Fig. 5-1

CHEM 008 Experiment 5

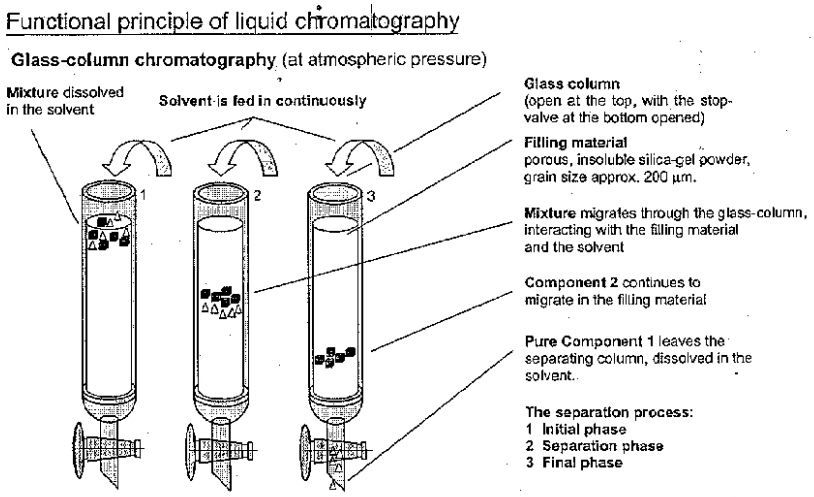
CHROMATOGRAPHY

Text Topics and New Techniques

Column and paper chromatography, visible spectroscopy

Discussion and Techniques

One of the most important aspects of chemistry is the analysis of mixtures. Usually, a mixture must be separated into pure compounds before chemical analysis can be attempted. Polluted water often contains several pesticides as well as chemicals such as PCBs and analytic techniques cannot identify one pesticide (e.g., DDT) when another is present unless a separation is first performed. Many mixtures are separated using some variation of a technique discovered by Michael Tswett (Russian botanist) early in the 20th century.

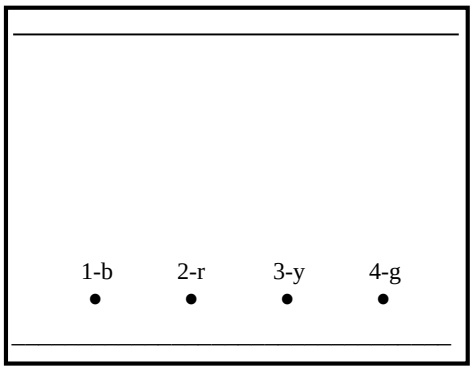


Tswett allowed a mixture of pigments extracted from plants to percolate down through a column of calcium carbonate. Solvent was added from the top as needed to cause continuous movement of the pigments down the column. Pigments that were more strongly attracted to the stationary phase (calcium carbonate) and had less affinity for the solvent, moved down the column more slowly than pigments that had greater affinity for the solvent and had weaker attraction for the stationary phase. Tswett observed that the pigments had separated into several differently colored bands as a result of the fact that they moved down the column at different rates. The term "chromatography" was coined to describe the phenomenon. In this experiment, you will perform a column chromatogram similar to Tswett's and a paper chromatographic separation of food colorings.

As indicated in the previous experiment in a discussion of gas chromatography, chromatography can be described as a dynamic extraction where the components of a mixture have the continuous ability to partition between a stationary phase and a moving phase. If the two phases are selected appropriately, different substances will spend different amounts of time in each phase and move through the system at different rates and separate. For column chromatography, one of the phases is the moving solvent that is added continuously from the top. The stationary phase is the absorbent that has been used to pack the column. Alumina and silica gel are the most commonly used absorbents. For paper chromatography, the paper (cellulose) is the stationary absorbent phase and the solvent moves up the paper and is the moving phase. Selection of the appropriate phases is the difficult and time consuming part of chromatography.

Once appropriate moving and stationary phases have been found, running the chromatograms can become routine. For column chromatography, it is common procedure to start with a non-polar solvent and gradually increase the polarity of the solvent as the substances move down the column. Non-polar substances usually move rapidly down with non-polar solvents and are eluted first. Polar substances do not move much until the solvent polarity is increased. For paper chromatography, many solvents are usually tested until one is found that separates the mixture into pure substances. Often, mixtures of solvents are needed to accomplish this purpose.

The paper chromatography experiments you will perform today utilize similar principles to those Tswett employed. A piece of paper, spotted with pure compounds and mixtures, is placed in a solvent as illustrated. Assume spots 1, 2, and 3 are pure compounds and spot 4 is an unknown mixture of the compounds. The solvent (moving phase) will move up the paper (stationary phase). When the solvent reaches the spots, the components of each spot have options. They can dissolve in the solvent and progress up the paper or they can stay adsorbed on the paper and resist movement. The choice depends on several factors including the polarities of the compounds, the solvent and the paper. The solvent and the stationary phase are selected so that the components spend some time in each phase and do not move right along with the solvent front or stay at the origin. If the solvent and the stationary phase are selected properly, different compounds will move up the paper at different rates and separate.



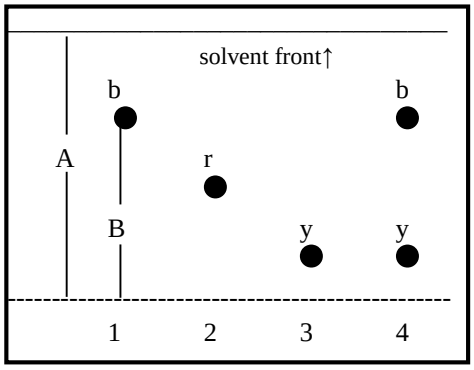


Figure 5-2 *b = blue r = red y = yellow g = green* **Figure 5-3**

The spots of compounds that are less strongly adsorbed on the paper will move up the paper faster than the spots of the more strongly adsorbed compounds. When the solvent front nears the top of the paper, the chromatogram is removed from the solvent and the solvent front marked with a pencil. To find out the composition of the fourth spot, two factors are considered, color and relative distance moved. To quantify the relative distances, the R_f (ratio to front) value of each spot is calculated.

$$R_f = \frac{\text{distance from origin to center of spot}}{\text{distance from origin to solvent front}} = \frac{B}{A} \text{ for spot 1}$$

In the chromatogram on this page, it can be seen that spot 4 (the green spot) has compounds with the same color and R_f values as pure compounds 1 and 3. This provides strong evidence (but not proof) that spot 4 contains compounds 1 and 3 and does not contain compound 2. Paper chromatography then serves as a separation technique and can also assist with identification if the possible compounds in a mixture are available in pure form.

Procedure

Chemical Hazard Summary: All chemicals may cause irritation of eyes, skin, and respiratory tract. Wear proper goggles and gloves. Avoid inhaling vapors/dust. The following notes are summaries and do not supplant reading the entire MSDS.

Magnesium sulfate is hygroscopic and is harmful if inhaled or absorbed through skin.

Hexanes is extremely flammable, is harmful if absorbed through skin, and causes eye, skin, and respiratory tract irritation. Inhalation can cause drowsiness and dizziness. Hexanes can impair fertility. Keep away from open flame and sources of ignition.

Acetone is extremely flammable and causes eye, skin, and respiratory tract irritation. Inhalation can cause drowsiness and dizziness. Keep away from open flame and sources of ignition.

Sand is a suspected carcinogen.

Silica gel is hygroscopic and causes respiratory tract irritation.

Erioglaucine (FD&C blue #1) is a suspected carcinogen.

Erythrosin B (FD&C red #3) is harmful if inhaled or absorbed through skin.

Tartrazine (FD&C yellow #5) is hygroscopic and may form explosive dust-air mixtures. Avoid creating dust. Keep away from open flame and sources of ignition.

Ethanol is a flammable poison that causes severe eye irritation, moderate skin irritation, and respiratory tract irritation. Ethanol has adverse reproductive and fetal effects. Keep away from open flame and sources of ignition.

2-Propanol is a flammable, hygroscopic, light and air sensitive, potential peroxide former that causes eye, skin, and respiratory tract irritation. Inhalation can cause drowsiness and dizziness. Keep away from open flame and sources of ignition.

You will work with a partner for all parts of this experiment.

Column Chromatography. For this part of the experiment, some pigments will be extracted from spinach and separated in a silica gel column packed in a Pasteur pipet. Visible spectra will be run on the separated pigments. Note that the solvent hexanes refers to a mixture of isomeric hexanes. *n*-Hexane also works but costs more. Low boiling petroleum ether should also work.

Using a mortar and pestle, grind about 5 g of spinach leaves and 2 g of anhydrous magnesium sulfate in 10 mL of an 80/20 mixture of hexanes and acetone. Grind until the solvent is dark green – this may take up to 5 minutes. Decant the liquid into a centrifuge tube and centrifuge the mixture until any remaining solids have gathered at the bottom of the tube and the top liquid portion is not cloudy. Carefully extract the top liquid portion from the tube with a large plastic pipet and transfer it to a clean test tube. Do not transfer any of the solids or the water that should be in the bottom of the centrifuge tube. Stopper the test tube and save the solution until the chromatography column has been prepared. Place the solid spinach and magnesium sulfate in the "Used Spinach and Magnesium Sulfate" container.

1.) The column will be a glass Pasteur pipet with a plug of glass wool held with a 3-fingered clamp on a ring stand. Avoid tightening the clamp so much that the pipet breaks.

2.) Using weighing paper as a funnel, put a small layer (about ½ cm) of sand on top of the glass wool. Gently tap the pipet to level the sand.

3.) Add silica gel to the pipet containing the sand and glass wool until about ⅔ full. Gently tap the pipet to level the silica gel.

4.) Add another small layer (about ½ cm) of sand on top of the silica and gently tap to level the top surface. Place an empty waste beaker under it.

5.) Label 2-4 test tubes with 1, 2, etc.

At this point, you should be prepared to continue the column chromatography until it is done. If you stop in the middle, the column may dry out and ruin your separation.

6.) Get about 15 mL of hexanes, 10 mL of 70% hexanes/30% acetone (by volume), and 10 mL of acetone.

7.) Carefully and slowly add 3 mL of hexanes to the top sand layer so that the sand layer is not appreciably disturbed. The solvent level should be near the top of the pipet (within ½ cm). Let the hexanes drain through the pipet column **until the level of the hexanes is near the top sand layer without letting the sand go dry**. If solvent is very slow, use the pipet bulb to gently push the hexanes through with air.

8.) **Do steps 8-14 in quick succession!** Once the hexanes are just covering the top sand layer, add about 2 mL of your spinach extract to the top of the column. Make sure you save a small amount of extract for the paper chromatography section.

9.) Allow the spinach extract to flow through the column until it just covers the sand and add 1 mL of hexanes to the top of the column. As the extract drains onto the silica gel, the pigments will begin to separate with the yellow carotene band getting ahead of the green chlorophyll band.

10.) Continue to add hexanes until the yellow band reaches the bottom of the column. Sometimes the yellow band is very narrow - don't miss it! If 5 mL of hexanes are not enough to move the yellow band to the bottom or you don't see a yellow band, use some of your 70% hexanes/30% acetone mixture to move the yellow band out.

11.) When the elutant is yellow, replace the waste beaker with test tube #1. Continue to collect in this test tube until the elutant is no longer yellow or the test tube is full. Use test tube #2 if necessary to collect additional yellow elutant.

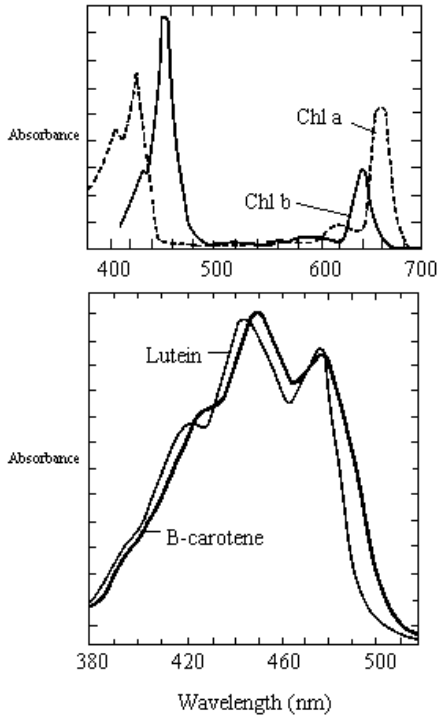
12.) When the elutant is colorless, put the waste beaker back under the column.

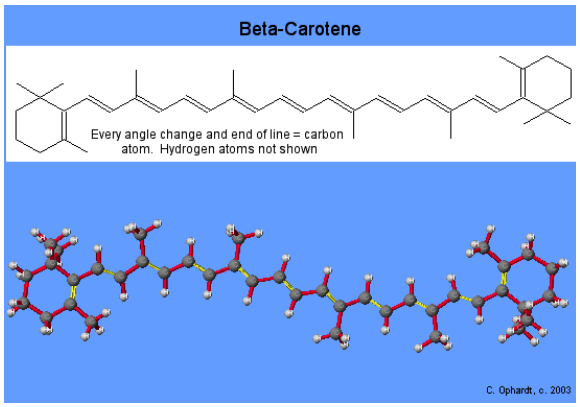
13.) Once the remaining hexanes are just covering the sand at the top of the column, add your 70%/30% mixture to move the green band down the column. Use all 10 mL of your 70%/30% mixture. Switch to acetone if you need more solvent to collect the green band.

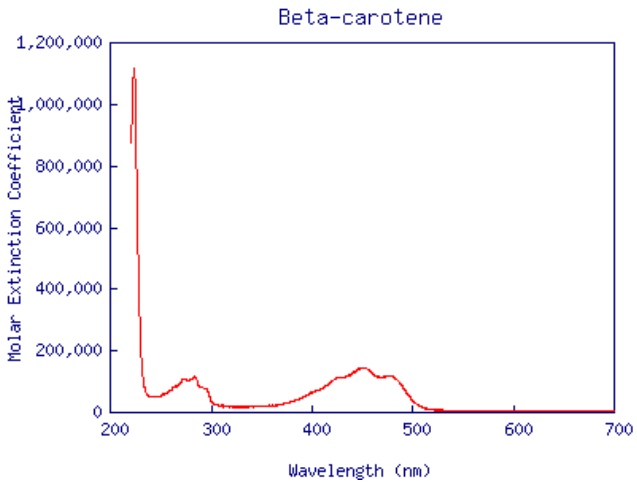
14.) When the green band starts to collect, replace the waste beaker with your next test tube. After collecting all of the green band in 1 or 2 test tube, put your waste beaker under the column and let the remaining solvent drain out.

15.) When you are done with the column, let it dry and toss it in the "Chemical Contaminated Items" container.

Run UV-visible spectra of the two collected colored bands from about 350 nm to 700 nm. The range will depend on the capabilities of your instrument and the cells used. If your cells are Pyrex the high energy limit (short wavelength limit) will be about 310 nm as Pyrex absorbs at higher energies. If the absorption values are too high (>2), dilute a portion of your sample with hexanes and rerun the spectrum. Compare your spectra to those presented. When done, pour all elutants into the "Organic Solutions" bottles in the hoods. Rinse the cuvettes thoroughly with acetone and let dry.




Beta-Carotene
Every angle change and end of line = carbon atom. Hydrogen atoms not shown
C. Ophardt, c. 2003


Beta-carotene
Molar Extinction Coefficient
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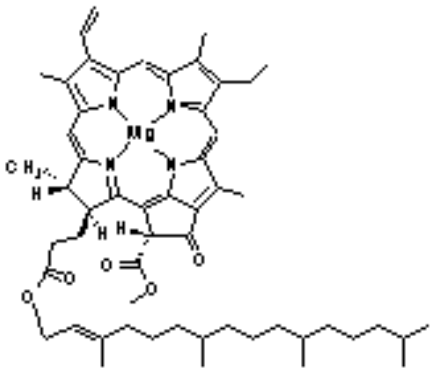
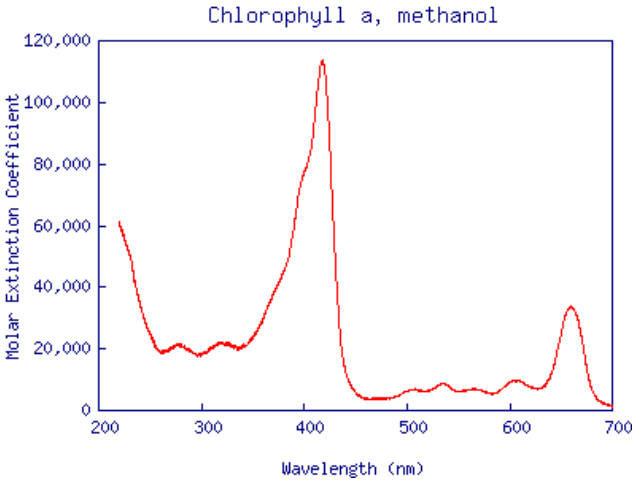
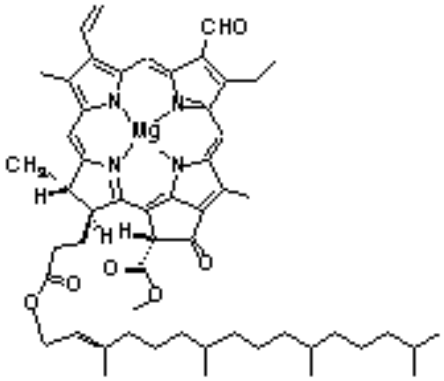
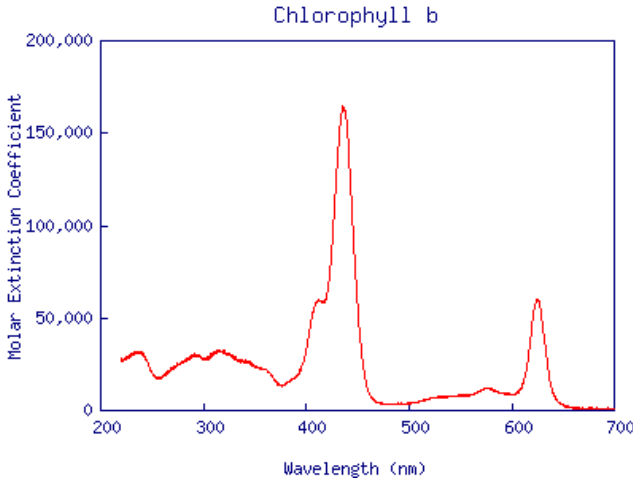
800,000
600,000
400,000
200,000
0
200
300
400
500
600
700
Wavelength (nm)




Chlorophyll a, methanol
Molar Extinction Coefficient
120,000
100,000
80,000
60,000
40,000
20,000
0
200
300
400
500
600
700
Wavelength (nm)


CHO
Mg

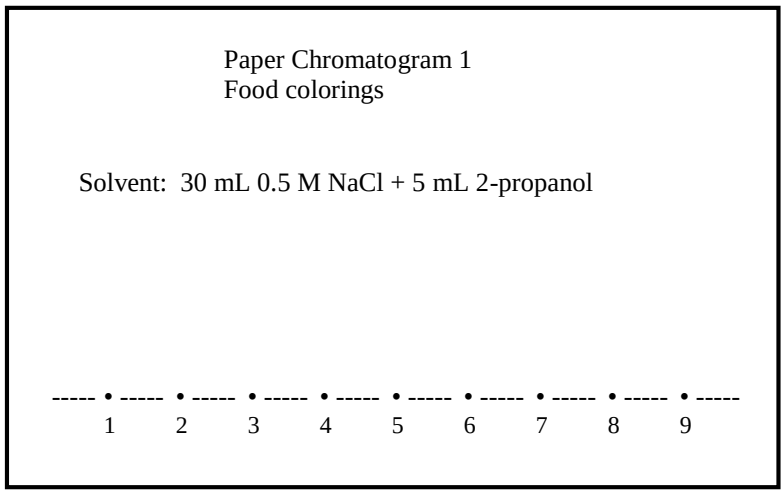

Chlorophyll b
Molar Extinction Coefficient
200,000
150,000
100,000
50,000
0
200
300
400
500
600
700
Wavelength (nm)

Paper chromatography. Food coloring chromatogram. Following *Figure 5-4*, draw a straight pencil line 2 centimeters from the bottom of a 10x20 cm piece of Whatman #1 chromatography paper and place pencil dots numbered from 1 to 9 at 2 cm intervals along the lines and 2 cm from each edge.

Dilute solutions of 4 food dyes will be available in vials. Dip the end of an open ended capillary tube into the Red # 3 solution and apply it quickly to a scrap piece of chromatography paper. Practice this until you can make spots about this size ●. Now use the capillary to put a spot of the Red # 3 on to spot 1. Use new capillary tubes to spot Red # 40, Blue # 1 and Yellow # 5 on their respective spots. Toss the used capillaries in the "Chemical Contaminated Items" container.

Put a drop each of commercial red, blue, green, and yellow food colorings into 4 depressions in a spot plate. Keep them in that order so you will know which color is in which depression. Dip the end of capillary tubes into each of the food colorings and spot them on the appropriate places on the paper. You may need to let the solvent dry and respot a few times to make the spots darker. Toss the used capillaries in the "Chemical Contaminated Items" container. Rinse the spot plate with water and dry.

For the ninth spot, take two brown M & M's and gently swirl them in a small beaker or flask with 1 mL of a 50/50 mixture of water and ethanol. Decant the solvent into a clean test tube before the solvent exposes the chocolate and toss the M&M's in the trash. Use a new capillary to spot the solvent on spot # 9. Because the coloring in this spot will be considerably more dilute than the previous 8 spots, you should allow the solvent to dry and respot at least eight times. Pour the solution into the "Organic Solutions" bottles in the hoods. Toss the used capillary in the "Chemical Contaminated Items" container.



1 - Red # 3 (erythrosine)
2 - Red # 40 (allura red)
3 - Blue # 1 (brilliant blue FCF or erioglaucine)
4 - Yellow # 5 (tartrazine)
5 - Red food coloring
6 - Blue food coloring
7 - Green food coloring
8 - Yellow food coloring
9 - Brown M & M shell

Figure 5-4

After the spotting (*Figure 5-4*) is complete, roll the paper into a cylinder and staple it so that the spots are on the outside and there is a small gap between the two ends (*Figure 5-5*). The ends of the paper should **not** overlap. Add 30 mL 0.5 M sodium chloride + 5 mL 2-propanol to a 600 mL beaker **and mix**. Gently put the paper cylinder (spotted edge down) into the beaker and cover the beaker with aluminum foil. Do not move or turn the beaker again. At this point, you should begin your second chromatogram but keep your eye on the first one. When the solvent front reaches about 2 cm below the top of the paper, remove the chromatogram. Mark the solvent front with a pencil and let the paper dry in the hood. Outline every spot with a pencil. Measure the distance to the solvent front and to the center of each spot and calculate and record each R_f value and the color of each spot. Save the aluminum foil. Pour solvent into "Organic Solutions" bottles in the hoods. Turn in the chromatogram with your lab report.

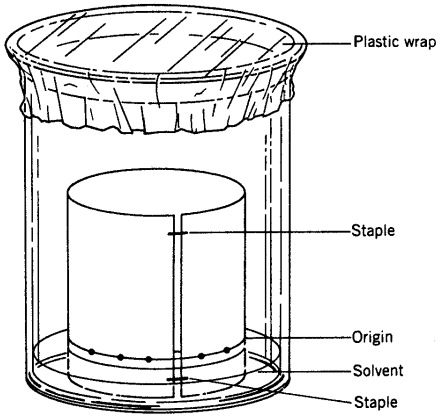


Figure 5-5

Spinach extract chromatogram. For the second chromatogram, a 9.5 x 14 cm piece of filter paper will be used. Draw a pencil line 2 cm from the bottom edge. Put a pencil dot 3 cm in from the edge and put two more dots at the 7 and 10 cm points. Using an open ended capillary, spot the 3 cm spot about 5 times with the spinach extract prepared for the column chromatography. Let the paper dry between spot applications. Put a spinach leaf down covering the 7 and 10 cm dots and lay a ruler down over the leaf on the line that is 2 cm up from the bottom. Use a quarter to roll over the leaf with pressing from approximately the 7 to 10 cm mark several times. Roll the paper into a cylinder and staple as with the previous chromatogram. Add 30 mL of a 90/10 hexanes to acetone solution to a 400 mL beaker. Insert the paper cylinder, cover with aluminum foil, and do not disturb until the solvent reaches about 1 cm from the top of the paper. Withdraw the paper, mark the solvent front with a pencil and allow it to dry. Outline every spot with a pencil. Measure the distance to the solvent front and to the center of each spot and calculate and record each R_f value and the color of each spot or band. Save the aluminum foil. Pour the remaining spinach extract and solvent into the "Organic Solutions" bottles in the hoods. Toss the spinach leaf. Turn in the chromatogram with your lab report.

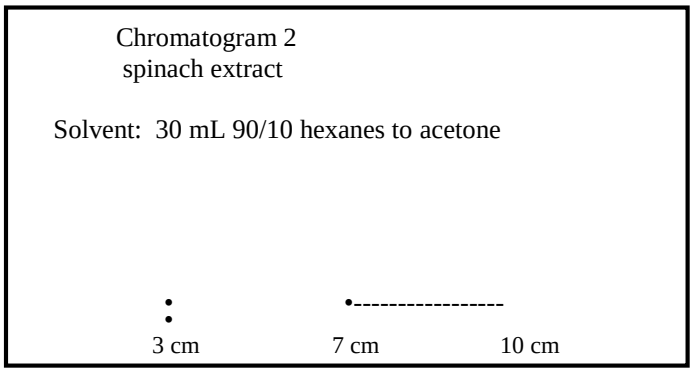


Figure 5-6

CLEANING UP. Toss all M&M's in the trash. Pour all of your hexanes and acetone solutions, the M&M extraction, and the sodium chloride/2-propanol into the "Organic Solutions" bottles in the hoods. Pour all of the food coloring into the sink with the faucet running. If you ran UV-vis spectra of your pigments, clean the glass cuvettes with acetone or hexanes and pour the solutions in the "Organic Solutions" bottles. Toss used Pasteur pipets and capillaries into the "Chemical Contaminated Items" container. Toss the spinach and magnesium sulfate mixture in the "Used Spinach and Magnesium Sulfate" container. Save the aluminum foil for subsequent sections. Clean your glassware with soap and water and do a final rinse with distilled water before drying it. Pour any acetone used for a final drying rinse of glassware into the "Organic Solutions" bottles. Wipe down your table top with a paper towel dampened with water.

Origin to solvent front distance _____

					Pigments								
Spot #	Dye	Color	Dist.	R_f	Color	Dist.	R_f	Color	Dist.	R_f	Color	Dist.	R_f
1	Red #3												
2	Red #40												
3	Blue #1												
4	Yellow #5												
5	Red f.c.												
6	Blue f.c.												
7	Green f.c.												
8	Yellow f.c.												
9	Brown M&Ms												

References

For food coloring information, see: http://vm.cfsan.fda.gov/~lrd/colorfac.html or look up each dye in Wikipedia or another online encyclopedia.

Hao T. Quach, Robert L. Steeper, and G. William Griffin, *J. Chem. Ed.*, **81**, 385, 2004.

Kurt R. Birdwhistell and Thomas G. Spence, *J. Chem. Ed.*, **79**, 847, 2002.

Prelaboratory Preparation - *Experiment 5*

First, be sure to list all the goals of the experiment. Read the reference on food coloring listed above. Include a statement about yellow #5 (tartrazine) in your introduction and comment on the significance if any if you find tartrazine in the food colorings or the M & M.

Observations

Report all relevant observations including the color and R_f of each spot, the order of elution on the column chromatograph and the spectra of the bands eluted.

Conclusions

This section should include the following:

1. Were the goals of the experiment achieved? Explain your answer.
2. Did you collect at least two different colored bands from the column chromatograph? Were you able to identify the bands using spectroscopy? Explain your answer.
3. Were the conditions used adequate for separating the different compounds used in food colorings? Which of the four FD&C dyes were used in the food colorings and the brown M&M? Were any other dyes used? Explain your answer.
4. Do the food coloring bottles and the M&M package give adequate warnings about the presence of tartrazine? Explain your answer?
5. Were the conditions used adequate for separating the different compounds present in the spinach extract? The R_f value for β-carotene should have been very high and the values for the chlorophylls considerably lower. Were you able to separate β-carotene from the chlorophylls and from other dyes if they appeared to be present? Explain your answer.
6. Comment on the applicability of column chromatography and paper chromatography. Are they good procedures for separating, purifying and identifying? Is sample size and important consideration?
7. Suggest changes you would make to any part of this experiment to improve it.