Experiment 2

Separation of Nontoxic Dyes by Paper Chromatography

Introduction

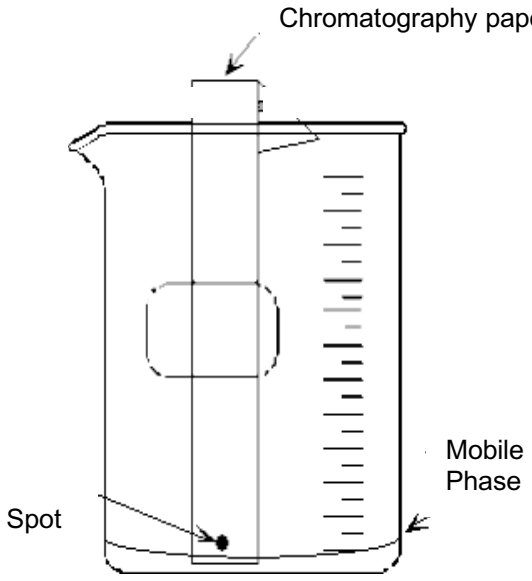
Chemical additives, both natural and synthetic, are used to color food and other materials such as inks, paints, and toys. The specific color seen in the item is due to the mixing of several different chemicals to give the desired hue. The specific number and types of chemicals present in a mixture can be determined by using a separation method called "Chromatography".

Chromatography Theory

Chromatography is an important separation technique that depends upon how strongly compounds in a mixture are attracted to a solid **stationary phase** versus their **solubility** (ability to dissolve) in a solvent, called the **mobile phase**. During the analysis, the compounds separate enough to determine the number of compounds in the mixture. These compounds will always separate the same way if the experimental conditions (temperature, mobile phase, stationary phase, etc.) are held constant since the ratio of the chemical's solubility in the mobile phase to the stationary phase attraction is constant for each compound. This ratio, called a **retention factor (R_f)** can be used to aid in identification of a compound. After the analysis is complete, the dried paper containing the separated compounds is referred to as the "**chromatogram**".

In paper chromatography, the stationary phase is a special paper like the paper used to make coffee filters. It has approximately the same polarity as water and when immersed in an aqueous solution, it attracts water molecules to its surface creating a layer of water molecules that lay on the surface of the paper. The mobile phase must then be very polar to overcome the attraction to the water-soaked stationary phase. In this experiment, we will use a sodium chloride solution which is extremely polar due to the "**ion-dipole**" forces present between the ions in the salt and the water molecules. Because this solution is even more polar than pure water, the polar dye molecules are more attracted to the salt water than the paper, so they stay in solution rather than remain sticking to the paper. The degree that they stay in the mobile phase will be based on the polarity of the dye molecule. The more polar the molecule, the further up it will travel on the paper before finally sticking to the paper rather than staying dissolved in the mobile phase.

The primary advantages of paper chromatography are its simplicity and the speed of the analysis. For the analysis of dyes, the method is relatively simple. A spot of dye is placed near the bottom edge of a piece of chromatography paper. The paper is placed in a beaker containing water below the level of the spot. As the mobile phase moves up the paper through capillary action, the less polar dye molecules will begin to stick to the paper. More polar molecules will stay in the mobile phase longer and deposit further up. If two or more dyes have been mixed, then the different dye molecules travel at different rates as the mobile phase moves up the paper. Over time, the dyes separate sufficiently to allow us to determine both the number of dyes present in the sample and to determine the identity of each one by calculating the retention factors.

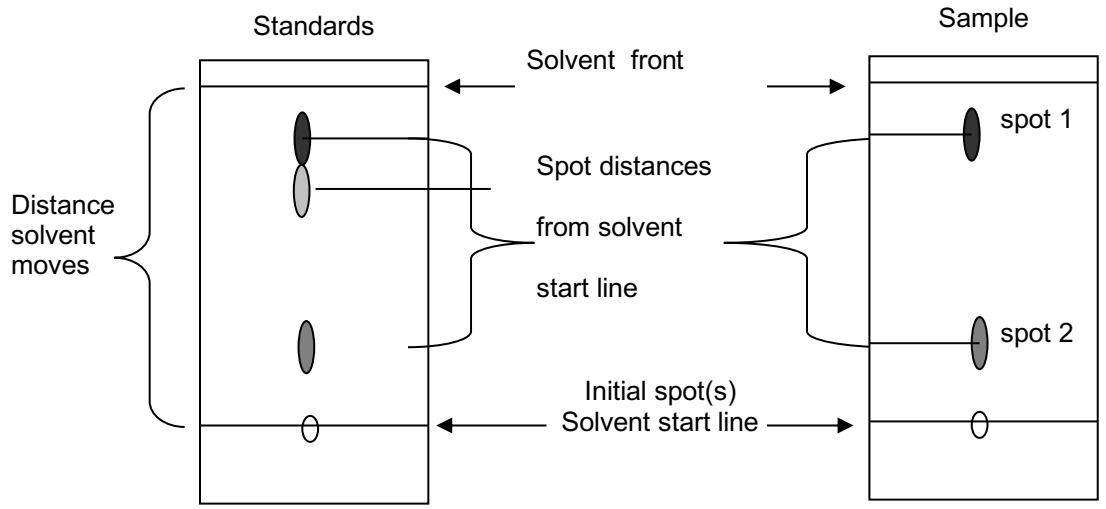


Identification of Unknown Dyes in a Mixture

Identification of the dyes in the mixture can be performed since each dye will move a definite distance on the paper in proportion to the distance that the solvent moves. This ratio is called the "**retention factor**", or **R_f**. The R_f is calculated by dividing the distance the dye moves by the distance the solvent moves. The R_f is dependent on both the type of solvent and the paper used, so you must use the same separation conditions when comparing R_f values.

$$R_f = \frac{\text{Distance dye moves (mm)}}{\text{Distance solvent moves (mm)}} = \frac{\text{Start line to spot (mm)}}{\text{Start line to solvent front (mm)}}$$

In the example below, each separated "spot" can be assigned a R_f value, which is characteristic of the dye under a particular set of chromatographic conditions (i.e. paper chromatogram, specific mobile phase or solvent, etc.). Identification of a dye is made by comparing the R_f value of the standard dye to that seen in the sample chromatogram (see figure 2 below).



Standard Chromatogram

Distance solvent moves: 86mm

Standards	Distance spot moves	R_f
Dye 1	56mm	0.65
Dye 2	78mm	0.91
Dye 3	24mm	0.28

Sample Chromatogram

Distance solvent moves: 84mm

Spots	Distance spot moves	R_f
Spot 1	76mm	0.90
Spot 2	23mm	0.27

In the analysis of the chromatograms, the sample contained Dye 2 and Dye 3 but did not contain Dye 1. Thus, not only can you positively identify which dyes are present, you can also see which ones are not in the sample. To completely match the sample to the standard, all of the spots must match. When using colored chemicals such as dyes, the color of the spot provides a confirmation that the dye is the same for both the sample and the standard.

In Your Experiment

There will be 2 nontoxic markers at each station, which you will use to see how solvent polarity changes the separation pattern of the dyes in each of the markers. To study the effects of the mobile phase on the chromatographic separation, you will first look at the separation of the dyes in the ink using water, and then a more polar solution of 5% NaCl. Be patient when running the chromatograms, as the data you generate will be used to determine the number of dyes making up the overall color of the pen and the relative polarity and solubility of these dyes.

Lab Precautions

Chemical Hazards

Aqueous 5% NaCl Solution
NFPA RATING: HEALTH: 1 FLAMMABILITY: 0 REACTIVITY: 0
EYE EXPOSURE: Flush with copious amounts of water for at least 15 minutes. Assure adequate flushing by separating the eyelids with fingers.

SKIN EXPOSURE: Rinse with water to remove.

Chemical Disposal

Dispose of both mobile phases in the liquid waste container in the lab.

Equipment Procedures

There is no new equipment associated with this lab.

Experiment 2: Procedures and Data Sheet
(Submit as part of your informal report)

Name: _____ Date: _____ Section: _____

TA Signature: _____

All data must be written in pen at the time it is collected. **Pencil is not allowed!!**
Record all measurements with the correct number of significant figures and units.
TA signature & TA initials on any changes made to the data are required or the data is invalid.

Part 1: Determination of R_f values for the nontoxic inks used in markers
Start this part of the lab before you do the concept review.
Use Figure 1 in the introduction to see the correct placement of lines and marking spots.

1. You will need 2 strips of chromatography paper approximately 11cm long. Prepare each strip by drawing a line on the paper 1.0 cm from the bottom with a **pencil**. Draw a second line 1cm from the top as well. (Do not use a pen because the ink may be soluble in water and interfere with the experiment by also separating and moving up the paper.)

2. Fold the paper lengthwise then unfold the paper and lay it flat with the pencil mark on the side facing you. Carefully make a dot from each pen on the pencil line on each side of the fold in the chromatography paper. The dot should be approximately this size: (●)

3. Record the color of each pen in pencil below each dot. Also record the colors on this sheet.

   Pen 1: Color_____

   Pen 2: Color_____

4. Fill a wash bottle with distilled water. Dispense 5 mL of distilled water into a 10 mL graduated cylinder. Pour the water into a 100 or 150 mL beaker.

5. Lower one of the spotted chromatography papers into the beaker until the bottom of the paper is in the water. When you insert the paper into the beaker, THE DOT OF DYE MUST BE ABOVE THE LEVEL OF THE LIQUID. Do not allow the spotted sample to touch the liquid or the sample may dissolve ruining your experiment. Make sure the paper stands upright on its own. Your paper may lean towards the side of the beaker, but the damp edge of the chromatogram should not come in contact with the beaker at any time during the separation.

6. Using a second beaker, repeat the process with 5 mL of the 5% NaCl solution.

7. The solvents will begin moving up the paper by capillary action. When the mobile phase reaches the 1 cm line at the top of the paper, remove the chromatogram from the beaker and lay it on a paper towel. Blot gently with another paper towel to stop the movement of the liquid through the paper. It takes approximately ½ hour for each chromatogram to develop.

8. The Solvent Front (see introduction) may not be a perfectly straight line or may be slightly above or below the top 1cm line that you drew. Before the chromatography paper has completely dried, mark the Solvent Front with a second pencil line.

9. Circle the colored spots in pencil immediately, as they often fade as they dry. Ideally the spot will be completely spherical, but often it is more of a streak. Make your circle around the darkest area of the spot as this is where most of the dye is located. (Fig 2)

10. Measure the distance traveled by the solvent from the bottom pencil line to the solvent front. This measurement will be recorded as "Distance traveled by the solvent" for use in the calculation of R_f for these standards. Record this measurement in the data table for pens.

11. Place a dot in the center of the darkest area of each circled spot and measure the distance from the bottom pencil line to the center of each spot. This measurement is the "Distance to spot" for each spot. Record each of these measurements in your data table.

12. When finished, dispose of both solutions in the waste container in the lab.

13. Staple your chromatograms to the data sheet below.

Chromatographic Data using water (you may not need all of the lines)

Pen 1: Distance to solvent front _____cm Chromatogram

Spot 1: Distance to spot _____cm Color of spot_____

Spot 2: Distance to spot _____cm Color of spot_____

Spot 3: Distance to spot _____cm Color of spot_____

Spot 4: Distance to spot _____cm Color of spot_____

Spot 5: Distance to spot _____cm Color of spot_____

Pen 2: Distance to solvent front _____cm

Spot 1: Distance to spot _____cm Color of spot_____

Spot 2: Distance to spot _____cm Color of spot_____

Spot 3: Distance to spot _____cm Color of spot_____

Spot 4: Distance to spot _____cm Color of spot_____

Spot 5: Distance to spot _____cm Color of spot_____

Chromatographic data using NaCl solution (you may not need all of the lines)

Pen 1: Distance to solvent front _____cm Chromatogram

Spot 1: Distance to spot _____cm Color of spot_____

Spot 2: Distance to spot _____cm Color of spot_____

Spot 3: Distance to spot _____cm Color of spot_____

Spot 4: Distance to spot _____cm Color of spot_____

Spot 5: Distance to spot _____cm Color of spot_____

Pen 2: Distance to solvent front _____cm

Spot 1: Distance to spot _____cm Color of spot_____

Spot 2: Distance to spot _____cm Color of spot_____

Spot 3: Distance to spot _____cm Color of spot_____

Spot 4: Distance to spot _____cm Color of spot_____

Spot 5: Distance to spot _____cm Color of spot_____

Experiment 2: Data Rubric (20pts)

Points

Data are neat and legible	5pts	_____pts
Significant figures (>80% correct)	3pts	_____pts
Units (>80% correct)	2pts	_____pts
All data are present and make sense	10pts	_____pts

Deductions (sliding scale based on TA discretion)

Lab area left unclean	-20pts	_____pts
Improper waste disposal	-20pts	_____pts
Disruptive behavior	-20pts	_____pts
Lab coat or safety glasses removed while in lab	-20pts	_____pts
Data sheet is missing TA signature	-20pts	_____pts

Comments: _____

Grade for Data Sheet **_____pts**

Experiment 2: Results Table
(Submit as part of your informal report)

Name: _____ Date: _____ Section: _____

All results must be written in pen. **Pencil is not allowed!!**
Record all results with the correct number of significant figures and units.
No marks or notes should be present on this page. Only the tabulated results are allowed.

Chromatographic Data using water

Pen 1 Color: _____

Pen 1 spots	Color	Rf from chromatogram
1		
2		
3		
4		
5		
6		

Pen 2 Color: _____

Pen 2 spots	Color	Rf from chromatogram
1		
2		
3		
4		
5		
6		

Chromatographic data using 5% NaCl

Pen 1 Color: _____

Pen 1 spots	Color	Rf from chromatogram
1		
2		
3		
4		
5		
6		

Pen 2 Color: _____

Pen 2 spots	Color	Rf from chromatogram
1		
2		
3		
4		
5		
6		

Experiment 2: Results Table Rubric (20pts)

Points

Tables are neat and legible	5pts	_____pts
Significant figures (>80% correct)	3pts	_____pts
Units (>80% correct)	2pts	_____pts
All results are present and make sense	10pts	_____pts

Deductions (sliding based on TA discretion)

Results to not match data	-20pts	_____pts
Plagiarism!!! Results are identical to another student	**-100pts**	**_____pts**
Other: _____		_____pts

Comments: _____

Grade for Results Table **_____pts**

Experiment 2: Calculations

Perform the following calculations & submit as part of your informal report.

You must be able to perform these calculations on your concept review.

<u>Determination of R_f of Chromatographic Spot</u>

Perform the following calculation for each spot in each chromatogram. Record the R_f values in the appropriate table in the results section.

$$R_f = \frac{\text{Distance dye moves (mm)}}{\text{Distance solvent moves (mm)}} = \frac{\text{Start line to spot (mm)}}{\text{Start line to solvent front (mm)}}$$

Experiment 2: Questions

Answer the following questions and submit as part of your informal report.

1. Which of the two solvents was better at separating the dyes in each of your two pens? Explain how you arrived at your answer for each pen.
   a. Pen 1:

   b. Pen 2:

2. Do your two pens contain dyes that are chemically similar to each other, or are the dyes in one of the pens significantly different from the dyes in the other pen? Explain the reasoning behind your answer.

3. What would have happened to your experiment if your mobile phase had covered the two pen spots that you placed on the chromatography paper? Briefly explain your answer.

4. How many different dyes are contained in each of your pens? Explain how you arrived at your answer for each pen.
   a. Pen 1:

   b. Pen 2:

5. Of the dyes contained in your pens, which color was most soluble in the mobile phase? Which color was most soluble in the stationary phase? Briefly explain each answer.
   a. Most soluble in mobile phase:

   b. Most soluble in stationary phase:

Experiment 2: Prelab Worksheet

(Submit via Brightspace BEFORE the start of your lab session)

Name: _____ Date: _____ Section: _____ Grade: _____

All information needed to complete this worksheet can be found in the pre-lab information and calculations sections of the lab manual. Read this introductory material first!

- Record all values with the correct number of significant figures and units.
- Place all answers on the line when provided.
- Show calculations for any numerical answers; **work must be shown to receive credit.**
- See any 102 TA in the help office before your prelab is due if you have any questions.
- Each question is worth 2 points.

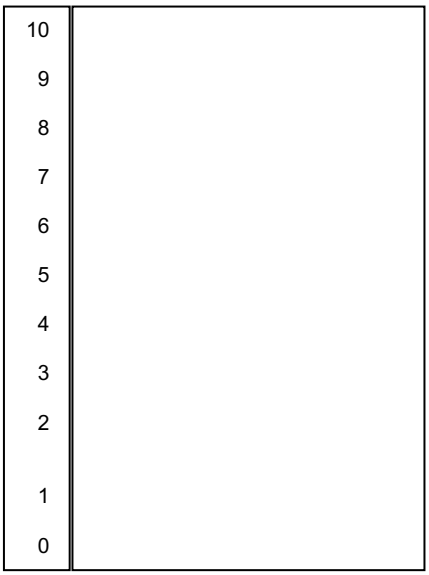
1. What is the purpose of chromatography?

_____

2. Draw a chromatogram in the box with a spot located at an R_f = 0.25. Use the numbers to the left of the chromatogram to estimate your values.

3. Draw lines to represent the solvent start and the solvent front. Label both lines.

4. Show the calculation of this R_f value.



5. What do you call the chromatography paper after the experiment is completed?

Answer: _____

6. Identify the 2 mobile phases used in the experiment.

Mobile Phase 1: _____

Mobile Phase 2: _____

7. Identify the stationary phase in the lab. Answer: _____

8. a.) Can you still use your data if the mobile phase in the beaker covers the spot of dye you are trying to separate? b.) What should you do if this happens?

a.) _____

b.) _____

9. Which is less soluble in the mobile phase, a spot at the top of the chromatogram or a spot at the bottom?

Answer: _____

10. Where do you dispose of the mobile phases in this lab?

Answer: _____