Experiment 6 —

Thin-Layer Chromatography

Pre-lab preparation (1) An introduction to TLC can be found at www.chemguide.co.uk/analysis/chromatography/thinlayer.html (ignore the little green note blocks within that text, and we'll be using screw-cap jars, not beakers.) (2) In a few sentences, describe how TLC works, and sketch what a TLC plate might look like after development with solvent. (3) Use your drawing to show how the R_f value is determined.

Thin-layer chromatography (TLC) is an extremely valuable analytical technique in the organic lab. It provides a rapid separation of compounds, and thereby gives an indication of the number and nature of the components of a mixture. TLC can also be used to identify compounds by comparison with known samples, to check the purity of a compound, or to monitor the progress of a reaction, an extraction, or a purification procedure.

This experiment will introduce you to the mechanics of TLC, and the chemical principles behind it. In the first part, you will separate the soluble components of spinach extract; in the second, you will analyze the compounds you separated by extraction in the last lab.

Principles of TLC. TLC is normally done on a small glass or plastic plate coated with a thin layer of a solid — the most common are *silica* (SiO_2) or *alumina* (Al_2O_3). This is the stationary phase. The mobile phase is an organic solvent or solvent mixture. The sample mixture is applied near the bottom of the plate as a small spot, then placed in a jar containing a few ml of solvent. The solvent climbs up the plate by capillary action, carrying the sample mixture along with it. Each compound in the mixture moves at a different rate, depending on its solubility in the mobile phase and the strength of its absorption to the stationary phase. When the solvent gets near the top of the plate, it is allowed to evaporate, leaving behind the components of the mixture at various distances from the point of origin. The ratio of the distance a compound moves to the distance the solvent moves is the $\mathbf{R_f}$ value (retention factor). This value is characteristic of the compound, the solvent, and the stationary phase.

In column chromatography, the sample is carried *down* a column of silica or alumina by solvent, and the separate components of the mixture are captured as they elutes from (exit) the column. This can be done by allowing the solvent to flow under the force of gravity, but this is slow. Today, organic chemists use a technique called "Flash Chromatography", in which the solvent is pushed through the column with a little air pressure. A related technique for especially difficult separations HPLC — High-Pressure Liquid Chromatography — that uses a very high-quality stationary phase and high solvent pressure to accomplish separations.

Silica and alumina are relatively polar stationary phases. Both have OH groups on their surfaces that interact strongly with polar compounds. Such compounds are adsorbed strongly and therefore move along the plate slowly, while non-polar compounds are absorbed only weakly and are therefore carried along the plate more quickly. Of course, solvent polarity also affects how fast compounds travel. Polar compounds are carried along quickly by polar solvents, but move slowly or not at all with non-polar solvents. Because non-polar compounds don't adhere strongly to the silica, they tend to move more quickly in most solvents. The table below lists several common chromatographic solvents in order of increasing dielectric constant, ε, which is a measure of bulk polarity. Since a solvent's chromatographic "eluting power" (ability to move compounds) is roughly related to its polarity, this is an approximate *eluotropic series*.

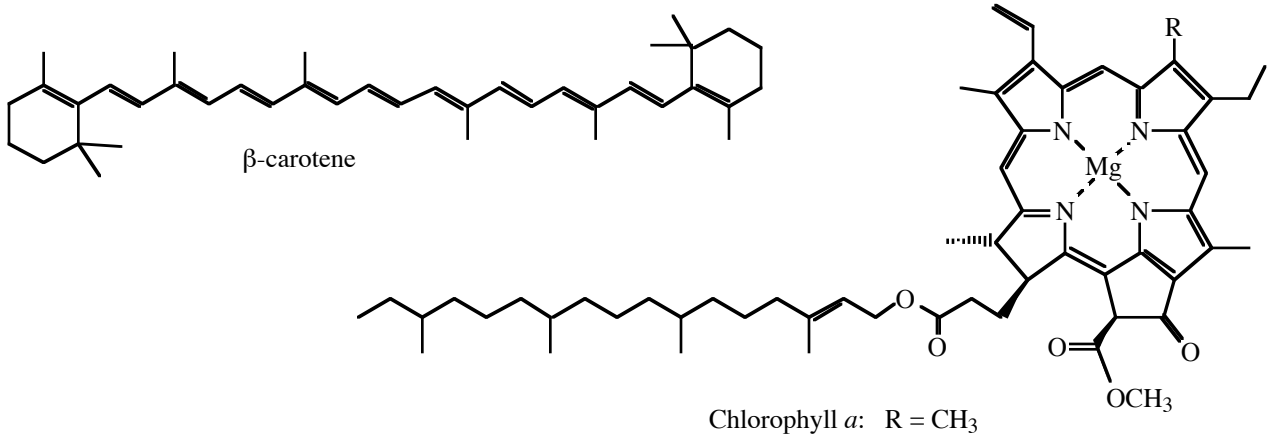
Eluotropic series

Solvent	*ε **	*Solvent*	*ε **
alkanes	2	isopropyl alcohol	18.3
benzene	2.3	acetone	20.7
diethyl ether	4.3	ethanol	24.3
chloroform	4.7	methanol	32.6
ethyl acetate	6.0	acetonitrile	37.0
dichloromethane	8.9	water	78.5

* dielectric constant (debyes)

(Data from JA Landgrebe *Theory and Practice in the Organic Laboratory*, 4th ed, p 68 and AJ Gordon, RA Ford *The Chemist's Companion*, pp 3 - 14.)

Experiment A. Plant pigments. Plants use a number of different pigments in their light-harvesting systems. These compounds belong to the chlorophyll and carotenoid classes. Representative members of these groups, chlorophyll (*a* and *b*) and β-carotene are shown below.



Chlorophyll *a*: R = CH_3
Chlorophyll *b*: R = CH=O

TLC will allow you to separate these pigments in a sample of spinach extract. You should be able to see spots from several carotenes, including **β-carotene**, **α-carotene**, whose endocyclic double bonds are shifted one position (out of conjugation) relative to the β isomer, and several oxygen-containing carotene derivatives called **xanthophylls**. All should appear as yellow or orange spots on the TLC plate. In addition, you should see spots corresponding to the green **chlorophylls *a*** and ***b*** as well as gray spots for **pheophytins *a*** and ***b***. Pheophytins are just the chlorophylls with the Mg^{2+} replaced by two H^+s.

Begin by going to Valentine and fetching a medium-sized wad of fresh spinach... just kidding... A solution of spinach extract in 1:1 acetone and petroleum ether will be provided. You're welcome. (This solution was prepared by adding the solvent mixture and sand to the spinach, then grinding it thoroughly with a mortar and pestle. The sand tears up the cell walls and allows the organic compounds to dissolve in the solvent. The dark green solution was then washed with water in a separatory funnel, dried, filtered, and stored in a cold, dark place (i.e., a refrigerator. We'll assume that the light does goes out when you close the door.)

Plastic-backed silica TLC plates (2.5 x 7.5 cm) will be provided. Be sure you handle these by the *edges*. Draw a light pencil line about 1/3 to 1/2 inch from the bottom of one plate. Estimate, don't measure — all you're doing is marking the starting point — it just has to be high

enough that your sample mixture is above the solvent level! You're going to use a capillary micropipet to make three separate spots along the pencil line, so make three evenly spaced "tick" marks with the pencil to indicate where you will place these spots. (Before you spot a real TLC plate, practice on a piece of filter paper — try to make as small a spot as possible.)

Now that you're proficient, go ahead and spot the TLC plate. Make the first spot as small as possible (1 mm in diameter or less). Next, make a wide spot by holding the capillary against the plate. Third, make as small a spot as you can, give the solvent a few seconds to evaporate, spot again, and repeat the process a few times to build up the concentration without widening the spot excessively.

Develop the plate with a 1:1 mixture of hexane and ethanol. This is a case where speed is more important than precision. Just pipet about 2 ml of each into one of the small screw-cap bottles provided, then cap and gently swirl to get the air inside saturated with solvent vapor. Use forceps to carefully insert the TLC plate, *cap the bottle*, and allow the solvent to rise until it gets close to the top of the plate. Be careful not to disturb the bottle. Remove the plate with forceps, mark the position of the solvent front with a pencil, and allow the solvent to evaporate. (Why doesn't it matter exactly how close the solvent gets to the top? Why *does* it matter that you mark exactly where the solvent front ended up *immediately* after you remove the plate?)

Which of the three gave the best separation? If something went seriously awry (compounds all ran to the edge, for example), try it again. If you can't easily see the spots, use more; if everything ran together in a big smear in every case, you may have spotted too much, so use less. Seek advice from your instructor or TA as necessary.

Circle all the spots that are visible (in case they disappear due to exposure to light and air). Make a sketch of the plate in your notebook, and note the colors of the various spots. Next, expose the plate to 254-nm UV light by using one of the hand-held UV lamps. **Caution: UV light is harmful to your eyes. (1) Keep your goggles on — they will absorb UV, and (2) Do not look directly at the light.** The silica TLC plates contain a fluorescent indicator that will glow green when exposed to 254-nm light. Many compounds will quench (decrease the intensity of) this fluorescence and appear as dark spots against the bright background. In addition, some *spots* may fluoresce and appear bright on exposure to UV light. Circle any new spots that show

up, and note whether the compounds are fluorescent (bright) or fluorescence quenchers (dark). Next, expose the plates to I_2 vapor for a few minutes by placing them in the bottles provided in the lab. Note any new spots that show up. Dispose of your used TLC plates in the waste bottle provided, *not in the trash!*

Next, use a scissors to cut one plate in half lengthwise, spot the spinach on each plate (use single or multiple spotting — whatever worked best the last time). Elute one with 3:1 ethanol/hexane and one with a 1:3 mixture. Remember, no fooling with grad cylinders — quick 'n' dirty — use dispo pipets and estimate. Use two separate jars, of course, but run them both at the same time.

While those are running, in your notebook, summarize the results of the first TLC by listing the R_f values of the chlorophyls, pheophytins, and carotenes/xanthophyls that you can identify based on spot color, and those of any other unknown spots that showed up. How many decimal places do you think are appropriate for an R_f value?

After your 1:3 and 3:1 are finished, do whatever is necessary to find the spots. (If nothing new showed up before with UV or I_2, then those steps aren't necessary, are they?) How do the R_f values change with solvent polarity? Perhaps certain compounds were more sensitive to the polarity change than others. What does this tell you about those compounds?

Spinach experiment and discussion adapted from DL Pavia, GM Lampman, GS Kriz, RG Engel *Intro to Organic Laboratory Techniques*, 2nd ed, pp 122 - 131; and AL Lehninger, DL Nelson, MM Cox *Principles of Biochemistry*, 2nd ed., 1993, Worth, pp 575 - 577.

Experiment B. Analysis of pure compounds from the Exp-5 extraction. How well did you do with your separation? Did you get three nice pure solids, or are your products contaminated with the other components of the mixture or with other unknown junk? TLC is an easy way to find out. You know enough about TLC at this point that we could say "just do it" and turn you loose. OK, maybe a little guidance is necessary. These are solids, so you'll have to start by dissolving a bit in a solvent. Use the smallest test tube that you can find. First, think

about how much you need. How much are you going to spot on the TLC plate? 10 ml? 2 ml? Certainly not. So don't make up way more than you need. A tiny bit dissolved in a few drops of solvent is more than enough.

Spot 'em side-by-side-by-side and develop with a mixture of ether and hexane. How much of each? It's up to you. Figure out what's necessary to do the job. Find the spots — you might see one major spot and perhaps a few less intense ones in each case. Mark *everything* that shows up, and sketch the plate in your notebook. Do the relative R_f values make sense in terms of the *structures* of the compounds? If you didn't get pure compound in one or more of your samples, what might have gone wrong? Did you accidentally get some ether layer in with your aqueous? Oooops. Aqueous mixed in with the ether? Forget to shake the sep funnel? Hmmm...

Now for the fun part. You may think you know what each of these compounds is, but we need to be sure. Measuring melting points is one way to find out. Refer to your prelab from Expt 5 — what are the melting points of the three pure compounds? Keep in mind that a 100.0000... % pure compound has a melting *point*; any real compound we handle in the lab has a melting range — the range may be a fraction of a degree, it may be a couple of degrees, it may be 10°C or more. In general, the less pure a compound is, the *lower* the temperature at which it melts, and the *broader* its melting range. So this experiment is an independent way to get an idea of the purity as well as identity of each compound.

To speed things along and get people finished and on their way, let's pool our resources. Get together with one or two other groups (no more, please!). If you can establish by TLC that all the samples of a particular compound are the same (how? Would it make more sense to compare R_fs or to spot them side-by-side on the same plate?), then you can pick the cleanest one for the melting point measurement. When you report the data make sure it's clear whether the mp is for *your* compound or that of another group.

To measure the melting points of your compounds, you'll use the MelTemps in the back of the lab. Load a bit of each sample into a capillary tube, bounce it to pack the solid, then heat it slowly and carefully until it melts. Be sure that the heating rate is no more than about 1°C per minute through the melting range. Record the temperature of the *onset* of melting, and the temp at which the last bits of solid finally "disappear". This is your melting *range*. Everyone should

have a chance to watch one or two samples melt — don't just assign the task to one person and forget about it.

While you're working on getting melting points, measure the mass of each compound, and calculate the % recovery. (You recorded the exact amount you started with, didn't you?) You may need to think about how best to determine the mass of that yellow solid in the round-bottom flask. Did you tare the flask? Can you scrape it all out? Can you maybe weigh it, get out as much solid as possible, clean it, then tare it? It's up to you. Just record what you did.

Save all the samples; we'll run IR spectra of these compounds later in the course. For storage, transfer the solids to one of the plastic storage vials provided. Label each with the names of both group members, the Experiment number, the date, and the *name and structure* of the compound. Put them in a safe place in your lab drawer.

Your "report" should be turned in at the end of the lab period. In addition your results section for Expt B (a record of what you did, sketches of your TLC plates, melting ranges, etc) briefly discuss (1) how you decided whose sample of each compound to use for the melting point experiment, and what the mp results imply about the identities and purities of the compounds. Is this what you expected based on each compound's purity as indicated by TLC? (2) If your group's TLC results indicate that one or more of the compounds is less than completely pure, what can you say about the impurities? Did your separation not go as planned? What went wrong? (If you kept a careful record of what you did in your notebook during Expt 5, perhaps that will provide some hints; if not, then you may need to be more detail-conscious with your record-keeping.)