

BACKGROUND Chromatography is a powerful technique for the separation and purification of both solids and liquids on relatively small scale (ideally <10g). Chromatographic techniques are also invaluable as analytical techniques for analyzing tiny quantities of material (as little as 10^{-9} g). This is one of the four major purification techniques. Advantages for chromatography are its power and generality (not limited to liquids or solids or the need for ionizability). A disadvantage is the limitation in scalability (has problems with hundreds of grams of material).

1. Recrystallization
2. Distillation
3. Liquid/Liquid Separation (Separatory Funnel Separation)
4. Chromatography

Every type of chromatography depends on the distribution of a substance between two phases, a **mobile phase** and a **stationary phase**. (In a river, the running water would be called the mobile phase and the riverbed the stationary phase...) In today's case, the mobile phase will be an organic solvent, the stationary phase a polar surface. A particular chemical will partition between being bound to the surface, where it doesn't move, and being dissolved in the solvent, such that it flows along. Thus different chemicals move at different speeds, depending on adsorption/solubility equilibrium. The more tightly the sample binds to the surface, the less it will move. Anything that impacts the sample's partition between binding to the stationary phase versus dissolving in the mobile phase will impact the sample's mobility.

Practical Summary: A sample "stuck" to a surface is "washed along" with a solvent. "Less sticky" things move faster!

Different types of chromatography use different binding principles for "sticking" to the stationary phase, and are useful in different contexts of science.

- Organic Chemistry (today): A polar surface binds polar organics
- Biochemistry
 - Size exclusion: surface pores can fit small molecules, exclude larger molecules
 - Charge: cationic surfaces bind anionic chemicals, anionic surfaces bind cationic chemicals
- Gas/Liquid Chromatography (Organic and Analytical Chemistry): the "stationary phase" is actually a nonvolatile liquid coating on the walls of a tube; the mobile phase is gas passing through the tube. Volatile chemicals are more likely to evaporate from the liquid phase and fly along in the gas phase. Less volatile chemicals are better retained in the liquid phase and thus move more slowly. Polarity can also be used to attract materials to the stationary phases.

ANALYTICAL TLC (THIN LAYER CHROMATOGRAPHY)

TLC chromatography uses glass or plastic plates coated with a thin layer of adsorbent as the stationary phase. Silica gel ($\text{SiO}_2 \cdot x\text{H}_2\text{O}$) and alumina ($\text{Al}_2\text{O}_3 \cdot x\text{H}_2\text{O}$) are the most common solid adsorbents. Both are polar, hydrogen-bonding adsorbents, with lots of polar, hydrogen-bonding "sticky sites". Samples are applied to the surface, and then the organic "eluent" (solvent) is applied and runs up the plate. (The flow of the eluent results from capillary action.)

The mobility of a particular chemical depends on its partition between the mobile phase (the eluent) and the stationary phase (silica gel). The more tightly the sample binds to the silica (the "stickier" it is), the less it will move. The less well it binds, the more it will dissolve in the solvent and flow up the plate.

1. **Sample Polarity and Sample Movement:** A typical ranking of sample polarity in terms of functional groups, all else being equal, is in the order shown. In practice, **a more polar sample will bind to the stationary phase better, and will not move as much.** A more nonpolar, less sticky sample will move faster and farther

- **Polarity Pattern:**
Carboxylic acids > alcohols > amines > ketones/aldehydes > esters > ethers > halocarbons > arenes > alkanes
- **Adding additional nonpolar hydrocarbon to a given molecule moves it in the non-polar direction (tiebreaker).** For example, C₄H₉OH will be more polar than C₇H₁₅OH.
- **Key: More polar sample → moves less. Less polar sample → moves farther.**

2. **Eluent Polarity and Sample Movement:** Eluents have the same order of polarity. But **while a sample's movement decreases with increasing sample polarity, a sample's movement increases with increasing eluent polarity.** A good-binding polar eluent competes for the sticky sites on the silica, and either "displaces" the substrate from the surface or else prevents the sample from binding to the sticky sites on the surface. The result is that the sample will have its adsorption/solubility partition moved away from the adsorbed side toward the dissolved side. The practical result is that increasing eluent polarity means that the substrate will move to a greater degree.

- **Eluent factor: The more polar the eluent, the faster and farther samples will move**

3. **Response Factors:** Movement is quantified by "R_f" (**R**esponse_factor) values: relative to where the sample begins, this is the distance the sample travels divided by the distance that the eluent travels. For a given surface, substrate, and eluent, the R_f is characteristic and is useful for identification. For a series of substrates, their relative R_f's reflect their relative polarities.
4. **Sample Visualization:** Visualizing samples is crucial, since most organics are colorless. UV (ultraviolet light) or a chemical colorizing dip will be used. (UV is ideal, but is only applicable to molecules with extensive systems of sp² atoms that are able to absorb UV light.)

Summary: For silica gel surfaces, which are polar, the following relationships are true:

1. When two samples are run under identical conditions, **the more polar sample will move less and have the lower R_f; the less polar sample will move more and have the higher R_f.**
2. When the same sample is run under two different solvent conditions, **any sample will have a higher R_f with the more polar eluent, and a lower R_f with the less polar eluent.**

COLUMN CHROMATOGRAPHY

While TLC is useful as an analytical tool, chromatography can also be used as a purification technique. But to separate larger than analytical quantities of chemical, a larger amount of solid surface material must also be used, and larger volumes of solvents must also be used.

The general idea is that if sufficient quantities of solvent are used, solutes will eventually "wash off" of the surface. The differential mobilities of different compounds allow them to come off at different speeds. Individual collection of the different fractions, followed by reconcentration, enables isolation of pure compounds from initial mixtures.

Eluent Polarity Ramping: In practice solvent polarity "ramping" is commonly used. In this technique, a relatively nonpolar solvent is used first, which is only able to selectively wash off mobile, nonpolar substrates, while leaving polar substrates behind ("stuck" to the solid surface). Then a more polar solvent is applied which is able to wash off the more polar substrate. In today's lab we will do a very abrupt increase in solvent polarity to make things go faster. But often this is done gradually.

Pressure is frequently used to push solvent through more quickly and speed up the process. We will use modest pressure in today's experiment.

In today's experiment, the samples will be colored, so it will be relatively easy to see them separating and moving. Most ordinary organics are colorless. When colorless organics, a series of different solvent fractions are collected in test tubes or flasks. Then the same visualization techniques that are used for TLC are applied to determine which fractions actually have chemicals

AUTOMATED "FLASH" COLUMN CHROMATOGRAPHY

While manual column chromatography is still widely used, many research labs now use automated chromatography. Automated "flash" chromatography involves special high-grade silica cartridges; the ability to program solvent mixtures in order to ramp up the eluent polarity; and a built-in ultraviolet detector.

The standard process is to dissolve the sample in some organic solvent; add some silica; and concentrate the mixture such that the sample is finely dispersed over the surface of the silica and blend can be easily poured. (This will have already been done for you in advance of today's lab.) Solvent is then delivered through the mixture and through a silica cartridge, after which fractions are collected in a series of test tubes. The contents of a tube can be analyzed by GC-MS (introduced later this semester) or NMR (also introduced later this semester). Test tubes containing a particular component can be combined and concentrated to provide the purified chemical.

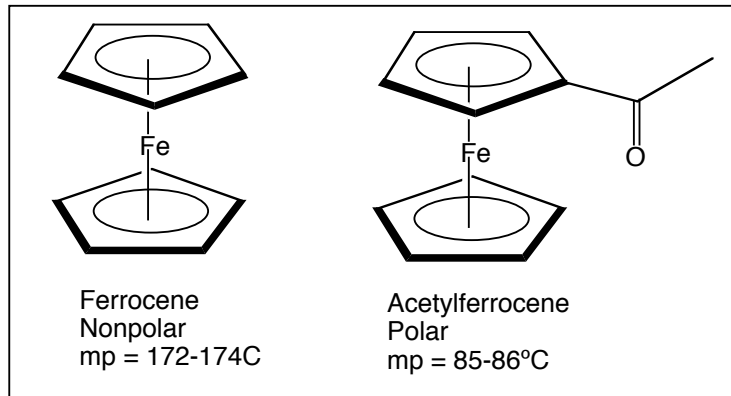
The Experiments

Part I: AUTOMATED “FLASH” COLUMN CHROMATOGRAPHY

1. Jasperse will demo this procedure. We'll either try to all squeeze in around the combi-flash machine and see how things work. Or else will split that up into two groups, half the lab early in the lab period, and then the rest will need to pause what they're doing and come over later to see how it works.
 2. Once actually started, a run will take about 15 minutes: two minutes to equilibrate and rinse out some of the tubes and columns, then ten minutes of actual collection in test tubes, then some rinsing.
 3. **Analyze and identify one of the unknown components by GC-MS.**
 - After a run is completed, the two or three different components of the unknown mixture will be located in different test tubes.
 - Each set of partners will be responsible to analyze and identify one of the unknowns.
 4. **Vial Preparation for GC-MS Analysis.**
 - Use a pipet to transfer ≥ 0.5 mL of solution from a test tube containing your sample into a GC-MS vial.
 - The samples are already plenty dilute from the solvent, so no further dilution is required.
 - Submit the vial to the GC-MS sequence, using the procedures you used in earlier week.
 - Be sure to remember which spot in the tray you are using!
 5. Unknown Candidates with Molecular weights are shown on the lab report hand-in page.
 - You should be able to identify your unknown directly from matching your sample's molecular weight with the sample with that molecular weight on the table.
 - So, super easy!
-

Part II: COLUMN CHROMATOGRAPHY**Separation of nonpolar Ferrocene(mp 172-174°C) from polar Acetylferrocene (mp 85-86°C).****Overview**

In this experiment, a small-scale chromatographic separation of non-polar ferrocene (mp 172-174°) and relatively polar acetylferrocene (mp 85-86°) will be attempted. These structurally interesting iron-containing molecules have been chosen for the experiment specifically because they are colored (unlike most organics). So their visible color will enable you to see the separation and chemical movement as it happens. The solid surface will be silica; the nonpolar solvent will be “petroleum ether” (a misnomer, it is a mixture of low-boiling alkane isomers); and the polar solvent will be 50/50 diethyl ether/petroleum ether. The column will be packed with silica gel. Then a 50/50 mixture of ferrocene and acetylferrocene will be poured on top. Nonpolar petroleum ether will wash off the nonpolar ferrocene while leaving the polar acetylferrocene behind. More polar 50/50 diethyl ether/petroleum ether will then be used to wash off the acetylferrocene. Both wash solutions will then be concentrated to give the purified samples.

**Preparing the sample:**

1. Weigh out approximately 0.1g of 50/50 pre-mixed ferrocene/acetylferrocene material onto some weighing boat. (Record the exact weight of the mixture).

Preparing the column:

2. Plug the end of a 4-mL “monster” pipette (the “column”) with glass wool. A long-nosed regular pipette serves as a good ramrod. (The “monster” pipets are on the middle table, and grab a big blue pressure bulb.)
 - a. Note: This is a special pipet, with thicker glass and a larger diameter than you normally use. Make sure you get the correct pipet.
3. Weight out ~1g of silica and add this to the pipet. (Doesn't need to be weighed carefully). (Use weighing boat to try to funnel it in). Your column should be approximately half full.
4. Securely clamp the column, and tap it to try to level the surface of the silica.
5. Take your dry sample (see above) and pour it (carefully!) onto the column, again using the weighing boat. Again tap the surface to try to level the material.

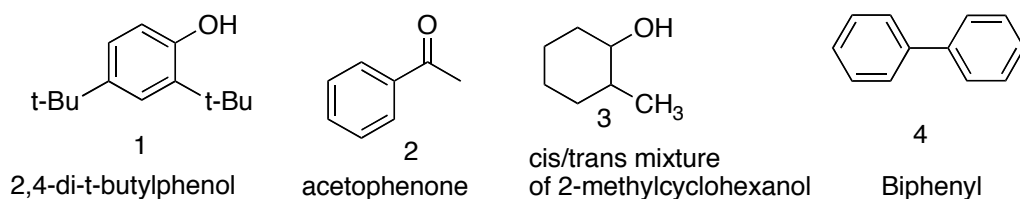
Applying the sample to the column:**Eluting the Nonpolar Substrate:**

6. Clamp down a 150-mL ground-glass jointed Erlenmeyer
7. Get 15 mL of petroleum ether and use it to wash the mobile, nonpolar substrate off from your column into the Erlenmeyer. Hopefully by the end the bottom portion of your column should be essentially white and there should be no yellow color coming off anymore.
8. Note: If you release the pressure on your big blue pipet bulb, while it is still held tightly onto your pipet, it will probably suck up all of the silica! Not a good plan. So after you have apply pressure to the big blue pipet bulb, be sure to take it off of the pipet before relaxing your grip and releasing the pressure. Otherwise, you'll have to start over.

9. Concentrate the solution by first adding a vacuum adaptor, and attach the vacuum hose. Gradually turn your vacuum open (turn counterclockwise to open) until it is wide open. Let it vacuum for around 10 minutes.
10. Take a melting point for your ferrocene. (You may need to use a bent microspatula to scrape off enough solid material to do the melting point.)

Eluting the Polar Substrate:

11. Clamp down another 150-mL ground-glass jointed Erlenmeyer with a small stir bar in the bottom.
12. Now use 10-15 ml of 50/50 diethyl ether/petroleum ether solvent to wash the more polar acetylferrocene off of the column and into your second Erlenmeyer. You should be able to see the colored band as it moves through the column. There will be some residual gray or brown color that will not wash off; these are impurities that we don't want anyway.
13. Concentrate the solution by under vacuum for 10 minutes.
14. Take a melting point for your acetylferrocene. (You may need to use a bent microspatula to scrape off enough solid material to do the melting point. It won't be much.)

Part III: TLC (Thin Layer Chromatography)4 Substrates, Unknown CandidatesGoals

- Use TLC mobility to observe the TLC behavior and rank the "TLC polarity" of the 4 reference substrates, from least polar to most polar. (Some may be essentially tied).
- Determine how each substrate can be visualized. Which are UV active, and which appear only when using a visualizing "dip"?
- Observe the dependence of TLC mobility on solvent polarity.
- Calculate R_f values
- Identify two unknowns by TLC.
- Learn general analytical TLC techniques.

Procedure

Work with a partner on this experiment. Each of you will need at least 4 TLC plates (two for each eluent). Each of you will identify two unknowns. Do not do the same unknowns as your partner.

Preparing the Plates:

1. Prepare the two plates for your first eluent: Use a pencil to mark 4 spots.
 - Give at least a 1 cm margin from the bottom. **Otherwise the sample may subsequently submerge, get dissolved away, and not get drawn up the plate.**
 - Avoid placing spots within 0.8 cm of the sides.
 - Use a pencil to mark placement of your original spots.
2. On plate 1, write **1**, **X** (the letter for your first unknown), **Y** (the letter for your second unknown) and **2**.
3. On plate 2, write **3**, **X** (the letter for your first unknown), **Y** (the letter for your second unknown) and **4**. (Both unknowns should be on each plate).
4. Then use the capillary tubes to apply your chemical solutions onto your TLC plates.
 - Spots should be neither too heavy nor too light (strong enough to be able to visualize, but **light enough to avoid overlap and chemical "tailing"**).
 - Before running your plates, check your second plate by UV. Biphenyl (**4**) should be easily visible. If not, your spot sizes may be too small. [Note: some spots are not very UV active, and may not appear. The point here is that if you don't even see #4, which is strongly visible, something is wrong. But don't worry if some of your spots show little or nothing by UV.]

To run the TLC's:

1. Place 3 full pipets of eluting solvent into a 50-mL beaker, and put a watch-glass on top to prevent solvent evaporation.
 - The eluent choices are 2% ethyl acetate/hexane, 10% ethyl acetate/hexane, and 20% ethyl acetate/hexane.
 - One partner should run both the 2% and 10% solutions. The other partner should run both the 10% and 20% solutions. (I want both of you to run 10% just to see how much scatter there is or isn't between two different scientists.)
 - You don't want to put in so much eluent that your spot will get submerged, in which case it will get dissolved away and not get drawn up the plate.
 - Ethyl acetate is an ester. The more ethyl acetate is present in the mixture, the more polar the eluent should be.
2. Carefully put your two TLC plate(s) into the beaker, and put the watch-glass back on top. The eluent will creep up the plate.
 - You can easily put both plates 1 and 2 in at the same time.
 - Avoid putting plates in crooked, or touching the walls.
 - You'd like the solvent line on the bottom to be level.
 - Note: the spots must not be submerged in solvent, or they will simply dissolve and not be drawn up the plate.
3. After the eluent has risen a significant distance, (it should **not** be allowed to get all the way to the top), remove the plate(s) and immediately mark with pencil the top distance that the eluent went.
 - If the solvent reaches the top, your sample can keep creeping up the plate and you will get falsely high R_f 's.
 - Note: It's important to keep the cover on your beaker. Otherwise evaporation competes with elution and you will get falsely high R_f 's.

Eluent choice: You will test two different solvent mixtures, as will your partner. One of you should run both the 2% and 10% ethyl acetate/hexane solvents; the other should run both the 10% and 20% ethyl acetate/hexane solvents. Since each of you will test two solvents, and for each solvent you will need 2 plates, that means each of you will be running 4 plates total.

Visualizing the spots:

1. Look at your plates under UV light, and circle with pencil the spots that you can see.
 - Spots may seem weaker than before; during the process the sample gets "stretched" out, so the signal intensity essentially gets diluted.
 - Not all samples will give UV spots.
2. After marking the UV active spots, take your plate to the "p-Anisaldehyde Dip" station. (Caution: 5% sulfuric acid!)
 - Dip your plate into the solution, using forceps
 - Try to let the excess liquid drain off
 - Then dry the plate with a heat gun.
3. Circle the new spots that appear, and record their color. (Some spots may differ in color, and the colors may have diagnostic value).

Calculate the R_f Values for All Your Spots: This will be the ratio of the distance traveled by your spot relative to the distance traveled by the solvent. You can qualitatively do this just by eye-ball analysis to get values to nearest tenth. (Ex. 0.1 vs 0.2 0.3 vs 0.4, etc.)

- Do not measure relative to the bottom of the plate. Measure relative to where the samples were applied. That's the starting line, because when the eluent climbs that far is when the race between eluent and sample to climb the plate begins.

Name: _____

Chromatography Lab Report

Part 1: Combiflash Chromatography

Fraction (1, 2, or 3...): _____

Identification of unknown (see Table): _____

Table 1: "Combiflash" Candidates:

<u>mw (g/mol)</u>	<u>Name</u>		<u>mw (g/mol)</u>	<u>Name</u>
106	Ethylbenzene		154	Biphenyl
108	Benzyl Alcohol		180	Stilbene
119	Benzotriazole		181	Methyl 3-nitrobenzoate
120	Acetophenone		184	Diphenylmethanol
134	Propriophenone		210	Benzil
135	Acetanilide		234	Dibenzylacetone
138	4-nitroaniline		244	Triphenylmethane
148	Butyrophenone		260	triphenylmethanol

Part 2: Column Chromatography Melting Ranges:

Nonpolar Ferrocene melting range: _____ Polar Acetylferrocene melting range: _____

Part 3: TLC1. Fill out the R_f data in the chart below for the two columns that you ran yourself (either first two, or last two).

- An eyeball estimate of R_f is satisfactory; one sig. fig. is probably appropriate (0.2, 0.3...)
- Remember that the R_f value for a spot will be fall somewhere from $0.0 \leq R_f \leq 1.0$, and is the ratio of the distance traveled by the spot (middle of the spot) relative to the distance traveled by the solvent, relative to where the spot began.
- Put a star above each of the two columns that you did yourself.
- Copy R_f data from partner and fill in those two columns.
- Note: You and your partner's R_f's may differ, but the relative ordering should be analogous.

2% Ethyl 10% Ethyl 10% Ethyl 20% Ethyl
Acetate/Hexane Acetate/Hexane Acetate/Hexane Acetate/Hexane

SubstrateR_fR_fR_fR_f2,4-di-t-Butylphenol **1**Acetophenone **2**2-Methylcyclohexanol **3**
(cis/trans, may give two spots)Biphenyl **4**

First Unknown

Second Unknown

2. Rank the observed polarity of the 4 samples **1-4**. In case of a close race, your eyes will be best for seeing which is a little faster. You may need to look at the results using more than just one eluent strength in order to be sure. Some might be too close together to tell with certain eluent strengths.

Most Polar: _____ > _____ > _____ > _____ Least Polar

3. Identify your two TLC Unknowns:

First Unknown: Letter _____ Identity _____

Second Unknown: Letter _____ Identity _____

4. Did increasing the polarity of your eluent increase or decrease your Rf's?
5. Given that the solid surface is polar, explain why polar **samples** have lower Rf values than less polar ones.
6. Explain why increasing **eluent** polarity increases the Rf value for a given sample?
7. In what order, from top to bottom, would you expect to see the spots for naphthalene (C₁₀H₈), butyric acid (CH₃CH₂CH₂CO₂H), and phenyl acetate (CH₃CO₂C₆H₅)?

Top

Middle

Bottom

8. In what order, from top to bottom, would you expect to see the spots for the following:

- | | |
|--|-----------------|
| • acetic acid=CH ₃ CO ₂ H | Top |
| • butanal=CH ₃ CH ₂ CH ₂ CHO; | 2 nd |
| • 2-octanone=CH ₃ COCH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃ | 3 rd |
| • decane=C ₁₀ H ₂₂ ; | 4 th |
| • 1-butanol=CH ₃ CH ₂ CH ₂ CH ₂ OH | Bottom |

9. If an eluent of too low polarity is used for the development of a TLC plate, will the sample spots be somewhere in the middle, way at the bottom, or way at the top of the plate?
10. If an eluent of too high polarity is used for the development of a TLC plate, will the sample spots be somewhere in the middle, way at the bottom, or way at the top of the plate?

Basic GC-MS Operation Compressed Draft 3 For Chem 355 Organic Unknowns Lab

Note: The following assumes that the hydrogen and compressed air gases have been turned on; that the machine has been warmed up; that the gc/ms program has been opened; that an appropriate “method” and “sequence” have been selected; and that Jasperse will shut things down.

Sequenced Data Acquisition: Using the Autosampler to Sequence Runs Automatically

Note: this assumes that Jasperse has already prepared and started a “sequence” (“Grignard..” for example, but you are trying to add your sample to the lineup. If you’re first in line, get Jasperse to come and help.

1. **Add your sample to the back of the line in the autosampler.**
 - Do NOT leave any open holes (unless the sample belonging in that hole is being sampled.)
 - Filling a “sample-is-in-the-injector-tray” hole will cause a system freeze.
2. **Open “edit sequence” by clicking the “edit” icon on the yellow panel** low on the computer screen.
 - This will open a spreadsheet that you can edit.
 - Add your names in the “name” box that goes with your vial number.
 - **Click OK.** Note: if you don’t click “OK”, the machine will freeze at the end of the current run. NEVER leave the spreadsheet page open unless somebody behind you is going to close it.

Data Processing/Analysis: Getting and Printing the GC Graph, % Report, and/or Mass Spec.

- Note: data analysis can be done while acquisition is ongoing.
 - Note: this assumes that the “**gcms data analysis**” software and appropriate analysis method are opened. In the data analysis page, check on the top blue line to see if it says “Enhanced data analysis-ADEFAULT-RTE.M...”, or “Grignards”, or something that fits the experiment for the week. If not, check with Jasperse or open it. (ex, Method > Load Method > Yes > ADefault-RTE.M > OK.)
3. **Open a data file** using the left mouse button to double click.
 - Your data file should be within the folder Organic Lab within the Data folder.
 - Data file will have the names “Vial-1” or “Vial-2”, so **remember which vial was yours.**
 4. **Printing GC Graph, % report, and retention times: Click Method>Run Method**
 - Repeat as many times as needed to provide prints for each student in your group.
 5. **Printing Mass Specs: Click the 2nd Hammer icon.**
 - Click the 2nd hammer icon as many times as needed to provide prints for each student in group.
 - Note: You don’t need to wait for a print to finish before clicking the hammer again. If you’ve got 5 partners, just click the hammer five times and the prints will come out one by one....

