Chemistry 105
The Chemistry of Color
Laboratory Meeting 6

Agenda

- Introduction to the Experiments, Homework 5 Help
- *Experiment: Isolation and Spectra of Plant Pigments*
- *Experiment: Fiber-Reactive Dyes*
- *Experiment: Paper Marbling*
- Data Analysis and Post Lab Questions

Chromatography

As we learned in discussion 2, many substances we encounter every day are mixtures. In many cases, it is desirable to separate such mixtures. The smelting of ores to obtain desired metals, the extraction of odoriferous compounds from plants for use in fragrances, and the distillation of alcohol are all examples of separation processes.

The separation of closely related substances is often difficult, but can be accomplished using family of separation techniques called chromatography. Chromatography literally means *color writing* in Greek and was coined by the Russian botanist M. S. Tswett to describe his process of separating plant pigments. Similar processes can be used to separate non-colored substances as well. Today, the term chromatography refers to any number of similar separation techniques.

Today, we will be using a process know as thin-layer chromatography (TLC). The basic theory behind this process is as follows. A mixture of substances dissolved in a solvent forms a mobile or moving phase. The mobile phase is passed over a stationary solid phase. Separation occurs because some substances are attracted to the stationary phase more than others. Substances with greater affinity for the stationary phase are retained longer than those with greater affinity for the mobile phase, thus causing the separation in space.

(*Source: Applebee, M. S. "Paper Chromatography: The Separation of Matter" 2003.*)

Isolation and Spectra of Plant Pigments

This experiment will use chromatography as well as visible spectroscopy, a technique we have used previously. As we will see in our next discussion, a plant may have a variety of pigments which contributes to its color at different stages. In this experiment, we will be investigating the absorption spectrum of several common plant pigments found in leaves:

1. chlorophyll a (bright green)
2. chlorophyll b (olive green)
3. carotenes (golden yellow)
4. xanthophylls (light yellow)

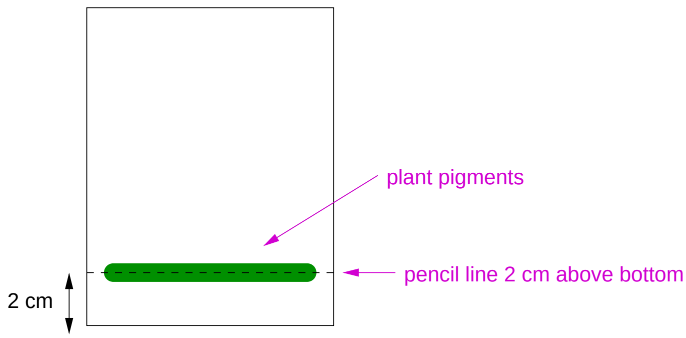
Each student pair will extract leaf pigments from spinach leaves and separate these pigments by paper chromatography.

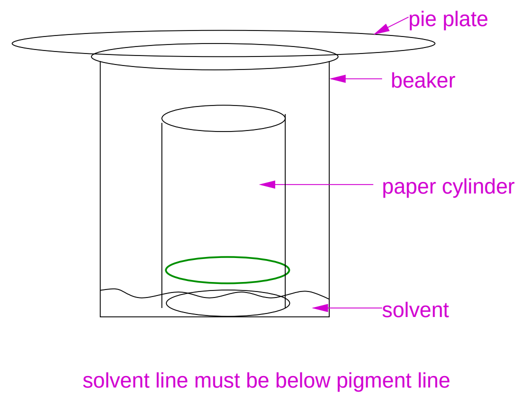
Extracting Leaf Pigments

1. Obtain several spinach leaves and tear into small pieces. Grind up this spinach leaves with about 5-10 ml of acetone (red bottles) using a mortar and pestle. Continue grinding for about 5 minutes. You may add more acetone solvent if necessary.
2. Filter this solution by pouring it through a glass funnel equipped with filter paper, and collecting the filtrate in a small beaker. (The instructor or one of the TAs will demonstrate how to fold the filter paper to place it in the funnel.)

Separation of Leaf Pigments

1. Obtain a 7" x 5.5" piece of chromatography paper. Touch it as little as possible so as not to get oil from your fingers on to the paper.
2. Draw a line in pencil about 2 cm from the bottom.
3. Using a capillary tube (a very thin glass tube), draw up a small amount of the plant pigment extract into the tube.
4. Transfer a small amount of the pigment to the line on the chromatography paper by BRIEFLY touching the capillary tube to the paper on your penciled line.
5. We will transfer the plant extract all along the penciled line. This will help us separate enough material for further testing. Continue to touch the capillary tube to the paper along the line, until you have a dark stripe of plant pigment extract along the line. Repeat this process several times to load the paper with a lot of pigment. Allow the solvent to evaporate in between subsequent additions of plant pigment.



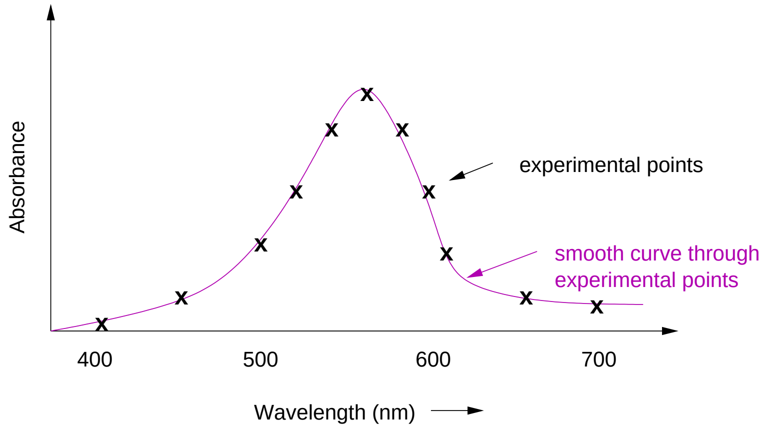


6. Roll up your chromatography paper as evenly as possible into a cylinder and staple the end together.
7. Place a thin layer of the PIGMENTS chromatography solvent into the 1 L beakers. The solvent should cover the bottom of the beaker, but not be higher that than about 1 centimeter or so.
8. Place your paper cylinder into the beaker as shown.
9. Cover the beaker with a glass pie plate and set in the hood.

10. The solvent will climb up the paper through capillary action (much like spilled liquids will climb up a paper towel). Wait until the solvent is near the top of the paper, or until you can clearly see the four serrated plant pigments, then remove the cylinder from the beaker and let dry. Confirm that your pigments have separated enough with the instructor or a TA before removing the cylinder from the beaker.
11. Pour your used PIGMENTS chromatography solvent into the designated waste container.
12. When your chromatography paper is dry, identify each pigment band by the color and the order of elution. The grayish line is leaf breakdown products and can be ignored. Cut out strips of paper containing each pigment band. Make diagonal or zig-zag lines if necessary to ensure that only one pigment is on each strip. **It is crucial to identify these correctly - mark each band with a pencil indicating the order of elution before cutting.**
13. Fold each strip in an accordion fashion and place it in the appropriate labeled beaker at the front of the room. There will be one beaker for each pigment. Be sure to put each strip in appropriate beaker for the pigment it contains. Every group will add their strips to these beakers, allowing us to collect a large amount of each of the separated pigments.**It is very important these are placed into the correct beakers - check with the instructor or TA before placing your strips in.**
14. Ethanol will be added to each of the beakers to extract the separated pigments from the paper. These solutions will be used to obtain the absorption spectrum of each pigment.

Absorption Spectra

1. You will be assigned one of the plant pigments. Your group will obtain the absorption spectrum for this pigment.
2. Your instructor or one of the teaching assistants will explain how to use the spectrophotometer.
3. Fill one cuvette about 1/2 to 2/3 full with ethanol. This is called the *blank* or reference solution. Choose one of the colored solutions available in the lab and and place it in another cuvette. This is your *sample*.
   (a) Set the wavelength selector to 400 nm.
   (b) If using one of the older beige spectrophotometers do the following (others can skip this step): Adjust the zero knob on the spectrophotometer as demonstrated by your instructor or T.A. The zero knob should be adjusted until the dial reads 0% transmittance. This should be adjusted without a cuvette in the spectrophotometer and with the cover closed.
   (c) Put your blank solution into the spectrophotometer. For the blue spectrophotometers: push the **0 Abs 100% T** button. For beige: Adjust the 100% transmittance knob until the dial reads 100% transmittance (0 absorbance).
   (d) Place the cuvette containing your sample into the spectrophotometer.
   (e) Record the absorbance readings in a table (separate sheet).
   (f) Change the wavelength to 425 nm and repeat steps c-f. Repeat for every wavelength up to 700 nm using 25 nm intervals.
4. Make a graph of absorbance as a function of wavelength. Use circles or x's for your experimental points, then sketch a smooth curve through your data, as show below. You may also use Excel.



5. Identify the color associated with the wavelength(s) of maximum absorbance. Record this in the table in the questions section.

Paper Marbling

This activity illustrates a number of chemical concepts. One is solubility or miscibility. Two materials are said to be miscible if they form a homogeneous mixture. A common example of this is water and ethyl alcohol (drinking alcohol). Two substances which are not miscible with one another are oil and water. These are said to be immiscible with one another. They form a heterogeneous mixture and can clearly be seem as two separate liquids in the mixture.

Solids that dissolve in a liquid are said to be soluble in that liquid. For example, table salt is soluble in water, but not terribly soluble in oil. In general, substances that are similar are miscible or soluble in one another. This leads to the common saying "like dissolves like" in chemistry. Polar molecules (those with a dipole moment) tend to be miscible with one another, but immiscible with nonpolar molecules (those without a dipole moment). Similarly, nonpolar molecules mix easily with other nonpolar molecules, but not with polar ones.

Two other concepts that are illustrated in this experiment are surface tension and adsorption. The surface tension of water allows the paper to "float" on the surface. Adsorption is the binding of atoms, molecules or other particles to a surface. This is not the same as absorption, where the molecules or particles are taken inside a bulk material (like a sponge). Rather, adsorption is limited to the surface. Adsorption of dyes or paints can be enhanced through the use of a mordant - a linker molecule that is adsorbed first onto the material to be colored.

Directions for marbling station will be given in class. You will make 4 marbled sheets. Use 2 types of paper. For each type of paper, make one marbled sheet using the mordant and one without.

Fiber Reactive Dyes

Wear Gloves!

Dye your T-shirt and a strip of multifiber ribbon. Use one color only for the ribbon. Use as many as you like for the T-shirt!

1. T-shirts are soaking in a solution of sodium carbonate. Test the pH of the solution and record it in

the Post Lab Questions area.

2. Wearing gloves, take the appropriate size shirt out of the bath and squeeze it out OVER the bath. Get out as much water as you can.
3. Wearing gloves, take the shirt over to a folding area and fold, twist, scrunch and rubber band as you see fit.
4. Check out the dyeing area. Make sure the plastic area where you plan to work is free of dye before laying your shirt down.
5. Wearing gloves, take your shirt to the dyeing area and apply dyes as you would like. Take into account how neighboring colors might mix. Apply dye in folds for more complete coverage. Do less this less if you would more white areas on the shirt.
6. Wearing gloves, place your shirt in a plastic zipper bag to take home.
7. Wearing gloves, clean up the plastic in the area where you worked, to leave it dye-free for the next person.
8. Let your shirt sit in the bag for at least four hours. Overnight of for 24 hours is fine as well. Rinse the shirt out very well. Wash with a small amount of synthropol, a special detergent which helps keep the dye soluble while rinsing.
9. If you get a lot of undesired bleeding into white areas of the shirt, wash repeatedly to get out as much as possible and dry flat on an old towel, rather than by hanging.

Questions

1. What was the pH of the sodium carbonate solution used for tie-dying? Is this acidic or basic? Why is it necessary to wear gloves?

2. Consider the principles of (a) density (b) surface tension and (c) polarity and miscibility to explain why the marbleizing paints float.

3. Describe the differences observed between the types of paper and the use of a mordant in marbling.

4. Consulting with other student groups, what are the best absorption wavelengths for each of the pigments? What colors of light do each of these correspond to?

Pigment	Color	Best Absorption Wavelength (nm)	Color Absorbed
chlorophyll a	bright green		
chlorophyll b	olive green		
carotenes	golden yellow		
xanthophylls	light yellow		

5. Using the absorption spectra of the chlorophylls, describe why spinach leaves are green.

6. Would you expect a plant to be able to absorb green light? Do you think it would be able to photosynthesize if exposed only to green light?

7. Why do you think plants have more than one type of pigment? What advantages might be gained by having multiple pigments?