

Thin Layer and Column Chromatography

Chromatography is a widely used chemical separation method that takes advantage of different affinities of compounds to a fixed or stationary phase and a mobile phase. There are many different types of chromatography; Thin Layer Chromatography (TLC), Column Chromatography, Gas Chromatography (GC), High Pressure Liquid Chromatography (HPLC), Gel Permeation Chromatography (GPC), and Flash Vacuum Chromatography (FVC) to name a few. In today's lab TLC and Column will be carried out. GC will be conducted later this semester. The fundamental separation principal behind each of these techniques is the same.

In all types of chromatography, there are two phases, mobile and stationary. The stationary phase remains fixed in place and the mobile phase moves through the stationary phase. The sample partitions itself between these two phases. If the compound has a greater affinity for the mobile phase, it will move along faster. If the sample has a greater affinity for the stationary phase it will spend more of its time on the stationary phase and move slower. If the sample is a mixture of compounds and the stationary and mobile phases are well-chosen, the various components of the mixture will have different affinities and move at different rates. This will separate the compounds. These compounds can be collected (preparative) or simply analyzed and identified. Column Chromatography is a preparative technique, TLC is not normally used in a preparative manner.

PRE-EXPERIMENT ASSIGNMENT

Study this chapter of the manual and the lab notes on the Chemistry Department web site. Do the first parts of your notebook writeup.

A student who has prepared for the Thin Layer and Column Chromatography experiments should be able to:

1. Define and explain: chromatography, column chromatography, thin layer chromatography (TLC), stationary phase, partition, mobile phase, elution, and R_f value.
2. Explain the samples and situations for which column and thin layer chromatography are useful. For example, thin layer chromatography can be used to identify components of a mixture (by comparing R_f values), monitor the progress of a reaction, check the purity of a sample, and (rarely) separate and purify compounds in very small quantities. What about column?
3. Draw, and label a drawing of, the apparatus used for thin layer and column chromatography experiments, and give the function of the components.

4. Draw the structure given the name, or give the name from the structure, of the compounds used in the day's experiment, and give the role of each (reactant, solvent, catalyst, etc.).
5. Understand and describe the procedure to carry out a TLC.
6. Understand and describe the procedure to carry out a Column.
7. Identify and explain safety considerations for this experiment.
8. Perform the day's experiment safely and successfully.

Quizzes given after the experiment has been performed may also include:

7. Given a developed TLC plate or a picture of one, calculate R_f values.
8. Give and interpret the procedure for running column and thin layer chromatography experiments, and predict the order of elution of compounds in these methods based on solvent and eluent polarity.
9. Predict the consequences of improper assembly and use of the apparatus (including solvent level effects, leaving off the top, and moving the chamber in TLC, and allowing the column to dry in column chromatography).

Safety Precautions^{i,ii}

Ferrocene causes irritation to the eyes and skin. Prolonged ingestion or inhalation may cause liver damage. Acetylferrocene is toxic if ingested or absorbed through skin. Do not eat or breathe these materials. Keep away from skin. Wear gloves. Wash hands at end of lab.

Dichloromethane (Methylene chloride) is a chlorinated organic material. Most chlorinated organics possess some negative health effects. High level exposure to dichloromethane can cause central nervous system depression; long term exposure may cause tumors. Handle in small quantities, minimize contact with liquid and avoid breathing vapors.

Toluene, ethanol, Hexane and methyl t-butyl ether (MTBE) are flammable organic liquids. Have no flames in lab. Avoid breathing vapors.

TLC and Column Procedure

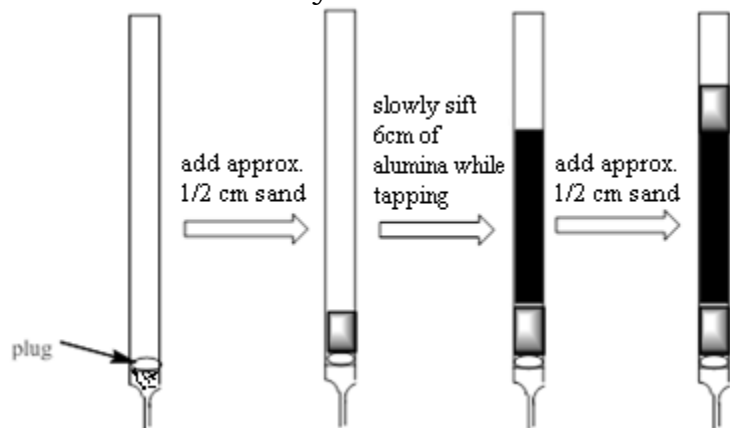
A 1:1 mixture of ferrocene and acetylferrocene will be separated using column chromatography. Different fractions of the solvent eluted from the column will be collected in separate watch glasses. The purity of the compounds contained within these fractions will be analyzed using TLC.

Weigh out approximately 0.02 g of solid Ferrocene: Acetylferrocene. (This is a solid mixture. Do NOT use the solutions from the TLC experiment.) Do NOT waste time getting exactly 0.0200 g of material. Record exact mass in notebook. Place in reaction tube and add about 10 drops of dichloromethane (methylene chloride). Set this aside. This is your F:AF solution.

Column Chromatography

A short pipette will be used as the column. Place small paper plug loosely in bottom of column. A small piece of Kim-wipe works well for this. Do not jam it down into tapered portion. Add approximately 1/2 cm of sand. Slowly, with constant tapping, add approximately 6 cm of dried Alumina to column. Be sure

to have alumina seat evenly. Do not waste time getting exactly 0.50 cm of sand and 6.00 cm of Alumina. Add approximately 1/2 cm of sand to top. Tap gently so bed seats evenly. Attach to ring stand using small three pronged clamp. The column is now ready to use.



Label and weigh two watch glasses. Place one under pipette column.

The solvents are volatile. When pipetting, be sure to keep pipette in strict vertical position (up and down, not sideways). Tilting pipette will cause liquid to drip or spray out.

Have 4 mL methyl t-butyl ether (MTBE), 4 mL hexane, and the F:AF solution labeled and within reach at your workspace. Have a pipette with a rubber bulb ready for each solution.

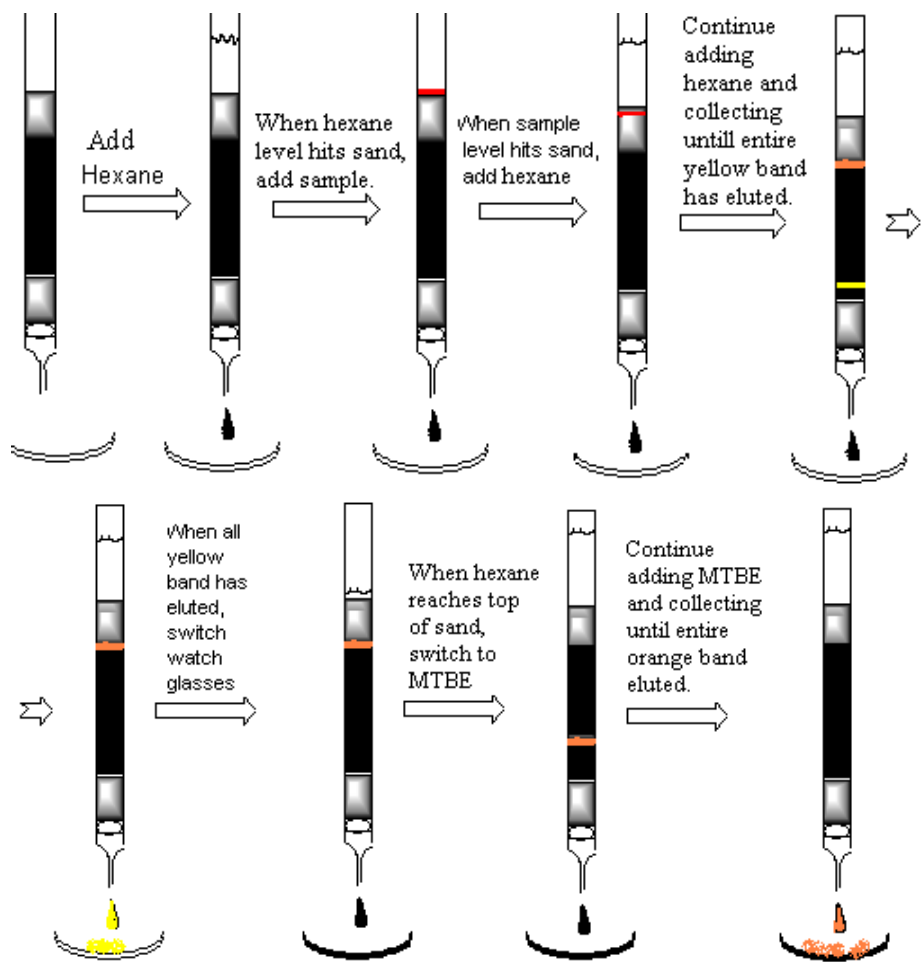
Once you begin the next step you may not stop until complete. DO NOT allow your column to run dry. Additions must be made quickly to avoid the column running dry and cracking. Be fully prepared before beginning the next step.

Running Column

Add approximate 2cm depth of hexane to top of column. When liquid level drains down and reaches top of sand, IMMEDIATELY add the entire F:AF solution to top of column. Try to add directly to top of column and not to drip down inside of glass pipette.

When the F:AF solution drops into sand, carefully add hexane to top of column. Continue adding hexane to prevent solvent level dipping below top of column. DO NOT allow column to run dry. Collect effluent in watch glass. You will notice color bands separating. Collect the yellow band in the first watch glass.

When all of the yellow band has eluted, switch watch glasses. When hexane level reaches top of sand, switch to MTBE. Continue adding MTBE to column to elute orange band.

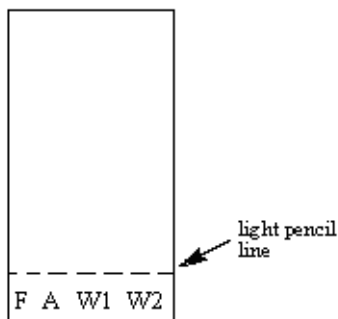


Spot mixture from first watch glass on TLC plate (W1). Spot mixture from second watch glass on TLC plate (W2). Place both watch glasses in hood to evaporate. After solvent has evaporated, weigh watch glasses again. The difference between the initial mass and the final mass is the mass of the component recovered. Calculate the percent recovery for Ferrocene, Acetylferrocene, and total mixture.

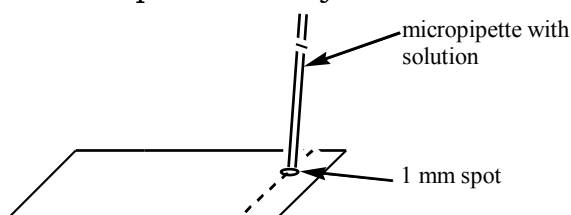
Thin Layer Chromatography

Prepare developing chamber by outfitting 100 mL beaker with a folded piece of filter paper and a watch glass or a piece of aluminum foil for a lid. Add developing solvent to chamber to a depth of no more than $\frac{1}{2}$ cm. The mobile phase for Ferrocene:Acetylferrocene is 30:1 Toluene : ethanol.

Obtain a pre-cut alumina coated TLC plate being careful to hold only by edges. Lightly draw a pencil line approximately 1 cm from the end of the plate. With a pencil lightly mark F, A and W1 and W2 for each sample. (Ferrocene, Acetylferrocene, Watch Glass 1 and Watch Glass 2). Each of these is a separate channel. Your TLC plate should look something like the following.

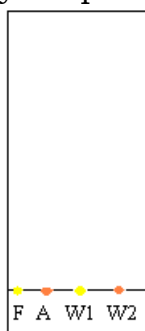


Draw up some of the pure ferrocene solution with a micropipette. Hold this pipette vertically over the plate. Aim the pipette over the pencil line made above. Touch the tip of the micropipette exactly on the line on the surface of the alumina-coated plate directly above the “F” mark to dispense an

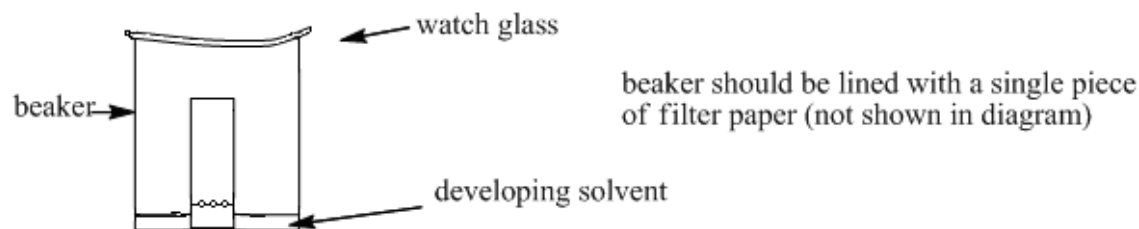


approximately 1 mm-sized spot of the solution. Wait a second or two for the solvent in the spot to evaporate, then touch the tip of the micropipette to the exact same spot. You may repeat this a few more times. Be careful to continue to place the pipette in the same exact area. This will make a very small and concentrated spot.

Using a different micro-pipette repeat with pure acetylferrocene solution and touching to “A” mark. As the first band is eluting from the column onto watch glass 1, using a new micropipette spot this onto the plate over the “W1”. Then repeat with the material in watch glass 2 over “W2”. Once this is complete your plate should look similar to the example below.



Place “spotted” TLC plate in developing chamber being sure that pencil line with spots is above the level of the solvent in the developing chamber. **DO NOT MOVE CHAMBER!!** This includes picking it up and walking with it to the instructor.



Allow developing solvent to migrate approximately $\frac{3}{4}$ up the length of the TLC plate. Do NOT allow the solvent to reach the end of the plate. Remove plate from chamber and IMMEDIATELY mark the position to which the solvent rose with a pencil line. This is called the solvent front.

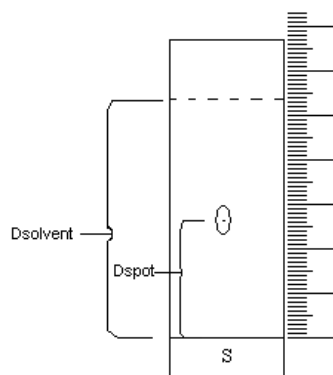
Allow the plate to air dry. The spots are visible but will fade with time. Circle the spots with pencil and place a small pencil dot in the exact center of each spot. When calculating Retention factor (R_f), measure to this dot.

Calculate the Retention Factors (R_f)

The retention factor (R_f) is a measure of the partitioning of the compound between the mobile and stationary phases. It is the ratio of the distances the spot and the solvent travel up the plate. The units cancel, so the R_f is a unitless value. The R_f value will always be between 0 and 1, $0 \leq R_f \leq 1.0$. A R_f value of 0 indicates the spot did not move at all. In this case the spot is very polar and has little to no affinity to the mobile phase. A R_f value of 1.00 means the spot traveled right with the solvent. In this case the compound is very non-polar and has little to no affinity to stationary phase.

Using a ruler, measure the distance from the start to the middle of the F spot. Remember to read to 2 decimal places. The distance between the smallest two lines on the ruler should be approximated. Measure the distance from the start to the solvent front (where the solvent stopped moving). Calculate the R_f as indicated. Remember your significant figure rules. Repeat for AF, W1, W2. If more than one spot is present in a channel, calculate the R_f for each spot within each channel. Interpret outcome. Information regarding how to properly read and analyze TLC results may be found in the on-line notes.

$$R_f = \frac{\text{Distance}_{\text{spot}} \text{ traveled up plate}}{\text{Distance}_{\text{solvent}} \text{ traveled up plate}} = \frac{D_{\text{spot}}}{D_{\text{solvent}}}$$



$$R_f = \frac{D_{spot}}{D_{solvent}} = \frac{2.63\text{cm}}{5.38\text{cm}} = 0.489$$

Attach the plate to your datasheet. Make a sketch of the plate in the data and observation section of your notebook.

CLEANUP

Excess and used solvents that contain any dichloromethane (methylene chloride) go in the halogenated liquid waste container in the hood. Solvents free of dichloromethane go in the non-halogenated liquid waste container in the same hood. Solid recovered ferrocene and acetylferrocene go in the non-halogenated solid organic waste container. Used Pasteur pipettes and capillaries go in the broken glass box. Wipe work space with sponge and remove any debris. Wash hands before leaving lab.

POST-EXPERIMENT ASSIGNMENT

Complete the datasheet and turn in before leaving class. Turn in the white notebook pages from your lab notebook. Staple multiple sheets together. Tear off rough edges.

Prepare for the TLC and Column chromatography portion of the next quiz. Remember that you may be asked questions on anything you should have learned from assigned readings, pre-experiment lecture, or doing the experiment.

ⁱ Fisher Scientific MSDS sheet Ferrocene <http://fscimage.fishersci.com/msds/03388.htm> and Acetylferrocene <https://fscimage.fishersci.com/msds/69220.htm> (September 14, 2011)

ⁱⁱ Fisher Scientific MSDS for Dichloromethane methylene chloride MSDS (September 14, 2011)