:

Using a greatly magnified diagram of a TLC plate, locate or define each of the following terms:

PreLab Exercise

- | | |
|---------------------|---------------------------|
| 1. Origin | 2. Stationary Phase |
| 3. Mobile Phase | 4. Solvent Front |
| 5. Component "spot" | 6. R_f value |
| 7. Development | 8. Visualization of spots |
9. What is meant by differential partitioning between stationary and mobile phase?
10. What is the recommended procedure for cleaning a TLC spotting capillary?

Introduction

Chromatography is the separation of two or more compounds or ions by the distribution between two phases, one which is moving and the other which is stationary. These two phases can be solid-liquid, liquid-liquid or gas-liquid. Although there are many different variations of chromatography, the principles are essentially the same. If you took Chem 15 at University Park, you may remember using paper chromatography to separate inks and food dyes. The cellulose paper was the stationary or solid phase and the 1-propanol/water mixture was the mobile or liquid phase. This chapter explores a very similar microscale technique for separating organic molecules, thin-layer chromatography.

Definition of
Chromatography

Thin-layer chromatography or TLC, is a solid-liquid form of chromatography where the stationary phase is normally a polar absorbent and the mobile phase can be a single solvent or combination of solvents. TLC is a quick, inexpensive microscale technique that can be used to:

- determine the number of components in a mixture
- verify a substance's identity
- monitor the progress of a reaction
- determine appropriate conditions for column chromatography
- analyze the fractions obtained from column chromatography

In paper chromatography, the stationary phase is a specially manufactured porous paper. The samples are added to one end of the sheet of paper and dipped into the liquid or mobile phase. The solvent is drawn through the paper by capillary action and the molecules are distributed by partition between the mobile and stationary phase. The partition coefficient, k , similar to the distribution coefficient for extraction, is the equilibrium constant for the distribution of molecules between the mobile phase and the stationary phase. It is this equilibrium that separates the components. Different inks and dyes, depending on their molecular structures and interactions with the paper and mobile phase, will adhere to the paper more or less than the other compounds allowing a quick and efficient separation.

Paper
Chromatography

TLC works on the same principles. In thin-layer chromatography, the stationary phase is a polar absorbent, usually finely ground alumina or silica particles. This absorbent is coated on a glass slide or plastic sheet creating a thin layer of the particular stationary phase. Almost all mixtures of solvents can be used as the mobile phase. By manipulating the mobile phase, organic compounds can be separated.

Theory

To thoroughly understand the process of TLC, as well as all types of chromatography, we must travel to the molecular level. All forms of chromatography involve a dynamic and rapid equilibrium of molecules between the two phases. As shown in Figure 7.1, there are:

1. **free** - completely dissolved in the liquid or gaseous mobile phase and
2. **absorbed** - stuck on the surface of the solid stationary phase.

Molecular Level

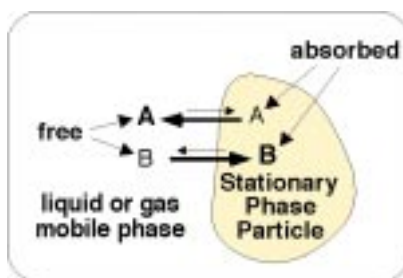


Figure 7.1: Mixture of A & B free in mobile phase and absorbed on the stationary phase.

Molecules are continuously moving back and forth between the free and absorbed states with millions of molecules absorbing and millions of other molecules desorbing each second. The equilibrium between the free and absorbed states depends on three factors:

- the polarity and size of the molecule
- the polarity of the stationary phase
- the polarity of the solvent

Thus, one has three different variables to change in chromatography. The polarity of the molecules is determined by their structures. By selecting different stationary and mobile phases, one can change the equilibrium between the free and absorbed states. It is important to understand chromatography at this molecular level because this allows one to choose mobile and stationary phases that will separate just about any mixture of molecules.

Partitioning

Different molecules partition differently between the free and absorbed state, that is the equilibria between these two states is not the same. In Fig. 7.2 below, molecule A is weakly absorbed, its equilibrium lies in the direction of the free state and there is a higher concentration in the mobile phase. Molecule B, on the other hand, is strongly absorbed, its equilibrium lies in the direction of the absorbed state, and has a higher concentration on the stationary phase.

Equilibrium

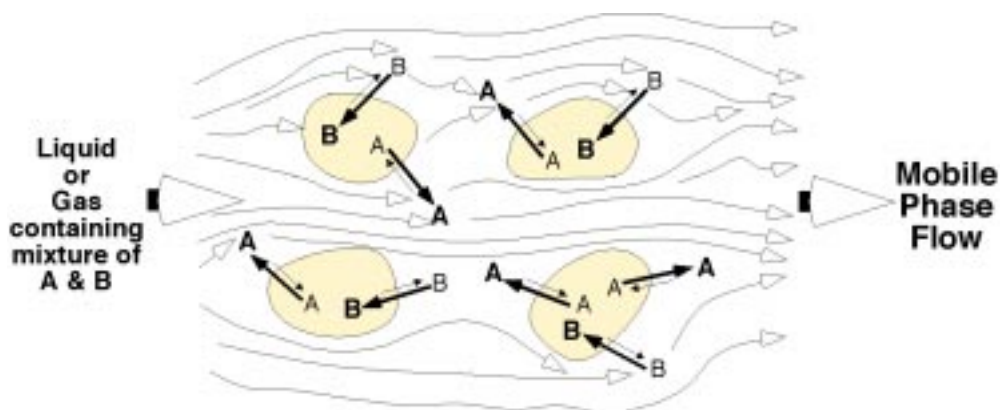
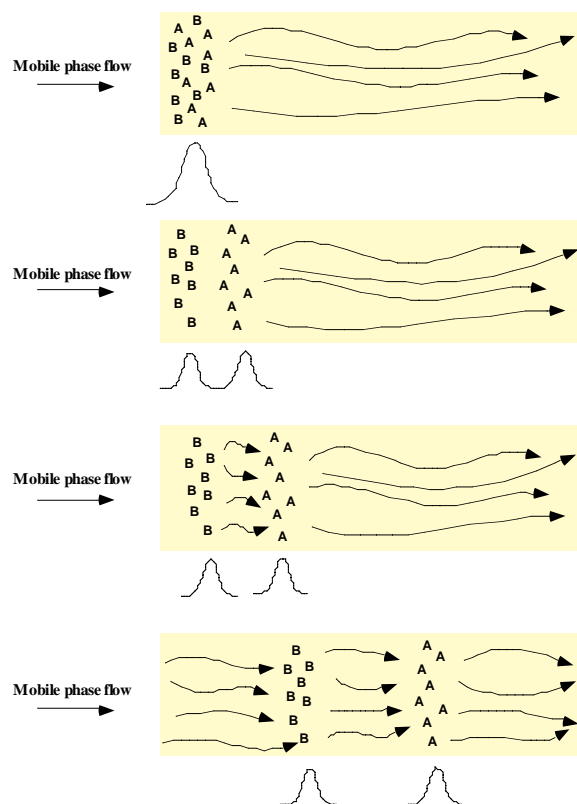


Figure 7.2: Dynamic equilibrium between A & B and the mobile and stationary phase.

Simply adding your mixture to a combination of a mobile phase and a stationary phase will not separate it into its pure molecular components. For this to happen, the mobile phase must flow past the stationary phase as depicted in Fig. 7.2. Since the A molecules spend more time in the mobile phase, they will be carried through the stationary phase faster and move farther in a given amount of time. Since B is absorbed to the stationary phase more than A, B molecules spend less time in the mobile phase and therefore move through the stationary phase particles more slowly. The B molecules don't move as far in the same amount of time.

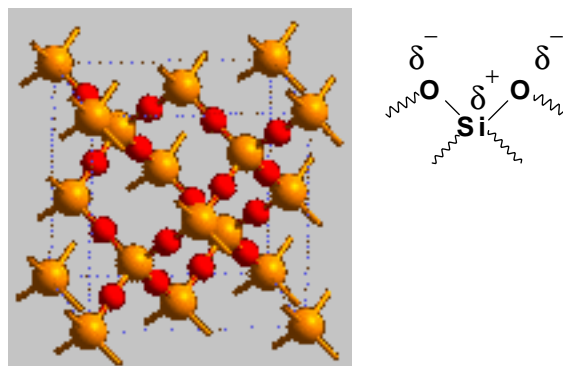
The consequences of this flowing mobile phase is that A is gradually separated from B by moving ahead in the flow. This separation process is depicted in Figure 7.3.



The separation of a mixture of molecules A & B.

Figure 7.3: Mixture of A & B separated by a moving mobile phase while being absorbed on the stationary phase.

In TLC, the stationary phase is typically alumina ($\text{Al}_2\text{O}_3 \cdot x\text{H}_2\text{O}$)_n or silica gel ($\text{SiO}_2 \cdot x\text{H}_2\text{O}$)_n. The covalent network of these absorbents create very polar materials. The structure of silica is shown below.



Silica

Figure 7.4: Structure of Silica ($\text{SiO}_2 \cdot x\text{H}_2\text{O}$)_n.

The electropositive character of the aluminum or silicon and the electronegative oxygen create a very polar stationary phase. Therefore, the more polar the molecule to be separated, the stronger the attractive force to the stationary phase. In some sense, the old adage “like-dissolves-like” can be applied here. The polar stationary phase will more strongly attract like or polar molecules. The equilibrium will be shifted as the molecules remain on the stationary phase. Nonpolar molecules will have a lower affinity for the stationary phase and will remain in the solvent longer. This is essentially how the partitioning separates the molecules. The equilibrium governs the separation, but the component’s attraction to the stationary phase versus the mobile phase determines the equilibrium. In general, the more polar the functional group, the stronger the bond to the stationary phase and the more slowly the molecules will move. In an extreme situation, the molecules will not move at all. This problem can be overcome by increasing the polarity of the mobile phase so that the equilibrium between the free and absorbed state is shifted towards the free.

Although alumina and silica are the most common stationary phases used for TLC, there are many different types. They range from paper to charcoal, nonpolar to polar, and reverse phase to normal phase. Several different types of stationary phases are listed according to polarity in Figure 7.5.

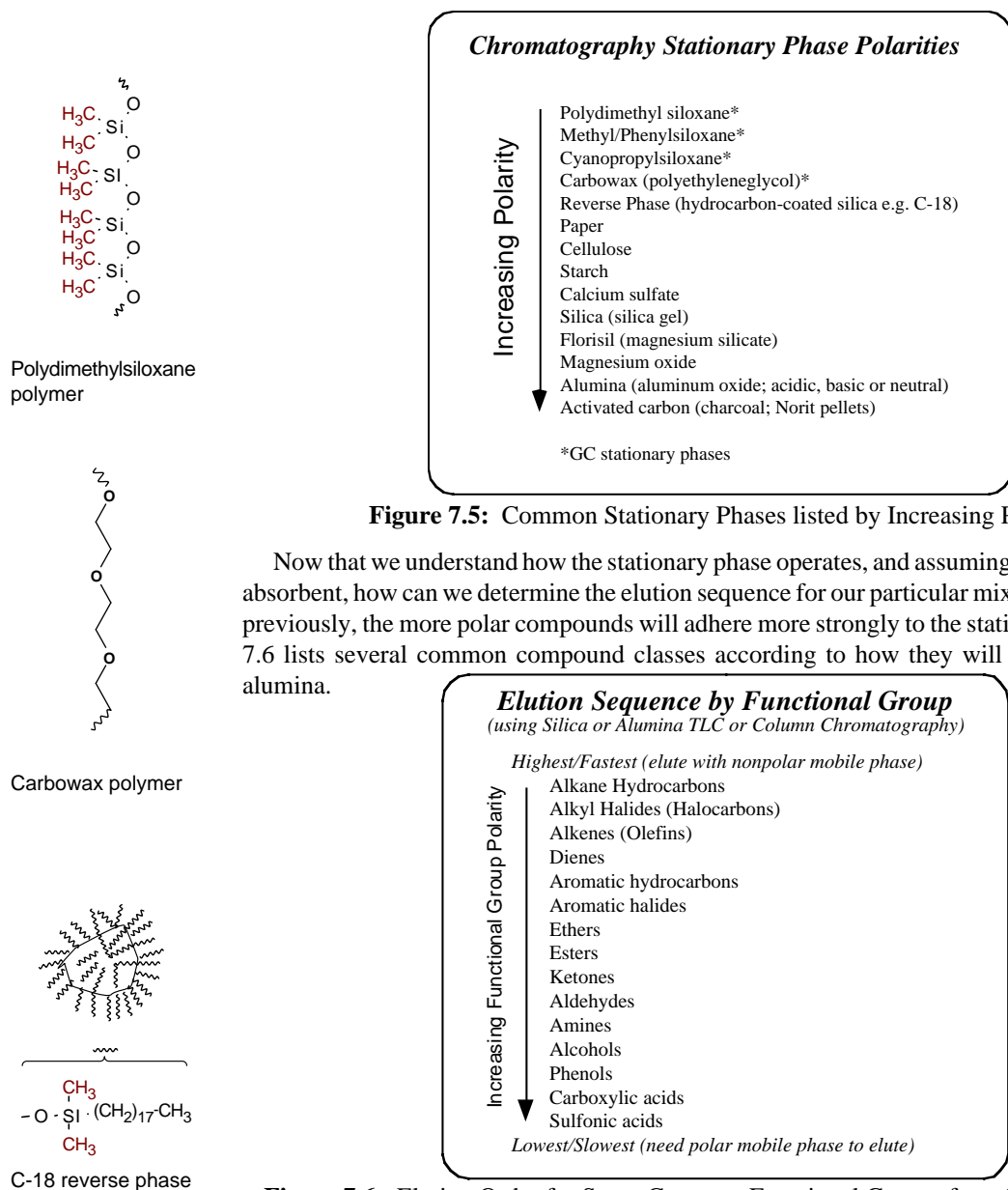


Figure 7.5: Common Stationary Phases listed by Increasing Polarity.

Now that we understand how the stationary phase operates, and assuming we are using a polar absorbent, how can we determine the elution sequence for our particular mixture? As mentioned previously, the more polar compounds will adhere more strongly to the stationary phase. Figure 7.6 lists several common compound classes according to how they will elute from silica or alumina.

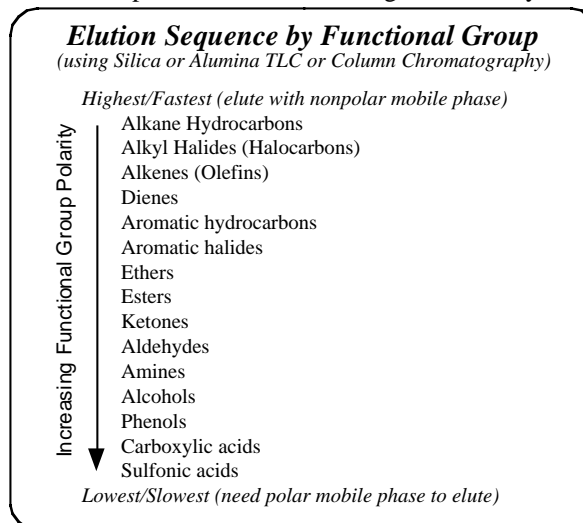
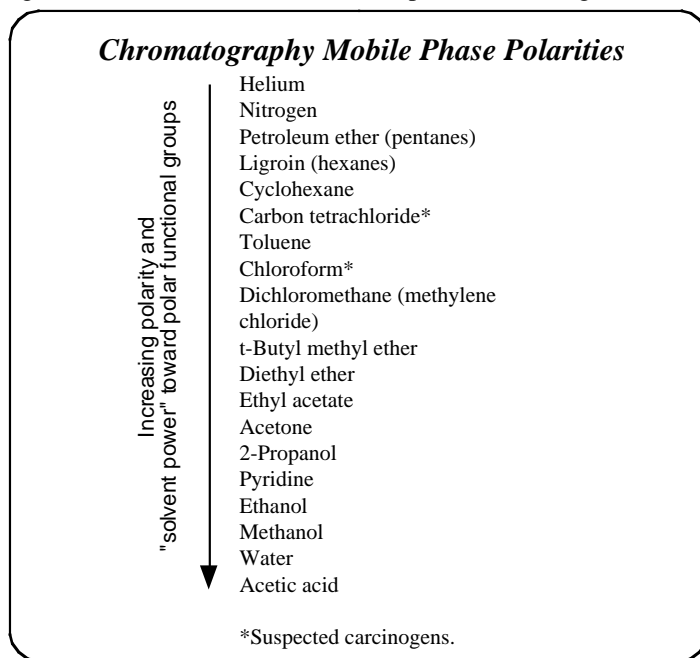


Figure 7.6: Elution Order for Some Common Functional Groups from Silica or Alumina.

You may be able to see a trend developing. In general, the more nonpolar the compound, the faster it will elute (or the less time it will remain on the stationary phase) and the more polar the compound the slower it will elute (or more time on the stationary phase). It is important to become familiar with the trend so that you will not have to rely on the chart. You should be able to recognize the functional groups and easily determine which one is more polar than another. However, it should be noted that chromatography is not an exact science. This chart should be used to help predict the order of elution, but only performing the experiment will give clear answers.

General Trend

As mentioned earlier, the mobile phase polarity can also be varied to effect the chromatographic separation. Figure 7.7 lists some common mobile phases according to increasing polarity.



Solvents

Figure 7.7: Common Mobile Phases listed by Increasing Polarity.

Finding a good solvent system is usually the most difficult part of TLC. If the mobile phase has not been previously determined, start with a nonpolar solvent such as ligroin and observe the separation. If the mixture's components do not move very far, try adding a polar solvent such as ether or ethyl acetate to the ligroin. Compare the separation to the previous plate. In most cases a combination of these two solvents is the best solution. If the spots stay at the bottom of the plate, add more of the polar solvent. If they run with the solvent front (go to the top too fast) then add a more nonpolar solvent. Unfortunately, rules are not full proof and some trial and error is involved in determining which solvent system is the best.

Spotting the TLC Plate

One advantage TLC has over other separation methods, is that it is truly a microscale technique. Only a few micrograms of material in solution is necessary to observe the solute on a TLC plate. Dissolve a few milligrams of material in a volatile solvent creating a dilute solution. Choose a volatile solvent that completely dissolves the sample. However, if it is partially soluble, since such only low concentrations are needed, normally you will be able to observe the compound.

TLC is truly a microscale technique.

Once the sample is prepared, a spotting capillary must be used to add the sample to the plate. The spotting capillaries must be extremely small. In fact, the opening at the end of a regular Pasteur pipet is too big for spotting a TLC plate. CG capillary columns donated by Restek, a chromatography company located in Bellefonte, are used to spot the plates. These columns are very small bore and can be cut into three inch sections to provide very good TLC spotting tubes. The solution can be drawn up the tube by capillary action (hence the name) and spotted on the plate

at the hash mark labeled in pencil. This is known as the origin and is shown in Figure 7.8. Since a TLC plate can run three, if not four mixtures at one time, it is very important to properly label the plate. Notice that pencil is always used to mark a TLC plate since the graphite carbon is inert. If organic ink is used to mark the plate, it will chromatograph just as any other organic compound and give incorrect results.

TLC plate

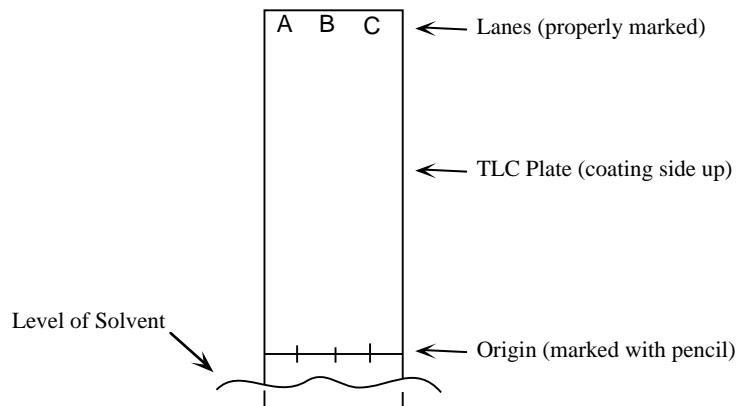


Figure 7.8: TLC Plate ready to be spotted.

To spot the plate, simply touch the end of the capillary tube to the *coated* side of the plate. The solvent should evaporate quickly leaving your mixture behind on the plate. You may have to spot the plate a couple of times to ensure the material is present, but do not spot too much sample. If too much solute is added to the plate, a poor separation will result. Smearing, smudging and spots that overlap will result making identification of separated components difficult.

Development

Once the dilute solution of the mixture has been spotted on the plate, the next step is the development. Just like paper chromatography, the solvent must be in contact with the stationary phase. Figure 7.9 shows a wide-mouth bottle commonly used to develop TLC plates.

Wide-mouth bottle
used for a
development
chamber.



Figure 7.9: Development Chamber.

The bottle is filled with a small amount of the mobile phase and capped with a cork. In addition, a piece of filter paper is put in the bottle to help create an atmosphere saturated with solvent. Use your tweezers to place the plate in the development chamber; oils from your fingers can sometimes smear or ruin a TLC plate. Also make sure the origin spots are not below the solvent level in the chamber. If the spots are submerged in the solvent, they are washed off the plate and lost. Once the solvent has run within a centimeter of the top of the plate, remove it with tweezers. Using a pencil, immediately draw a line across the plate where the solvent front can be seen. The proper location of this solvent front line will be important for later calculations.

Never use ink to label TLC plates.

Visualization

Some organic compounds are colored. If you are fortunate enough to be separating organic molecules that are colored such as dyes, inks or indicators, then visualizing the separated spots is easy. However, since most organic compounds are colorless, this first method does not always work.

Always use visual clues.

In most cases observing the separated spots by UV light works well. TLC plates normally contain a fluorescent indicator which makes the TLC plate glow green under UV light of wavelength 254 nm. Compounds that absorb UV light will quench the green fluorescence yielding dark purple or bluish spots on the plate. Simply put the plate under a UV lamp, and the compounds become visible to the naked eye. Lightly circle the spots, so that you will have a permanent record of their location for later calculations..

UV light

Figure 7.10: UV Lamp used to visualize spots.

Another useful visualizing technique is an iodine (I₂) chamber. Iodine sublimates and will absorb to organic molecules in the vapor phase. The organic spots on the plate will turn brown and can be easily identified. Also circle these observed spots, since the color stain will eventually fade from the plate. Sometimes, a combination of both a UV lamp and iodine is needed to observe all the spots. Some compounds are not “UV active”, that is, they do not absorb light at the wavelength of 254 nm. Using both methods will ensure correct identification of all the spots on the TLC plate.

Iodine

R_f Values

In addition to qualitative results, TLC can also provide a chromatographic measurement known as an R_f value. The R_f value is the “retardation factor” or the “ratio-to-front” value expressed as a decimal fraction.

The R_f value can be calculated as:

$$R_f = \frac{\text{distance spot travels}}{\text{distance solvent travels}}$$

This number can be calculated for each spot observed on a TLC plate. Essentially it describes the distance traveled by the individual components. If two spots travel the same distance or have the same R_f value then it might be concluded that the two components are the same molecule. For R_f value comparisons to be valid; however, TLC plates must be run under the same exact conditions. These conditions include the stationary phase, mobile phase, and temperature. Just as many organic molecules have the same melting point and color, many can have the same R_f value, so identical R_f values doesn't necessarily mean identical compounds. Additional information must be obtained before this conclusion can be made. It is important to restate that this number is only significant when the same chromatographic conditions are used. Figure 7.11 shows a diagram of a typical TLC plate and how the distances are measured to calculate the R_f value.

R_f calculation

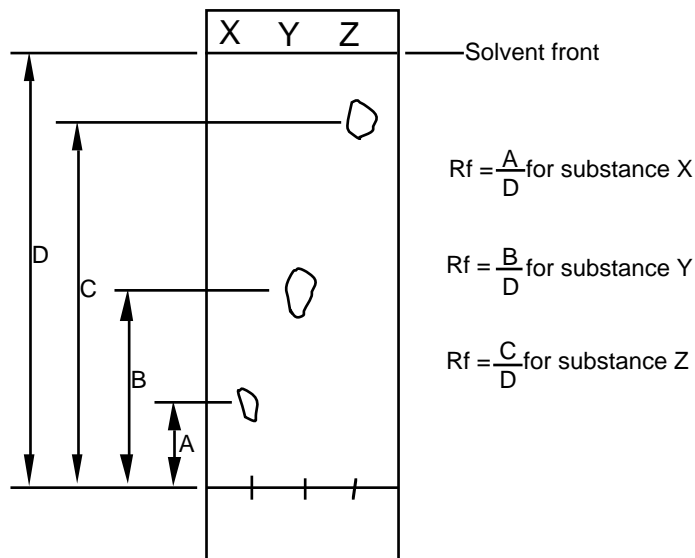


Figure 7.11: Calculation of R_f value.

Preparative Plates

TLC can be used on a microscale to monitor a reaction and determine if the product or products were successfully produced using only microgram quantities of materials. It is difficult to separate gram quantities using TLC and therefore column chromatography is used at this scale (see Chapter 8). However, larger TLC plates, called a Preparative Plates, can be used for separations of milligram quantities of materials because they are coated with thick layers (1-3 mm) of stationary phase. Once the plate is developed, the spots are scraped off the plate along with the absorbent. Each separate component is then extracted from the stationary phase with a polar solvent.

The polarity arguments discussed in this chapter can be applied to many different types of chromatography including: Column Chromatography (Chapter 8), Gas Chromatography (Chapter 11), and High Performance Liquid Chromatography (HPLC). You will be applying these principles to column chromatography in the next chapter.

TLC Experiment

Analgesics are substances that relieve pain. The most common of these is aspirin, a component of more than 100 nonprescription drugs. In the present experiment analgesic tablets will be analyzed by thin-layer chromatography to determine which analgesics they contain and whether they contain caffeine, which is often added to counteract the sedative effects of the analgesic.

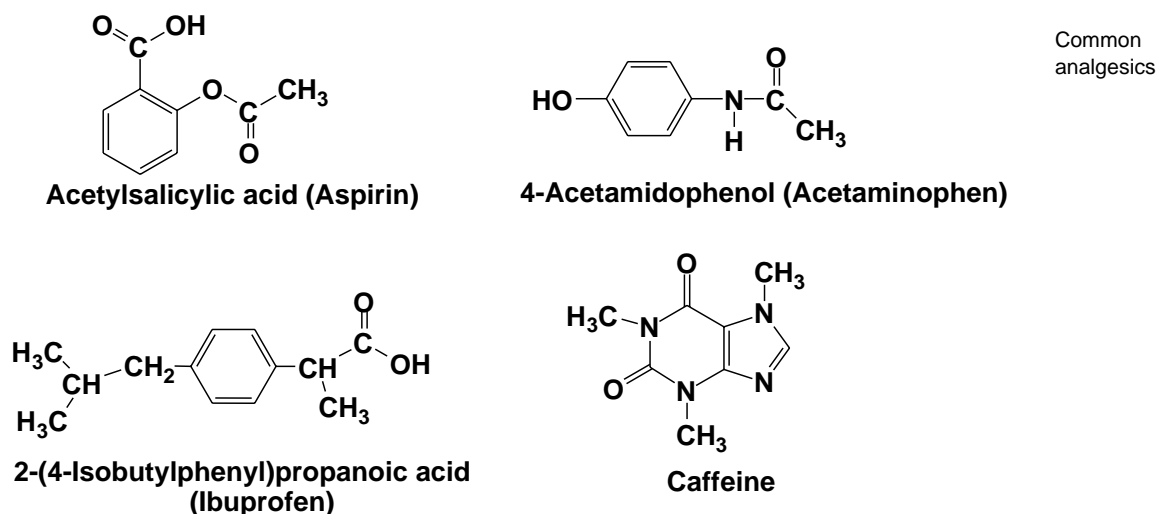


Figure 7.12: Some common ingredients in commercially available painkillers.

In addition to aspirin and caffeine the most common components of analgesics are, at present, acetaminophen and ibuprofen (Motrin). Phenacetin, the P of the APC tablets and a former component of Empirin, has been removed from the market because of deleterious side effects. In addition to one or more of these substances each tablet contains a binder, often starch or silica gel. To counteract the acidic properties of aspirin, an inorganic buffering agent is added to some analgesics. Inspection of labels will reveal that most cold remedies and decongestants contain both aspirin and caffeine in addition to the primary ingredient.

To identify an unknown by TLC, the usual strategy is to run chromatograms of known substances (the standards) that are likely to be present in the unknown and the unknown at the same time. If the unknown has one or more spots that correspond to spots with the same R_f 's as the standards, then those substances are probably present.

Proprietary drugs that contain one or more of the common analgesics and sometimes caffeine are sold under the names of Bayer Aspirin, Anacin, Datril, Advil, Excedrin, Extra Strength Excedrin, Tylenol, and Vanquish.

Standards

Procedure 1•

TLC Separation of Analgesics

Choose one of the analgesics and prepare a sample by crushing 1/4 of a tablet, adding this powder to a reaction tube or small vial along with a few drops of ethanol, and then mixing the suspension. Not all of the tablet will dissolve, but enough will go into solution to spot the plate. The binder, starch or silica, will not dissolve.

From the side shelf, acquire just a few drops of the 1% or 2% solutions of the four standard analgesics: *aspirin*, *acetaminophen*, *ibuprofen*, and *caffeine* in shorty vials or corked small test tubes, properly labeled. Work with the three other students on your side of the bench so that each of you get one vial of a standard and share it with the others.

Using a lead pencil (not a pen) and a ruler, mark two plates as shown below. First, draw a light pencil line across the plate about 1 cm from the bottom of two TLC plates. Make three equally spaced vertical dashes on this line. Then label the lanes at the top of the plate.

Problems
Properly labeled
TLC plates.

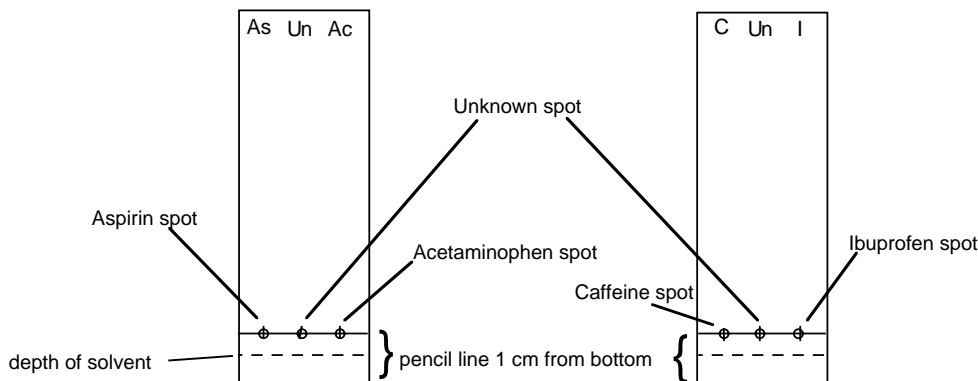


Figure 7.13: An example of a properly spotted TLC plate.

Using separate spotting capillary tubes from your desk, practice spotting on a paper towel using a pure solvent such as dichloromethane. After filling the capillary by dipping it in the liquid, touch it quickly to the towel so that the spot is no larger than 1 to 2 mm diameter. (The smaller the spot, the better the final TLC analysis.) After the solvent evaporates, you can apply more material in the same spot by, again, quickly touching the surface at the same place.

Now spot each of the plates on the middle vertical dash with your unknown tablet extract and on the outside with two standards as shown. Make each spot as small as possible, preferably less than 2 mm in diameter. Examine the plate under the UV light to see that enough of the compound has been applied by observing a visible dark purple dot; if not visible, spot more.

Develop your spotted TLC plates in one of the 250-mL wide-mouth bottles found in your desk. Make sure it is clean and dry. Use a large piece of filter paper in it to saturate the atmosphere with solvent vapor. Pour the 50:50 hexane:ethyl acetate developing solvent into the bottle to a depth of about 1/2 cm. Using forceps, carefully place the two TLC plates into the bottles so that they are leaning against the wall of the bottle, but not touching each other. Be sure that the spots are not below the solvent level or they will wash away into the solvent.

After the solvent has risen to the top of the plate or at least within a cm of the top, remove the plate with forceps and immediately mark the solvent front with a pencil. Allow the solvent to completely evaporate. Examine the plate under the UV lamp to see the components as dark spots against a bright orange or green-blue background. Outline the spots with a pencil. The spots can also be visualized by putting the plate in an iodine chamber which can be found on the side shelf. After a few minutes sitting inside the closed bottle, compound spots turn brown. Mark your spots with a pencil even after development in iodine vapor because the iodine color fades with time. Calculate the R_f value for the spots and identify the components in the unknown as shown in Figure 7.11.

Problems

NOTE: If the higher R_f compounds run into the broad band of “grunge” at the top of the plate, use 60:40 hexane:ethyl acetate. Don’t be disappointed if your TLC analysis doesn’t come out perfect the first time and you have to repeat the procedure. This is common. With practice, good results can be easily obtained.

Tape the properly labeled TLC plates in your notebook using the wide sticky tape available on the side shelf. Cover the whole plate with tape.

Cleaning up. Solvents should be placed in the appropriate organics waste container in your hood. Return unused iodine to the side-shelf supply bottle. **The spotting capillaries are not disposable!!** They may be cleaned by dipping the ends into acetone and blotting the ends with a paper towel. The acetone will move by capillary action (pardon the pun), carrying the residue of solute(s) with it. Gently blow a stream of nitrogen through the capillary to ensure complete drying. Store them safely in the test tube labeled for spotting capillary storage in your drawer for use in other experiments.

Cleaning Up

Final Report

In your *RESULTS AND DISCUSSION* section, explicitly show how you calculated the R_f values, give the commercial name of the painkiller you chose, and identify its ingredients. Discuss how observed R_f 's corresponded to predicted R_f 's.

PostLab Questions:

- 1) Arrange the following in order of increasing R_f on thin-layer chromatography: octanoic acid, butyraldehyde, 2-octanone, decane, and 1-pentanol.
- 2) Why is it necessary to run TLC in a closed container and have the interior saturated with the solvent vapor?
- 3) What will be the result of applying too much compound to a TLC plate?

InLab and PostLab Sections - Final Report:

Accuracy and completeness of <i>OBSERVATION /DATA</i> section	16	
<i>RESULTS /DISCUSSION</i> - Overall organization, readability, completeness	16	
Calculation of R_f 's for standards and unknown components	16	
Correct identity of the components of your chosen commercial "painkiller"	24	
Discussion of how observed R_f 's corresponded to predicted R_f 's.	16	
PostLab Questions	12	
<i>Total for Final Report</i>	100	
