# Experiment 3 – TLC Analysis of Spinach Pigments

## Learning Objectives

- Understand principles behind solid-liquid extraction, liquid-liquid extraction, and thin-layer chromatography (TLC)
- Critical analysis of extraction & TLC technique
- Analyze data to assess components of extract and success of experiment
- Understand the role polarity plays in extraction and TLC

---

**How to Prepare for this Lab + Assignments -** Follow Canvas Exp 3 Module…

## Before Lab

- Read this PDF – background, procedure, safety, pre-lab and in-lab questions
  - Option to listen to **Podcast** = Caitlin reads the lab ☺
- Attend **lab lecture** and take notes on **templates**
- Practice the lab online *via* **Slugs@home** - sites.google.com/ucsc.edu/slugshome/home
- **Pre-lab questions** incorporated into **Pre-lab Quiz** – check Canvas for due date

## Lab Notebook Preparation – *Required before lab*

- Use the **worksheet** to prepare your **lab notebook**, one day at a time…
- **Purpose:** brief summary of the main lab goals and structures of citrus oil components
- **Reagent Table** – add chemical properties; Wikipedia is a reliable source for chemical info
- **Procedure with Diagrams** – hand-drawn using procedure in this PDF, Slugs@home, & class notes
  - Instructions, sketches, & labels for all **equipment, chemical names** with **amounts**, & **transfers**
  - Format: Break it up with flow charts, bullet-points, comic strip, and/or whatever works for you!

## During Lab

- Check the **safety rules** to dress for lab and arrive a few minutes early to **Thimann Labs**
- **Pre-lab talk:** tips for success and open Q&A
- Show your **lab notebook pages** to your TA
- Perform the experiment with a partner, fill out data & observations in **lab notebook**

## After Lab – each partner submits separate, individual assignments

- Upload **Notebook Pages** to Canvas by midnight on lab day – graded on completeness / participation
- Complete & upload the **Lab Report** on GradeScope (GS) – due date on Canvas
  - Guidelines at end of this document

## BACKGROUND PART 1: Spinach Pigment Extraction

The most prominent types of spinach 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 tetrapyrrole cousins of other biologically important molecules such as vitamin $B_{12}$ and the heme found in hemoglobin. There are two main types of chlorophylls present in higher plants, **a** and **b** (**Figure 1**). 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 aldehyde group, CHO, in **b**.

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. Think about that for a minute: *every carbon in sugar comes from $CO_2$ in the air (woah)!*

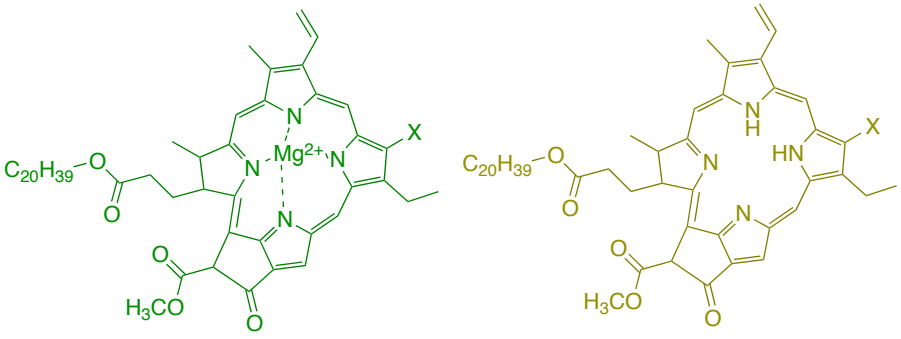



**Figure 1.** Structures of chlorophylls and pheophytins.

**Chlorophylls** are labile compounds, meaning that 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 2**), 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 play an important role in photosynthesis. They are auxiliary pigments in the light-harvesting process and protect the chloroplasts against photooxidation.

**$\beta$-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 $\alpha$-carotene, an isomer of $\beta$-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 2**).

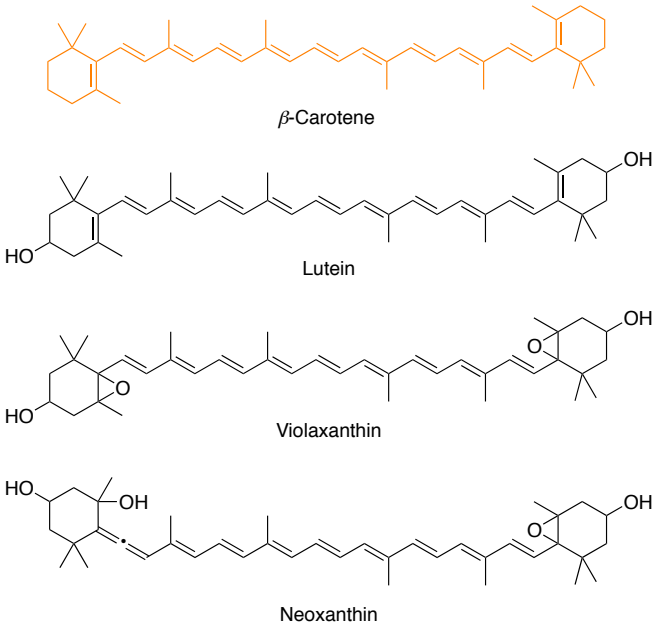



**Figure 2.** Structures of select carotenes and xanthophylls.

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 methanol-hexanes extract (solution of pigments) is separated from spinach pulp (mainly cellulose) by gentle vacuum filtration. The extract is washed several times with water to remove water-soluble pigments, which would interfere with TLC analysis. The extract is then dried with sodium sulfate ($Na_2SO_4$) and filtered. Depending on the concentration and amount of solvent remaining, it may be necessary to concentrate further using a rotary-evaporator (rota-vap).

## BACKGROUND PART 2: Thin-Layer Chromatography (TLC)

**Thin layer chromatography (TLC)** is a versatile and rapid form of qualitative analysis used regularly in synthetic organic chemistry labs. Analyses of complex mixtures can be successfully carried out in minutes with relatively inexpensive equipment. The composition of a mixture can be assessed relative to known standards. In TLC the separation takes place on a thin layer of **solid stationary phase** spread on a solid support such as a glass, aluminum, or plastic plate. A **liquid mobile phase,** an organic **solvent,** moves along the plate.

The principles behind this technique are not terribly different from GC. Separation is based on selective affinity for mobile and stationary phases. In GC, the mobile phase is a gas and the stationary phase is a liquid. In TLC, the mobile phase is a liquid and the stationary phase is a solid. 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 polarities to determine the optimal separation of the pigments found in spinach extracts on a TLC plate.

TLC separation of a mixture is based on relative polarity of components. The sample used for TLC is a nonvolatile liquid or a solution in a volatile solvent, in our case spinach pigments in hexane. It is applied to a polar silica ($SiO_2$) plate, the **stationary phase**, with the aid of a capillary tube. The sample is spotted about 1.5 cm from the lower edge of the plate, referred to as the **origin** (**Figure 3**). *The origin is marked with a pencil, never with ink because the components in ink may separate during the run and interfere with analysis.* 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 solvent should be lower than the spot on the plate, or else the sample will dissolve in the solvent. The chamber is capped to avoid evaporation of the solvent and to ensure liquid-vapor equilibrium inside.

The sample components are selectively carried up the TLC plate by the solvent *via* capillary action. Different compounds travel at different speeds because they have specific interactions with the polar stationary phase. The TLC chamber is kept very still while the plate is running to allow the components to travel in one straight lane. Various solvents and solvent mixtures are used to determine optimal separation conditions. 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. Regardless of solvent polarity, the separation order is the same because the stationary phase is always polar.

*Non-polar compounds have a lower affinity for the polar stationary phase and travel farther from the origin than polar compounds.*

In **Figure 3**, the original sample spot has been separated into two spots after interaction with the mobile phase, indicating that the sample contains at least two different compounds.

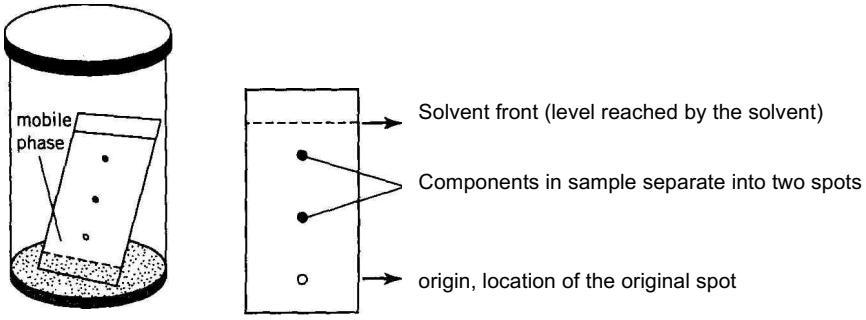



**Figure 3.** TLC chamber and developed plate

**Absorption** 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 **absorbent.** Absorption is strictly a surface process that depends on electrostatic forces between absorbent and sample. These forces arise from intermolecular forces including **dipole-dipole** and **ion-dipole interactions** as well as **H-bonds.** The surface of the absorbent is far from being perfectly smooth; it has crevices and crests with centers of positive and negative charge density. The solute binds to the absorbent through electrostatic attraction between its own centers of charge density and those on the surface of the absorbent. The places on the surface of the absorbent where the sample binds are called **binding sites**. Ions and molecules with permanent dipole moments readily bind to the absorbent.

The most commonly used absorbent 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 $SiO_2$ particles used for TLC have an average diameter of about 0.025 mm (teeny tiny!).

To understand how separation occurs on the surface of the absorbent, we should look closer at the interactions between the **absorbent** and **solutes** and between the **absorbent** and **mobile phase. Figure 4** displays a close-up view of a TLC plate cross-section showing the interactions on the surface of the absorbent. 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 absorbent, *solute and mobile phase molecules must compete* with each other to bind to the absorbent; the stronger the interactions, the tighter the binding. In **Figure 4a**, the thickness of the lines between the shapes and absorbent indicates the strength of the interaction. The oval molecules have a very strong interaction with the absorbent 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.

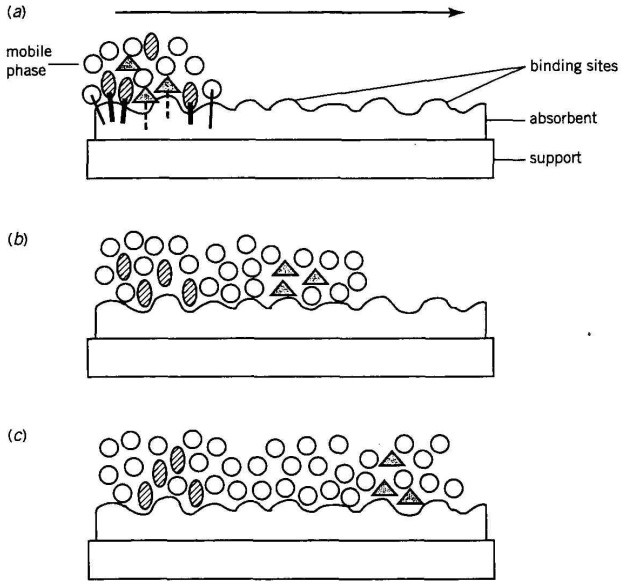



**Figure 4.** Separation of two compounds on a TLC plate (ovals and triangles). The arrow indicates the direction of the mobile phase flow (see text).

As the mobile phase ascends along the plate, *the mobile phase molecules close to the absorbent compete with the solutes for the binding sites.* If the interaction between mobile phase and absorbent is stronger than the interaction between absorbent and solutes, such as in the case of the triangle molecules, the mobile phase displaces the solute molecules from their binding sites, moving them farther away from the surface of the absorbent. As the mobile phase keeps flowing, these solute molecules are carried to a new location on the plate (**Fig. 4b**). Because the mobile phase has stronger interaction with the absorbent, 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. 4c**).

If the interaction between solute and absorbent is stronger than the interaction between mobile phase and absorbent, 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 absorbent. However, the stronger the interaction between absorbent and solute, the more difficult it is for the solvent to move the solute. As shown in **Figure 4c**, the oval molecules, which display the strongest affinity for the absorbent, stay very close to the place where they were originally spotted.

## Selection of solvent conditions

**Table 1.** Solvents and relative polarity

| Solvent | Dielectric constant |
|---|---|
| Hexanes | 1.89 |
| Cyclohexanes | 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 |

The *polarity of the mobile phase* plays a crucial role in the separations. 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. Solvent mixtures are very commonly used 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. A 7:3 mixture of hexanes / ethyl acetate is more polar than a 9:1 mixture, for example.

## TLC and Functional Groups

**Table 2.** Functional Groups and Polarity

| 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–$COO^-$ |

As we have seen, the separation in absorption 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 absorbed more tightly than esters (R—COOR'). Alcohols (R—OH) have a stronger interaction with the absorbent 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. The number of carbons in a molecule have a significant affect on polarity; more carbons make a compound less polar. In general, compounds that are H-bond donors (alcohols, phenols, carboxylic acids, amines, etc.) absorb more strongly than those that are only H-bond acceptors such as ethers, esters, ketones, etc.

To illustrate the relation between polarity and the success of the separation, consider three hypothetical mixtures, each containing two compounds of different polarity. In **Figure 5**, both compounds spotted on plates **A** and **B** are nonpolar, for example, an alkene and an aromatic hydrocarbon. A polar solvent does not separate the nonpolar mixture well (**Fig. 5 A**). Both compounds travel similar distances since the polarity of the solvent overrides the polarity of the plate. A nonpolar solvent, on the other hand, separates the two components (**Fig. 5 B**) due to greater interaction between non-polar solute and solvent molecules. The less polar alkene less polar travels farther than the slightly more polar aromatic hydrocarbon.

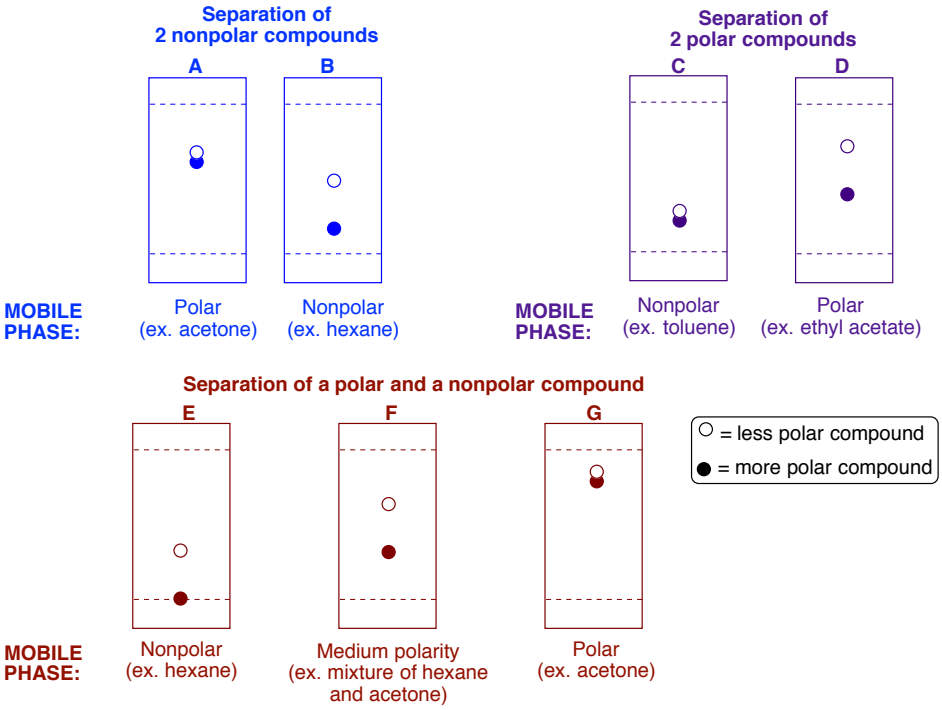



**Figure 5.** Separation of hypothetical mixtures.

The mixture second mixture on plates **C** and **D** contains two polar compounds, for example, an ester and an alcohol. Neither compound moves with a nonpolar solvent (**Fig. 5 - C**) since the polar compounds have a greater affinity for the polar plate. Both travel and separate when a polar solvent is used (**Fig. 5 - D**). The less polar ester travels farther than the more polar alcohol, which sticks to the plate.

The third case is most applicable to the separation of spinach pigments with a mixture of compounds of very different polarities. This type of mixture is best separated by medium polarity solvents, or a mixture of non-polar and polar 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 the more polar phenol stays at the origin (**Fig. 5 - E**). It is ideal for both compounds to move at least slightly from the origin to ensure complete separation, especially in the case of spinach pigments where there are more than two components. Using a solvent of medium polarity increases the distances traveled by both compounds (**Fig. 5 F**). A polar solvent causes both compounds to move at similar speeds and no separation is achieved (**Fig. 5 G**). The polarity of the solvent overrides that of the plate and both compounds travel upward, with the non-polar compound moving slightly more.

## TLC CALCULATIONS: 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 in 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 absorbent or solvent system should be tried.

The **$R_f$ value depends on** several variables…
- the thickness of the absorbent
- the nature of the stationary phase and its degree of activation
- the mobile phase
- the amount of material applied

## BACKROUND SUMMARY

Spinach pigments are extracted from fresh leaves with methanol and hexanes. Water-soluble pigments are removed from the solution by liquid-liquid extraction. The solution of pigments in hexanes is analyzed by thin-layer chromatography (TLC). The less polar carotene pigments travel farther up the TLC plate than the more polar xanthophylls. Chlorophylls and pheophytins are of medium polarity and travel more than the xanthophylls but less than the carotenes. Students investigate four different mobile phases (solvent mixtures) and determine their power in separating **chlorophylls (green), xanthophylls (yellow), pheophytins (green-gray), and carotenes (yellow-orange).** Optimal separation is achieved with a mobile phase that separates spinach pigments into 4-8 colorful, distinct spots on the TLC plate.

Supplemental Materials

- Filtration, extraction, and drying: Mohrig, J. R. *Techniques in Experimental Organic Chemistry, 4th Edition,* Chapters 9 – 11.
- Exp 3 Slugs@home labsite & Canvas / JoVe pre-lab videos
- Experiment adapted from Palleros, D. R. “TLC Analysis of Vegetable Extracts,” Experimental Organic Chemistry, **2000**. Wiley: Hoboken. p. 190-196.

**PROCEDURE** - spinach provided in the lab

## PART 1. Solid-liquid and liquid-liquid extraction

Weigh 10 g of fresh spinach leaves and chop them using scissors. Obtain 12 mL of methanol using a graduated cylinder and pipet. Place the spinach in a large mortar along with the methanol and crush the leaves with the pestle gently for two 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 into a 250-mL Erlenmeyer flask labeled "methanol-water" and set aside. The contents of this flask will eventually be discarded.

FUME HOOD PHASE 1: 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. Transfer the filtrate into a labeled screw-cap test tube, add 5 mL of water, and bring the contents back to your benchtop. Clean the funnel immediately before proceeding to the next step or it will make any future experiment turn green! Use a small amount of ethanol as instructed by your TA to initially rinse the funnel into the waste before washing in the sink.

FUME HOOD PHASE 2 (liquid-liquid extraction): When enough students are done with PHASE 1, take your turn back at the fume hood. 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 ($Na_2SO_4$) to the test tube to dry the organic layer. Cap the test tube and swirl occasionally back at your benchtop. After about 5 minutes, filter the suspension in the fume hood by using a clean and dry micro-funnel (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) if necessary 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.

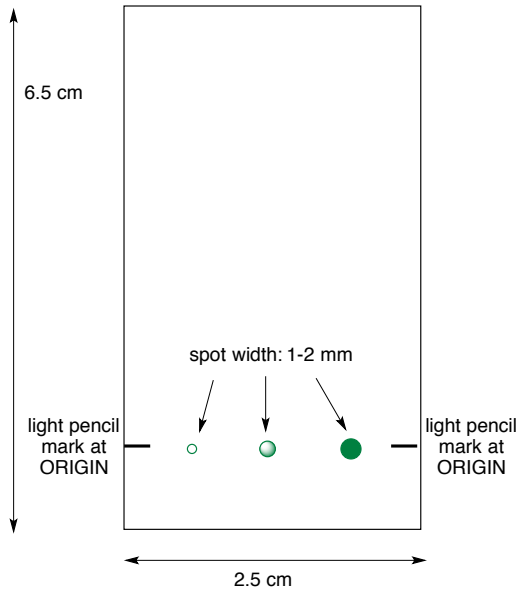
## PROCEDURE PART 2. TLC Analysis

*Locate, but DO NOT REMOVE, the TLC jars in the fume hood* containing each mobile phase (solvent)…

- 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 absorbent side of the plate at a distance of about 1 cm from the bottom edge.




### Determine 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 in the extract (not a melting point tube); 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 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 and using the same capillary tube, 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. *Conserve - only one capillary tube is needed per group for the entire lab!*

FUME HOOD PHASE 1: Using tweezers, hold the plate next to the jar to ensure the *spots are above the solvent level* of the 9:1 Hex / EtOAc 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 Hex / EtOAc. *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 it 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, or orange? See p. 6-7 for structures: **chlorophylls (green),** the **xanthophylls (yellow),** the **pheophytins (green-gray),** and **carotenes (yellow-orange).** Notice and record 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 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.

## Analyze the Extract

FUME HOOD PHASE 2: 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 remaining 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 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**<br>**This has the potential to be a very messy experiment so take proper precautions.**<br>**Clean and dry your work stations please.** | |
|---|---|
| *TLC – Solid Waste* | - All used TLC plates (return unused to TA)<br>- Sodium sulfate, used cotton, capillary tubes, pipets<br>- Let remains of spinach leaves dried in hood in mortar, then put in waste, NOT in sink or trash<br>- Capped, labeled vial of spinach extract |
| *Organic Solvents – TLC Waste* | - Contents of "methanol-water" flask<br>- Mobile phase from TLC chambers (only if asked)<br>**- Rinse all glassware, including mortar & pestles, with ethanol into the waste before washing in the sink.** |
| *General Clean-up* | - Remove gloves when washing glassware<br>- Wash glassware and mortar & pestles with soap & water after rinsing with solvent. TA's will perform a final ethanol wipe.<br>- Clean vacuum filter funnel immediately after using.<br>-*Thoroughly wipe down bench tops* |
| **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:
   - Explain the differences in stationary and mobile phases (states of matter);
   - State the property on which each type of separation is based.

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 $R_f$?

---

Take the **Canvas Exp 3 pre-lab quiz** *before* your enrolled section.

- The quiz incorporates the questions below - the questions may be reworded.
- **Be prepared with your responses to the pre-lab questions *before* starting the quiz.**
- There is a 20-minute time limit on the quiz and you get two attempts.
  - Complete each quiz in one sitting- you can't save and come back later.
  - If you choose to re-take the quiz, your grade will be the highest of the two attempts.

**Though we encourage collaboration in this class, this is an individual quiz.**

- The responses should be a product of your original work so that you are assessed on *your* understanding of the material.
- Sharing your quiz or the correct responses in any format (screenshots, email, CHEGG, social media, text, carrier pigeon, etc.) is in violation of the UCSC academic integrity policy.
  - Students in violation of this policy will go through the Academic Misconduct process.
    - *It's not worth the risk and is the least favorite part of Caitlin's job!*

# LAB REPORT

## Abstract

Refer to the writing worksheet for guidance in writing one paragraph: purpose, methods (NOT the procedure!), results, and conclusion.

The main result in this experiment is the 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?

## In-Lab Questions

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

2. Draw the TLC plates to scale. 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). 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 table from the worksheet 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.

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)?

5. Describe three procedural mistakes you actually made in the lab (aside from spilling) and/or what could have gone wrong (check out the mistakes on Slugs@home). Include at least one mistake from TLC. Explain what went wrong, how or why it affects the experimental results, and how to correct the mistake – does it require restarting the lab or can the experiment be saved?