Experiment 3 – TLC Analysis of Spinach Pigments

Reading Assignment
Mohrig Chapters 9 – 11 (filtration, extraction, and drying)
Watch the videos on spinach extraction and TLC online

Thin layer chromatography (TLC) is a form of qualitative analysis called **adsorption chromatography** used almost daily in synthetic organic chemistry labs. The composition of a mixture can be assessed relative to known standards and separation of the mixture is roughly based on polarity. In brief, a small portion of the sample is placed on the **stationary phase** and is carried up the TLC plate by the **mobile phase**. *A polar compound will interact more with the polar stationary phase and will not travel as far up the plate. Conversely, a less polar compound will move farther up the plate.* Notice that the principles behind this technique are not terribly different from GC. Separation is based on polarity in both cases – relative boiling points can be estimated from polarity. Mobile and stationary phases are still employed, except that the states of matter are different. The principles of TLC will later be applied to *column chromatography*, where larger sample volumes will be physically separated and isolated based on polarity of the components. In this lab, students will use solvents of different polarity (mobile phases) to determine the optimal separation of the pigments found in spinach extracts.

TLC is one of the most versatile and rapid separation techniques available to the organic chemist. Analyses of complex mixtures can be successfully carried out in a matter of minutes with relatively inexpensive equipment. In TLC the separation takes place on a thin layer of **solid stationary phase** spread on a solid support (a glass, aluminum, or plastic plate) as a **liquid mobile phase,** also simply called the **solvent,** moves along the plate. Separation can be achieved by partition, adsorption, ion-exchange, or size-exclusion processes. In the organic chemistry laboratory, however, most applications of TLC are based on adsorption and polarity. Thus, in our discussion we will primarily concentrate on TLC as **adsorption chromatography.**

The sample used for TLC is a nonvolatile liquid or a solution in a volatile solvent. It is spotted on the adsorbent with the aid of a capillary tube at a distance of about 1.5 cm from the lower edge of the plate, referred to as the **origin** (**Figure 1**). After the solvent has evaporated from the spot, the plate is placed in a chamber that contains the mobile phase of choice to a height of about 0.5 cm. The chamber is capped to avoid evaporation of the solvent and to ensure liquid-vapor equilibrium inside. The solvent rises on the TLC plate through capillary action, pulling along the components present in the sample. Different compounds travel at different speeds because they have specific interactions with the stationary phase. When the solvent has traveled 80-95% of the length of the plate, the plate is removed from the chamber, and the level reached by the solvent, called the **solvent front,** is marked. The solvent is allowed to evaporate, and the plate is analyzed.

In **Figure 1**, the original sample spot has separated into two spots after interaction with the mobile phase, indicating that the sample contains at least two different compounds.

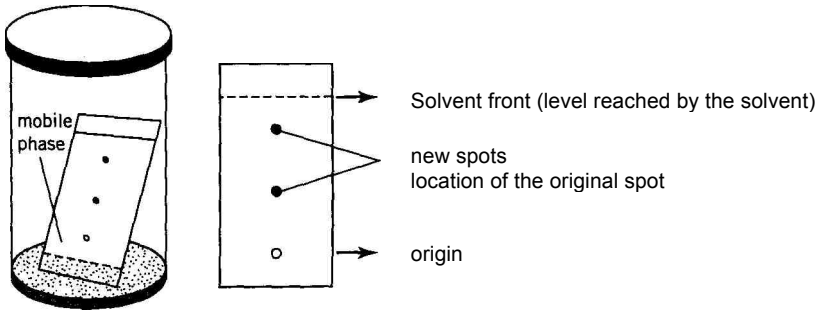

Figure 1. TLC Plate and developing chamber.

Adsorption is a process by which molecules of a gas, liquid, or solid in solution (**solute**) interact with the molecules *on the surface of a solid,* called the **adsorbent.** Adsorption is strictly a surface process that depends on electrostatic forces between adsorbent and sample. These forces arise from **dipole-dipole** and **ion-dipole interactions,**

and **H-bonds.** The surface of the adsorbent is far from being perfectly smooth; it has crevices and crests with centers of positive and negative charge density. The solute binds to the adsorbent through electrostatic attraction between its own centers of charge density and those on the surface of the adsorbent. The places on the surface of the adsorbent where the sample binds are called **binding sites**. Ions and molecules with permanent dipole moments readily bind to the adsorbent.

The most commonly used adsorbent in TLC is **silica gel.** Silica gel is obtained by hydrolysis of silicates. It has polarized Si—O and O—H bonds that interact with dipoles in the solute. It can also form H-bonds, especially with H-bond donors such as alcohols (R—OH), phenols (Ar-OH), amines (R-NH_2), amides (R-CO-NHR'), and carboxylic acids (R—COOH). The types normally used for TLC have an average diameter of about 0.025 mm with a wide distribution; some particles are as small as 0.005 mm and others as large as 0.045 mm.

To understand how separation occurs on the surface of the adsorbent, we should look closer at the interactions between the **adsorbent** and **solutes** and between the **adsorbent** and **mobile phase.**

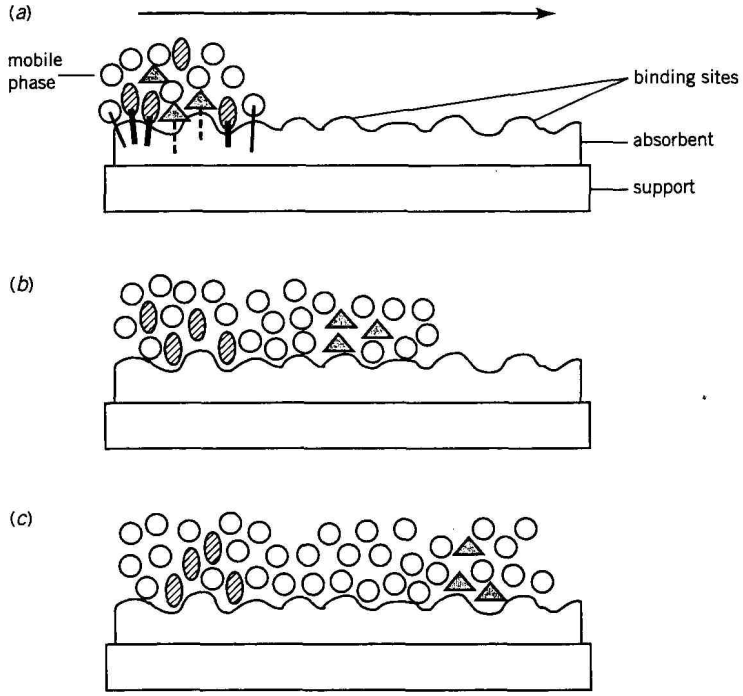

Figure 2. Separation of two compounds on a TLC plate (ovals and triangles). The arrow indicates the direction of the mobile phase flow (see text).

Figure 2 displays a close-up view of a TLC plate cross-section showing the interactions on the surface of the adsorbent. The sample has two different components, represented by ovals and triangles. The solvent molecules are shown as circles. Because there is a limited number of binding sites on the surface of the adsorbent, *solute and mobile phase molecules must compete* with each other to bind to the adsorbent; the stronger the interactions, the tighter the binding. In **Figure 2a**, the thickness of the lines indicates the strength of the interaction.

The oval molecules have a very strong interaction with the adsorbent as indicated by a very thick line, while the triangles have a weaker interaction as shown by the dashed line. The mobile phase molecules, on the other hand, have a stronger interaction than the triangles, but a weaker one than the ovals. As the mobile phase ascends along the plate, *those mobile phase molecules close to the adsorbent compete with the solutes for the binding sites.* If the interaction between mobile phase and adsorbent is stronger than the interaction between adsorbent and solutes, such as in the case of triangle molecules, the mobile phase displaces the solute molecules from their binding sites, moving them farther away from the surface of the adsorbent. As the mobile phase keeps flowing, these solute molecules are carried to a new location on the plate (**Fig. 2b**). Because the mobile phase has stronger interaction with the adsorbent, the triangle molecules do not bind efficiently and keep traveling along the plate at high speed. At the end of the chromatographic run, the triangles are found near the solvent front (**Fig. 2c**).

If the interaction between solute and adsorbent is stronger than the interaction between mobile phase and adsorbent, the mobile phase still moves the solute in the direction of flow. This is a result of mass action; the mobile phase molecules, being much more numerous than the solute molecules, eventually displace the solute molecules from

their binding sites, even if the solute has stronger affinity for the adsorbent. However, the stronger the interaction between adsorbent and solute, the more difficult it is for the solvent to move the solute. As shown in **Figure 2c**, the oval molecules, which display the strongest affinity for the adsorbent, stay very close to the place where they were originally spotted.

Selection of solvent conditions

One parameter that plays a crucial role in the separations is the *polarity of the mobile phase.* The dielectric constant is usually taken as an indicator for polarity; however, other indices (based on chromatographic separations) have been devised. Some useful solvents for TLC are given in **Table 1** in order of increasing polarity.

The use of solvent mixtures is very common in TLC analysis. The polarity of the mobile phase can be changed within a wide range by mixing solvents of different polarities. For example, mixtures of hexane and ethyl acetate with increasing proportion of the latter provide a series of solvents of increasing polarity.

Table 1. Selected Solvents in Order of Increasing Polarity

Solvent	Dielectric constant
hexanes	**1.89**
cyclohexane	2.02
toluene	2.38
diethyl ether	4.34
ethyl acetate"	6.02
methylene chloride"	8.93
acetone	20.7
methanol	32.7
water	80.1

"On the basis of chromatographic data, ethyl acetate is considered more polar than methylene chloride.

Family of compounds	Structure
aliphatic hydrocarbons	R–H
alkyl halides	R–X
unsaturated hydrocarbons	R–CH=CH–R
aromatic hydrocarbons	Ar–H
aryl halides	Ar–X
ethers	R–O–R
esters	R–COOR
ketones	R–CO–R
aldehydes	R–CO–H
amides	$R–CO–NH_2$
amines	$R–NH_2$
alcohols	R–OH
phenols	Ar–OH
carboxylic acids	R–COOH
amino acids	$H_3N^+–CHR–COO^-$

Table 2. Families of Organic Compounds in Order of Increasing Polarity

TLC and Functional Groups

As we have seen, the separation in adsorption chromatography is largely based on differences in polarity and the ability to form H-bonds. Compounds capable of donating an H-bond adsorb to silica more strongly than similar compounds with no H-donor capabilities. For example, carboxylic acids (R—COOH) are adsorbed more tightly than esters (R—COOR'). Alcohols (R—OH) have a stronger interaction with the adsorbent than ethers (R—O—R'). A list of different types of compounds in order of increasing polarity is given in **Table 2**. This list should be regarded only as a rough guide.

In general, compounds that are only H-bond acceptors (such as ethers, esters, tertiary amines, nitrocompounds, etc.) are absorbed less strongly than H-bond donors (alcohols, phenols, carboxylic acids, amines, etc.).

To illustrate the relation between polarity and the success of the separation, we will now consider three hypothetical cases (**Figure 3**). In all cases, the mixture contains two compounds of different polarity. In the first case, both compounds are relatively nonpolar, for example, an alkene and an aromatic hydrocarbon. In this case a polar solvent does not separate the mixture (**A**); instead it moves both compounds similar distances. A nonpolar solvent, on the other hand, separates the two components (**B**). The alkene (less polar) travels farther than the aromatic hydrocarbon (more polar).

In the second case the mixture contains two polar compounds, for example, an ester and an alcohol. Neither compound moves with a nonpolar solvent (**C**), but they are moved and separated by a polar solvent (**D**). The less-polar ester travels farther than the more-polar alcohol.

Finally, the third case is a mixture of two compounds of very different polarities. This type of mixture is relatively easy to separate using nonpolar or medium polarity solvents. For example, a mixture of an aromatic hydrocarbon and a phenol can be separated using a nonpolar solvent; the aromatic hydrocarbon moves farther, and separates from the more polar phenol (**E**). Using a solvent of medium polarity increases the distances traveled by both compounds but they are still separated from each other (**F**). Finally, if a polar solvent is used, both compounds move with similar speeds and no separation is achieved (**G**).

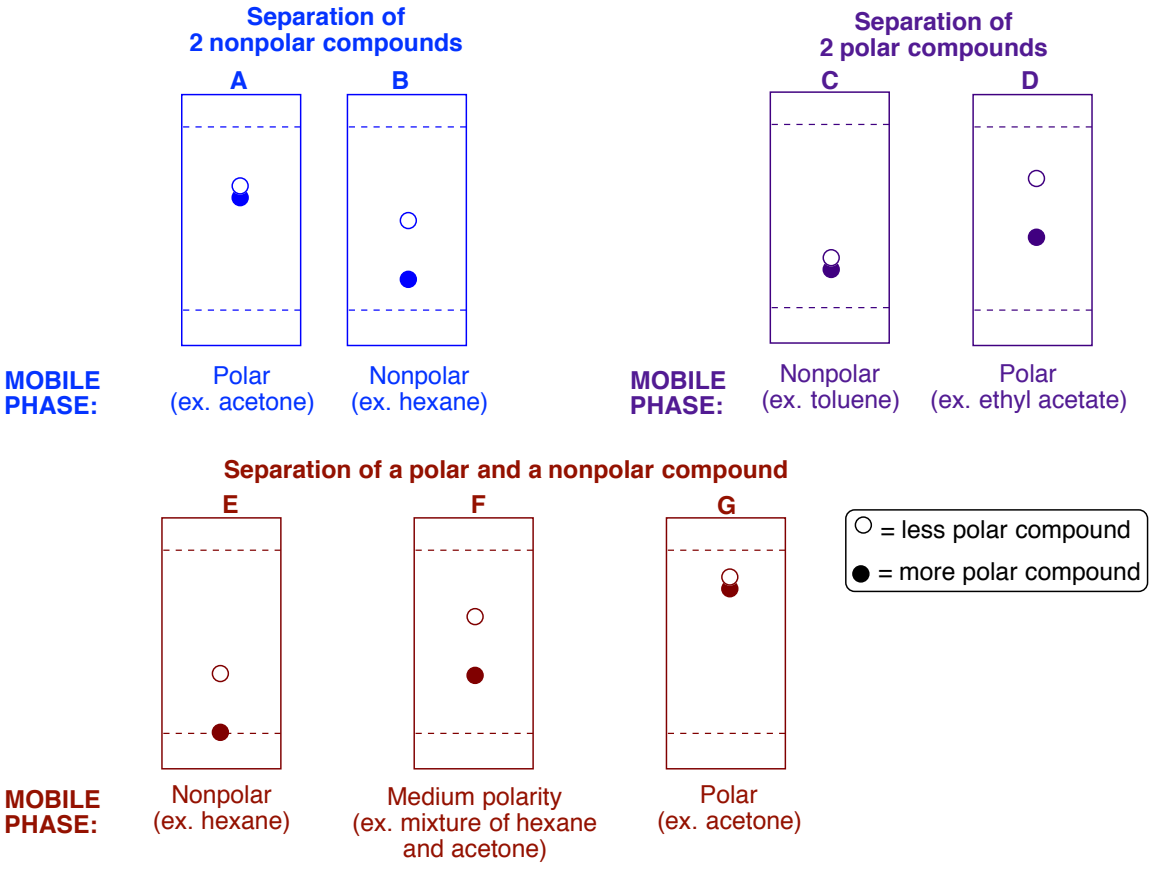

Figure 3. Separation of hypothetical mixtures.

Spotting the plates

A solution of the sample to be analyzed is made in a suitable solvent. The solvent must dissolve the sample completely to ensure an accurate representation of the sample's composition. Volatile solvents such as acetone and methanol are particularly recommended because they evaporate rather quickly once the sample is spotted on the plate.

Ordinary capillary tubes used for melting point determinations and commercial Pasteur pipets are too wide to be used as spotting devices for TLC. Narrow capillary tubes should be used instead. A small aliquot of the solution is taken with a capillary tube (with both ends open) and applied on the TLC plate by touching the adsorbent with the tip of the tube lying flat. Gentle pressure is applied to deliver the liquid, which penetrates the adsorbent. When the size of the spot is about 3-4 mm in diameter, the tube is raised to stop the flow of liquid. The solvent is allowed to evaporate and another application is made, if necessary.

The TLC plates used in the 8L labs are 6.5 x 2.5 cm (**Figure 4**). Spots should be applied at a distance of about **1 cm from the bottom edge of the plate, and about 7 – 8 mm from the sides of the plate**. If more than one sample is analyzed per plate, a distance of about 7 – 8 mm should separate the spots. A mark made with pencil on both sides of the plate should indicate the **origin line** where all the spots are applied. *All marks on the TLC plates should be made with pencil, never with ink because the components in ink may separate during the run and interfere with the TLC analysis.*

A question frequently asked by the students is, "How many applications should we do per spot?" The number of applications per spot would ideally be 1. The actual number depends on the concentration of the solution to be analyzed and the nature of the components. Beyond the overly general principle that the lower the concentration of the sample, the larger the number of applications, no other prediction can be made, because the optimum amount of material required for a good separation varies from sample to sample. It also depends on the mobile and stationary phases used. The recommended concentration range for the solutions is 0.1-5%. One should perform trials with different numbers of applications (1-10) until the optimum number is found. In an ideal situation, at the end of the run the spots should be clearly delineated, circular in shape, and without streaks. If more than 10 applications are needed to see the spots, the sample should be concentrated to avoid repeated applications that may damage the surface of the adsorbent.

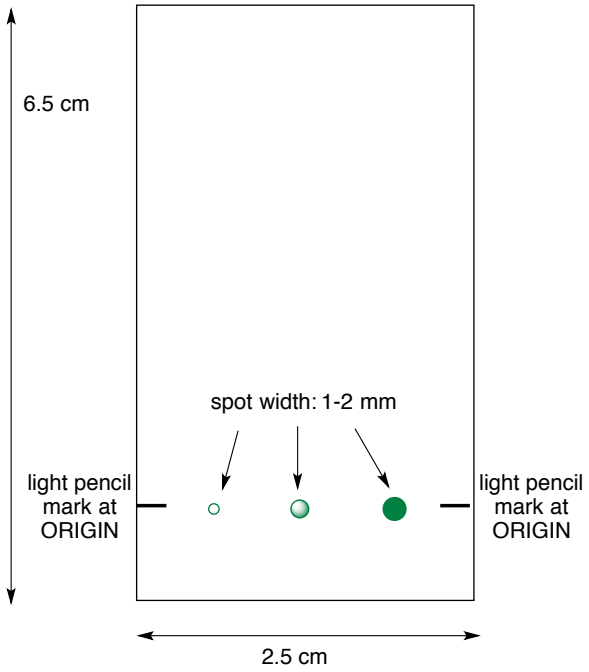

Figure 4. Diagram of TLC plate

Analyzing the Chromatogram

Once the spots have been visualized, the distance traveled by the spot from the origin is measured along with the distance traveled by the solvent front. The ratio between these two distances is called **ratio to the front** or **retention factor (R_f)**:

R_f = (Distance traveled by spot) / (Distance traveled by solvent)

The distance traveled by the sample is measured from the origin to the middle of the spot. With very large and ill-defined spots, as for example, spots with "tails," R_f values are meaningless because the middle of the spot varies with the amount of sample applied to the plate. If the spot streaks or runs with a tail, it is an indication that **too much** sample was applied. A decrease in the volume of sample spotted should be tried first; if this does not correct the problem, then a different adsorbent/solvent system should be tried.

The ratio to the front depends on several variables such as:

- the thickness of the adsorbent;
- the nature of the stationary phase and its degree of activation;
- the mobile phase;
- the amount of material applied.

Spinach Pigments – Experiment 3 Background

The most prominent types of plant pigments are **chlorophylls, carotenoids, flavanoids,** and **tannins.** Chlorophylls contain a ring system formed by four pyrroles linked by four methine bridges, a Mg^{2+} ion in its center, and a long nonpolar hydrocarbon chain ($C_{20}H_{39}$). Chlorophylls are cousins of other biologically important molecules such as vitamin B_{12} and the heme found in hemoglobin; they all have a tetrapyrrole ring system. There are two main types of chlorophylls present in higher plants, **a** and **b** (**Figure 5**). Chlorophyll **a** is more abundant than chlorophyll **b** by a ratio of 3 to 1. The only structural difference between them is that a methyl group in **a** has been replaced by a formyl group, CHO, in **b**.

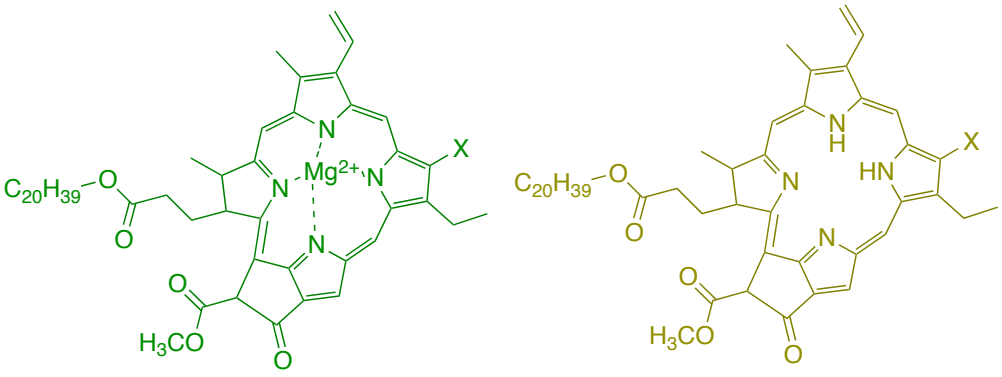

Figure 5. Structures of chlorophylls and pheophytins.

Chlorophylls are found in the chloroplast in association with small proteins. These protein-chlorophyll complexes are crucial in photosynthesis. The chlorophyll molecule acts as an antenna for visible radiation. The light absorbed is used to make carbohydrates and oxygen using carbon dioxide and water as raw materials.

Chlorophylls are labile compounds. In the presence of acids the central Mg^{2+} ion is easily replaced by protons and **pheophytins** are produced. In the summer months the leaves produce and degrade chlorophylls at a fast rate. Every fall, as the daylight dwindles, less chlorophyll is produced but its degradation continues, giving way the colors of autumn: yellows, reds, purples, and browns. These colors are largely due to the other leaf pigments, such as carotenes, flavins, and tannins (**Figure 6**), which are masked by an abundance of chlorophyll during the summer months.

Carotenes are polyunsaturated hydrocarbons that belong to the family of terpenes. Carotenes have 40 carbon atoms per molecule. They are found in the chloroplasts in association with chlorophylls and proteins where they, too, play an important role in photosynthesis. They are auxiliary pigments in the light-harvesting process and protect the chloroplasts against photooxidation.

β-Carotene, found in carrots, sweet potatoes, and green leaves, is one of the most abundant members of the family, and the precursor of vitamin A. Other members include α-carotene, an isomer of β-carotene with only half of its vitamin A activity, and lycopene, a red pigment found in tomatoes and watermelons that has no vitamin A activity. Carotenes are easily oxidized, especially in processed food, and must be protected from light and air to maintain their dietary value. The oxygen-containing products derived from carotenes are called **xanthophylls.** Xanthophylls and carotenes form the **carotenoid** family. The most abundant xanthophylls found in green leaves are lutein, violaxanthin, and neoxanthin (**Figure 6**).

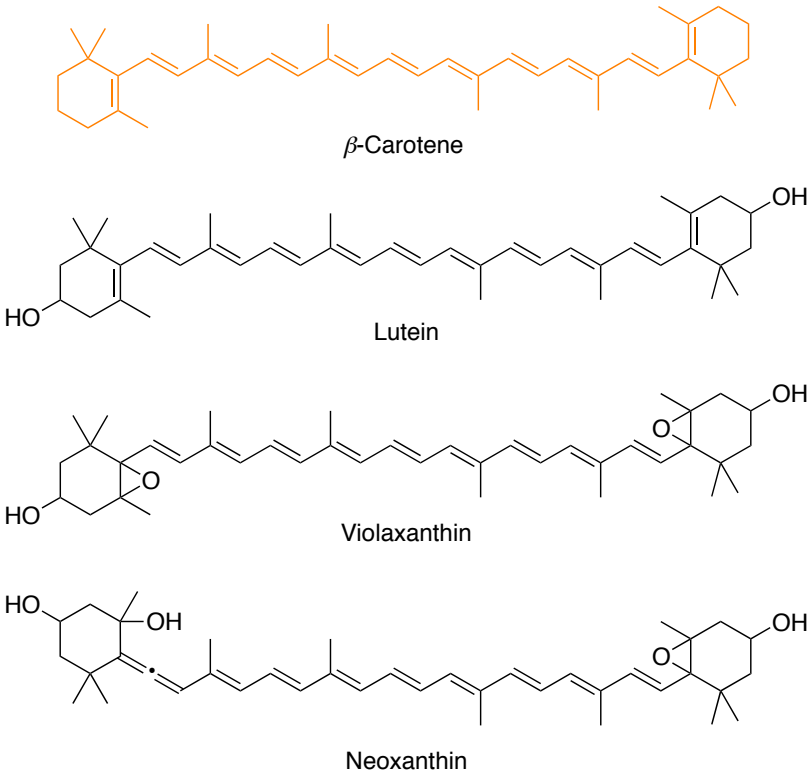

Figure 6. Structures of select carotenes and xanthophylls.

In this experiment students will isolate pigments from spinach leaves via solid-liquid and liquid-liquid extraction. The procedure given below can be applied with minor modifications to the extraction of chloroplast pigments (chlorophylls and carotenes) from almost all types of leaves. It can also be used to isolate the pigment from red, yellow, or green bell peppers. As already mentioned, the pigments from green leaves normally include carotenoids (β-carotene, lutein, violaxanthin, and neoxanthin), chlorophylls (a and b), and pheophytins (a and b).

Pheophytins may be present in the leaves, but they may also be generated during the extraction process. As the cell membranes are broken, naturally occurring acids from the cytoplasm and other cell organelles come in contact with chlorophylls (from the chloroplasts), producing pheophytins.

The extraction is performed by first crushing the leaves with methanol, and then with a mixture of methanol and hexanes (two immiscible solvents). The first extraction with methanol breaks the cell membranes and removes water. This extract is discarded because it is poor in pigments. The second extraction with methanol-hexanes removes the pigments that go preferentially to the hexane layer. Methanol helps in detaching the pigments from their cellular complexes with proteins.

The extracts will be analyzed using TLC plates with silica gel as the stationary phase. You will investigate four different mobile phases and determine their power in separating the **chlorophylls (green), the xanthophylls (yellow), the pheophytins (green-gray), and the carotenes (yellow-orange).** You will observe that not all the mobile phases are able to move the pigments from the origin and other mobile phases will move only certain components.

Notebook Preparation

- *Purpose*: one-sentence description plus the scheme, which begins with the spinach leaves. Include the extraction solvents over the arrow (read below) and write the names of the potential pigments on the right side of the arrow.
- *Reagent Table*: hexanes, methanol, and ethyl acetate (bp, density, and hazards). Qualitative analysis in this experiment – no need to include masses. Include a list of materials (chemicals, glassware, equipment, etc.) underneath the table.
- *Procedure*: Hand-written, step-wise procedure in your own words; diagrams, pictures, flow chart encouraged
- *Safety & Clean-up* – Copy the table at the end of the procedure into your notebook.

Bring your own spinach leaves (roughly a large handful). Any unused spinach brought into the lab must be discarded (don't expect to take it home and eat it!).

PROCEDURE – students work individually

Vegetable Extract

Weigh 10 g of fresh spinach leaves and chop them using scissors. Place the spinach in a large mortar, add 12 mL of methanol, and crush the leaves with the pestle for about 3 minutes. This can get messy if you are careless. Use common sense in your crushing technique! With the aid of the pestle or a spatula, squeeze the spinach against the sidewall of the mortar to remove as much methanol as possible. Use a glass stir rod to decant the liquid using a pipet into a 250-mL Erlenmeyer flask labeled "methanol-water" and set aside. The contents of this flask will eventually be discarded.

The following steps should be performed in a fume hood. Extract the remains of the leaves with a mixture of 15 mL hexanes and 5 mL methanol, crushing the tissue with the pestle for about 2-3 minutes. The extract should be deep green. Leaving behind as much solid as possible, use a glass stir rod to decant the liquid (a mixture of hexanes and methanol) directly into a Buchner funnel set up for vacuum filtration in the fume hood. The vacuum should be low to prevent too much solvent from evaporating. Use an additional 2 mL of hexanes to rinse the spinach a final time and aid the transfer. Collect the filtrate directly in a small filter flask and discard the filter paper. Clean the funnel immediately before proceeding to the next step or it will make any future experiment turn green! Use a small amount of methanol as instructed by your TA to initially rinse the funnel into the waste before washing in the sink.

Still in the fume hood, transfer the filtrate into a screw-cap test tube and add 5 mL of water. Mix, vent, and allow the layers to separate. If a deep green upper organic layer is not apparent, add 5 mL of hexane, being careful not to overflow the test tube. Remove the lower aqueous layer using a long-stem pipet with bulb and transfer into the Erlenmeyer flask labeled "methanol-water." Wash the remaining hexane layer with 5 mL of water (add 5 mL of water, mix, vent, separate). Collect the aqueous layer along with any emulsion present in the "methanol-water" flask. Add a small spatula-full of granular sodium sulfate to the test tube dry the organic layer. Cap the test tube and swirl occasionally. After about 5 minutes, filter the suspension by using a clean and dry micro-funnel (about 2.5 cm in diameter) and a cotton plug. Collect the filtrate in a dry 50-mL round-bottom flask. Your TA will operate the rotary-evaporator (rota-vap) to evaporate the solvent from the extract until the volume is approximately 2-3 mL (size of a nickel). The color of the final extract should be deep green. Transfer the liquid by pipet to a labeled scintillation vial or test tube.

TLC Analysis

Locate, but DO NOT REMOVE, the TLC jars in the fume hood containing solvents:

- Hexanes
- 9:1 Hexanes-Ethyl Acetate (9:1 Hex/EtOAc)
- 7:3 Hexanes-Ethyl Acetate (7:3 Hex/EtOAc)
- Ethyl Acetate

Keep the jars covered with their lids except when opening to add your plate. ***Students are not permitted to add or remove any solvent from the TLC jars.*** Consult TA for assistance with adjusting solvent levels if necessary.

Obtain four silica gel TLC plates (2.5 x 6.6 cm). *Be extremely careful not to bend the plates or to scratch the silica surface (the chalky side). Handle plates by the edges.* Label them with the name of solvent on the very top of the plate with light pencil markings. Make two small pencil marks on either side of the plate to indicate the origin on the adsorbent side of the plate at a distance of about 1 cm from the bottom edge (see lecture handout).

Determining the Optimum Number of Applications

The "9:1 Hex/EtOAc" plate will be used to determine the optimum number of applications needed to visualize the separation of pigments. On this plate, the sample will be applied in three different lanes, each one with a different number of applications (1, 4, and 7). Dip a narrow capillary tube (not a melting point tube) in the extract; the liquid will rise through capillary action. Apply the liquid to the plate by gently and *briefly* touching the plate with the tip of the capillary tube. Raise the capillary tube to stop the flow of liquid when the diameter of the spot is about 1-2 mm. The spot should be very tight and small and at least 0.5 cm from the side edge of the plate. On the same plate, apply a second and a third spot at a distance of about 0.5 cm from each other. The number of applications for the second spot should be 4, and for the third spot 7. Allow the solvent to evaporate between applications. Failure to do so will result in very large spots.

Using tweezers, hold the plate next to the jar to ensure the *spots are above the solvent level* of the 9:1 Hexanes-Ethyl Acetate jar. If the spots are too low, the sample will dissolve in the solvent and contaminate other experiments. *Gently lower and place* (DO NOT drop) the TLC plate in the chamber with 9:1 Hexane-Ethyl Acetate. *Without moving the jar*, allow the solvent to run up the plate to a height about 0.5-1 cm from the top of the plate. ***Keep TLC chambers covered and in the fume hood***. Remove the plate from the chamber (use tweezers). *Immediately mark the solvent front with pencil* and allow the solvent to evaporate in the fume hood before bringing to your bench.

Sketch the TLC plates to scale in your lab notebook, paying special attention to the colors of the spots. Are they yellow, pale green, deep green, gray, orange? Key for spot identification (structures on p. 5-6): **chlorophylls (green), the xanthophylls (yellow), the pheophytins (green-gray), the carotenes (yellow-orange).** Notice any color spot at the origin. This analysis

should be done without delay because the colors of the spots fade very quickly (within hours). Determine how many applications gave you the best results, or in other words, clear and well-delineated spots without streaking. If all spots are too faint to be seen, the extract should be concentrated by further evaporation of the solvent. If the spots run with tails, too much sample has been applied. Either reduce the number of applications or dilute your extract with hexanes.

Analyzing your Extract

Spot the extract on the remaining plates, using the optimal number of applications determined on the 9:1 plate. Only one lane is necessary for each of the remaining plates. *Develop all the plates at the same time (to the best of your ability).* Calculate the R_f values for each pigment in each solvent. Decide which solvent gives the best separation (most spots). Be sure to write down in your notebook all the data that are necessary to reproduce your R_f values (stationary phase, solvent, number of applications). Draw the plates in your notebook and indicate colors. Check first with your TA then dispose of all plates in the solid waste. Students who keep their plates will lose points. Dispose of your TLC plates and **consider the in-lab questions before leaving the lab.**

Clean-up and Waste

This has the potential to be a very messy experiment if you're not careful. You may not be able to see the dark green extracts on the black counters, but it's there!
Clean and dry your work stations.
When you dry with paper towels, *make sure it's a clean wipe, not a green wipe!*
That's how you know it's clean ☺

General Clean-up	-*Thoroughly wipe down bench tops* - Wash glassware and mortar & pestles (see below) with soap & water. TA's will finish clean-up with methanol. - Clean vacuum filter funnel immediately after using.
Organic Solvents – TLC Waste	- Contents of "methanol-water" flask - Mobile phase from TLC chambers (only if asked)
TLC – Solid Waste	- All used TLC plates (return unused to TA) - Sodium sulfate and cotton plug - Capillary tubes - Pipets - Let remains of spinach leaves dry in hood in mortar, then put in waste, NOT in sink
Reclaimed Hexanes	- Contents of the rota-vap trap
Safety: Hexane and ethyl acetate are flammable. Keep TLC jars in the fume hoods.	

Pre-lab Questions

1. Compare and contrast GC and TLC according to the following criteria:
   - State the classification of each chromatographic method;
   - Explain the differences in stationary and mobile phases (states of matter);
   - State the property that each type of separation is based on;

2. Arrange the following solvents/solvent mixtures in order of increasing polarity: hexanes, ethyl acetate, 7:3 hexanes-ethyl acetate, 9:1 hexanes-ethyl acetate.

3. Suppose a non-polar compound is spotted on a TLC plate. Which solvent from #2 will move the compound the farthest from the origin? Which solvent from #2 will move the compound the least from the origin?

4. What would happen if a TLC plate was placed in a jar where the solvent level was above the level of the origin (sample spot)? What should you do if you inadvertently spotted your plate too low (aside from consulting your TA)?

5. Why should ink be avoided in marking TLC plates?

6. Arrange the following compounds in order of increasing polarity: lutein (L), neoxanthin (N), β-carotene (C), violaxanthin (V). Which should display the highest R_f? Which should display the lowest R_f?

In-Lab Questions

1. What roles does methanol play in the first two extractions?

2. Draw the TLC plates both in your lab notebook and then again in your final report. Indicate colors (brownie points for using crayons or colored pencils in the report) and identify as many pigments as possible on each plate (chlorophylls, xanthophylls, pheophytins, and carotenes – see p E4-3). Recall that not all pigments will appear as separate spots on all plates.

3. Calculate the R_f values for each spot on each plate and tabulate your results. Include the pigment identification (in-lab #2) for each spot. There is no need to calculate the R_f of a 'smear', but note in the table that a smear did occur. As you re-create the following table in a word processing document, it may be appropriate to 'merge' cells when spots overlap. Alternatively, you can list the same R_f value for several pigments if they overlap.

Table x. TLC Analysis of Spinach Pigments

Solvent → ↓ Pigment	Hexanes (R_f)	9:1 Hex/EtOAc (R_f)	7:3 Hex/EtOAc (R_f)	EtOAc (R_f)

* More rows may be needed.

4. Briefly discuss the overall separation power of the solvent using the points below based on your results. Consider the polarity of the solvents and pigments in your discussion.

(a) Explain the differences observed in the plates run with hexanes and 9:1 hexane-ethyl acetate.
(b) Was ethyl acetate a good solvent to separate the pigments?
(c) Which solvent was best to separate just the carotenes from the other pigments?
(d) Which was the optimal solvent for separation of the most pigments (greatest number of individual spots observed)?

Abstract

Consult the technical writing guidelines to write a draft of the abstract after completing the in-lab questions and before leaving lab, time permitting. Either way, plan ahead to get help with the abstract by going to TA office hours.

The main result in this experiment is determination of the **optimal TLC solvent**, being the one that separated spinach extract into the most spots. Retention factors (R_f) should not be included in the abstract. Use one sentence to comment on whether results were as expected – what polarity of solvent would you expect to best separate the spinach pigments based on their polarity?

Palleros, D. R. "TLC Analysis of Vegetable Extracts," Experimental Organic Chemistry, **2000**. Wiley: Hoboken. p. 190-196.

Exp 3 Extraction and Analysis of Pigments From Spinach Leaves Name _____

Section Day _____ Time _____ TA Name _____

CHEM 8L GRADING RUBRIC – Use as cover page for report

SECTION	INSTRUCTOR COMMENTS	POINTS ASSIGNED
IN-LAB QUIZ		**/ 5**
LAB REPORT		
ABSTRACT One paragraph, four-six sentences: Purpose, procedure, main result(s), and conclusion(s).		/ **20**
INTRODUCTION Each pre-lab question is addressed complete sentences. Structures and calculations are hand-written, where appropriate.		/ **30**
RESULTS The main results are stated, as outlined in the in-lab questions, using complete sentences.		/ **40**
NOTEBOOK PAGES Proper format: reaction scheme, chemical info table, procedure, waste and clean-up procedure.		/ **20**
NEATNESS AND ORGANIZATION Proper order and format (see syllabus for full descriptions of each section).		/ **5**
LAB TECHNIQUE & CLEAN UP Lab space left clean, proper technique, instructions followed, checked in with TA before leaving.		/ **5**
	LAB REPORT TOTAL	**/ 125**