CHEM FAX!

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AP Chem unit 7

Candy Chromatography

AP* Chemistry Investigation
A Guided-Inquiry Wet/Dry Experiment

Introduction

Delve into essential chromatography concepts covered on the AP Chemistry exam with this investigation! A small percentage of the population experiences allergies or sensitivities to tartrazine—one of the seven approved FD&C food dyes. Because many sweets contain FD&C food dyes, this demographic avoids sweets or carefully monitors available food ingredient labels. In order to obtain "pretty" colors, manufacturers often create various mixtures from the approved dyes. As a result, this poses a dilemma for those with tartrazine allergies. Start making connections between the commercial uses of food dyes and their structures and properties with this lab. Select various colored M&Ms™ and use chromatography to determine which contain the FD&C dye, tartrazine. Is tartrazine the sole ingredient in your selected candy samples? Or, is tartrazine in a mixture with other FD&C dyes?

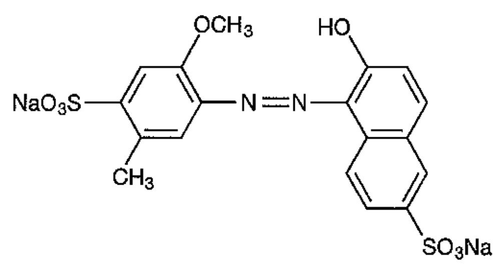
Concepts

- Chromatography
- R_f values
- Polar vs. non-polar
- Intermolecular forces
- Commercial chemistry

Background

Food dye chromatography is an incredibly useful method to explore separation of a dye mixture in this lab class. The concepts covered will strengthen understanding of intermolecular forces and solubility and will introduce the world-wide application of chromatography while bridging chemistry and consumer goods. Candy manufacturers add approved food dyes, known as FD&C dyes, to candy coatings, which are rendered safe for human consumption. Did you know that only seven food dyes are approved by the USDA as safe for human consumption in the U.S.? Just seven. Where, astonishingly, hundreds of color additives were utilized to make food more visually appealing just a century ago. Later, however, food safety regulators prohibited the use of many of these colorants due to their harmful toxicity levels to humans.

All FD&C-approved food dyes are charged, water-soluble organic compounds that bind to natural ionic and polar sites in large food molecules, including proteins and carbohydrates. Notice the organic structure of FD&C Red 40 in Figure 1.



FD&C Red 40
Molar mass = 496 g/mol

Figure 1.

There are extensive series of alternating single and double bonds (also known as conjugation) with a large molar mass when compared to smaller, organic molecules like benzene (78.11 g/mole). The resultant beautiful, intense colors of the FD&C dyes are due to the molecule's conjugation. Notice the bonding sites on the FD&C Red 40 molecule? These bonding sites play a key role in paper chromatography.

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Chromatography is one of the most useful methods of separating and purifying organic compounds. There are many different types of chromatography but most depend on the principle of adsorption. The two important components of chromatography are the adsorbent and the eluent. Absorbents are usually solid materials that will attract and adsorb the materials to be separated. The eluent is the solvent, which carries the materials to be separated through the adsorbent. Chromatography works on the concept that the compounds to be separated are slightly soluble in the eluent and will spend some of the time in the eluent (or solvent) and some of the time on the adsorbent. When the components of a mixture have varying affinities for the eluent, they can then be separated from one another. The polarity of the molecules to be separated and the polarity of the eluent are very important. Changing the polarity of the eluent will only slightly affect the solubility of the molecules but may greatly change the relative attraction for the adsorbent. Affinity of a substance for the eluent versus the adsorbent allows molecules to be separated by chromatography.

The choice of the eluent is the most difficult task in chromatography. Choosing the right polarity is critical because this determines the level of separation that will be achieved. Different samples will spend varying amounts of time interacting with the paper and the solvent. Through these different interactions, the samples will move different distances along the chromatography paper. The distance a sample moves along the chromatography paper is compared to the overall distance the solvent travels—this ratio is called the R_f value or *retardation factor.*

Experiment Overview

Complete the following homework set before lab day. The dry portion of this experiment—the homework set—solidifies the chromatography separation technique and knowledge needed for lab day (wet portion). On lab day, determine if those with allergies or sensitivities to the FD&C dye tartrazine are able to consume various colors of candy coated chocolates by determining its presence in the candies. Each student group will analyze two candy samples and compare their results to a control—tartrazine sample. Results will be shared with the class to arrive at a conclusion—which candies are safe to consume? Turn in any graphs or figures you were asked to create and use a separate sheet of paper, if necessary.

Name: ____________________________

Pre-Lab Homework Assignment

1. Observe a student's chromatography experimental set-up for FD&C dye mixture separation in Figure 2. Answer questions a–d. For free demonstration videos on food dye chromatography, visit flinnsci.com.

Chromatography strip was folded at the top, hanging over the flask lip. Starting at the top of the strip, the student measured 20 mm and folded the paper across the width of the strip.

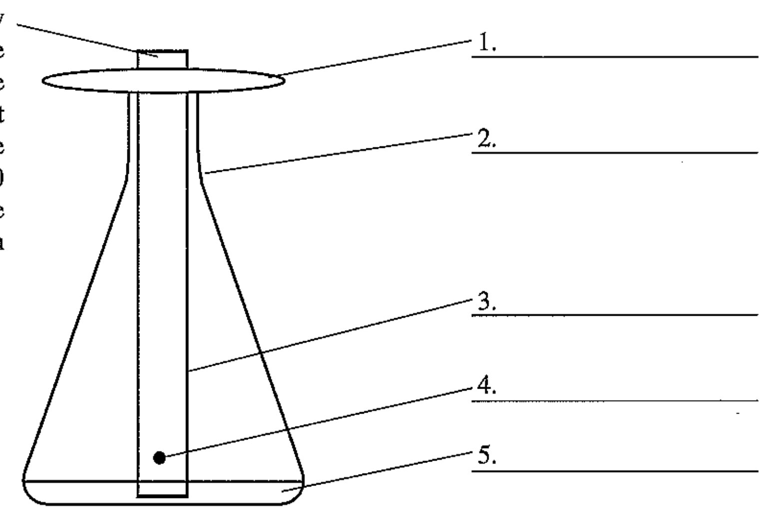


Figure 2.

   a. Label the Figure 2 experimental set-up from the available word bank: *Erlenmeyer flask (250-mL), eluent, chromatography paper (152 mm tall and 19 mm wide), watch glass, dye sample.* Define the terms in this word bank.

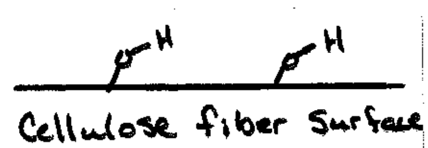
   b. Prior to applying the dye sample, the student made sure to place the sample marker above the eluent, approximately 15 mm from the bottom, marked with a pencil. Why is the eluent level below the sample marker?

   c. Using a toothpick, the student spotted the chromatography strip with a given FD&C dye mixture on the sample marker three times, placed the chromatography strip into the Erlenmeyer flask containing an eluent, and covered the flask with a watch glass. Why was the flask covered with a watch glass?

   d. After development of the chromatogram, about 20–30 minutes, the student removed the strip from the set-up and with a pencil lightly drew a line to mark the distance the solvent traveled—called the solvent front. Why is this step important?

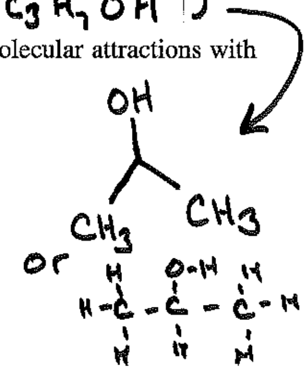
2. An eluent solution of aqueous 2% sodium chloride was used in the experimental set-up question 1. Answer questions a–c

   a. Chromatography paper is very hydrophilic. It is made from a long chain of glucose molecules, a polymer called cellulose. In the molecular diagram below, draw the intermolecular forces between [redacted] 2 water molecules and the unit of cellulose.



   b. Identify the intermolecular force between sodium chloride and water molecules in the 2% eluent and draw a particulate diagram of this representation. Repeat this exercise with an eluent of 2% isopropyl alcohol. (C_3H_7OH)

   c. Observe the FD&C Red 40 molecule in the Background section of this lab. Predict the intermolecular attractions with the two solvents from question 2a.



3. See the student's developed chromatogram in Figure 3. She traced the shape of the dye band, which is the streaked mark the dye leaves at it travels. Answer questions *a–d.*

   a. Label the stationary phase, mobile phase, solvent front in Figure 3.

   b. Calculate the R_f value. R_f = distance traveled by sample/distance traveled by solvent.

   c. In a separate experiment, the student measured the dye band traveling distance as 2.8 cm. In contrast to the results in Figure 3, what can you conclude about the intermolecular attractions to the eluent and paper in this second experiment?

   d. If the student were to witness two dye spots/bands on the resulting chromatogram, what does it tell us about the composition of the sample.

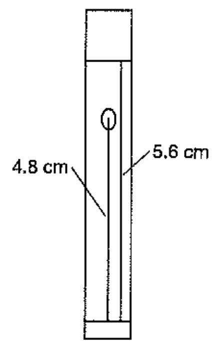


Figure 3.

4. Predict what would happen in the following scenarios:

   a. The student used a black marker instead of pencil as a sample marker on the chromatography paper.

   b. The student used filter paper instead of the given chromatography paper for dye separation.

5. The student analyzed a final FD&C dye mixture sample and recorded her results in the data table below. Select the best eluent, i.e. which eluent provided the best dye mixture separation based on R_f values.

Solvent	**Red 40 R_f**	**Blue 1 R_f**	**Yellow 5 R_f**
8%, sodium chloride solution	0.10	0.73	0.21
0.5%, sodium chloride solution	0.26	0.86	0.49
2%, isopropyl alcohol solution	0.71	0.94	0.92

Total time for chromatograms to develop ranged from 25–30 minutes. Based on the data collected for the 8% and 0.50% sodium chloride solutions, the more dilute solvent separated the mixture of dyes better. The three dyes were more distinguishable from one another in the 0.50% solution than in the 8% solution. This was evident with the overlapping of the Yellow 5 and Red 40 dyes with the 8% solvent.

Based on the data collected for the 2% isopropyl alcohol solution, two separate dye spots were visible: blue and yellow on top, and red on the bottom. The Blue 1 and Yellow 5 dyes overlapped resulting in a blue front followed by a green line then a yellow tail end. The blue-yellow color band traveled straight for a portion of the time, and then the edges began trailing while the middle continued at a "faster" rate.

Really the start of wet LAB

6. Select two candy coated chocolate samples and use paper chromatography to determine which ones contain tartrazine. As a control, obtain a sample of tartrazine from your instructor and analyze it by paper chromatography. Helpful tips:
   a. Think safety first. Make sure you have the proper PPE available to perform this lab, i.e., goggles, apron and gloves.
   b. Make a list of the equipment and glassware needed for this lab.
   c. Based on your answer to question 5, select the eluent and eluent concentration to be used in candy chromatography.
   d. Tartrazine is an FD&C dye, and is also a synonym. Which FD&C dye is tartrazine? Flinn Scientific has a full library of SDS documents on flinnsci.com.
   e. What information do you need from the control and how will this data be compared to the results?
   f. To extract the colors from the candy coated chocolates, place each in a spot well plate and squirt about 0.5 mL (keep the dye concentrated) of deionized water. Use the toothpick to apply it to the chromatography paper.
   g. The developed chromatogram will look very similar to Figure 4. This is an example of how to measure a chromatogram that does not provide even, circular sample spots.

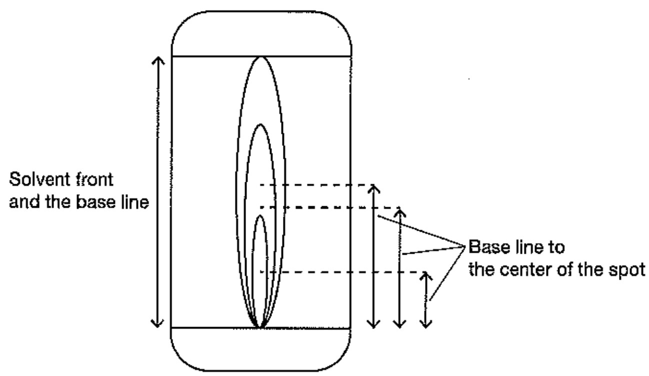


Figure 4.

Safety Precautions

The FD&C dyes are slightly hazardous by ingestion, inhalation, and eye or skin contact. Yellow No. 5 may be a skin sensitizer. All dyes are irritating to skin and eyes. Avoid contact with eyes, skin and clothing. Wear chemical splash goggles, chemical-resistant gloves and a chemical-resistant apron. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

Disposal

Consult your instructor for appropriate disposal procedures.

Sample Student Procedure

1. The materials needed for this lab are listed below:

   Watch glasses, 3

   Erlenmeyer flasks, 3

   Chromatography paper, 3

   Candy coated chocolate samples, 2

   Tartrazine sample, 1 mL

   Spot plate, 12 well

2. Position the chromatography paper strip so it is 152 mm tall and 19 mm wide. Note: Handle the paper by the edges so the analysis area is not accidentally compacted or contaminated.
3. Using a ruler and a pencil, draw a faint line 15 mm from the bottom of the paper across the width of the strip. Measure 9.5 mm from the edge and place a dot on the line. This is the starting point for the sample.
4. Using the same ruler, measure 20 mm from the top of the strip and fold across the width of the strip. This will allow the strip to hang on the lip of the flask.
5. Repeat steps 2 and 3 for a two more paper strips.
6. Obtain two candy coated chocolates and tartrazine sample.
7. Add 1–2 drops of DI water to two wells on the well plate. Add each color candy to the separate wells. Allow enough soaking for the color to dissolve in the water droplets. Using a clean toothpick, spot the chromatography strip by placing a toothpick into the candy wells and then touching the tip of the toothpick gently onto the designated pencil dot. Allow the sample to dry. Perform the same technique with the tartrazine solution. Repeat the procedure two to three more times. Note: This step is necessary to increase the concentration of the sample but do not allow the size of the spot to increase.
8. While the samples are drying, obtain three 250-mL Erlenmeyer flasks and watch glasses to cover the tops of the flasks.
9. Pour 20 mL of the assigned 0.5% chromatography solvent into each flask. Cover the flasks with the watch glasses.
10. Once the chromatography papers are dry, remove the watch glasses from the tops of the flasks. Carefully hang the chromatography strips into the flasks with the sample end down.
11. Carefully place the watch glasses back on the top of the flasks. Allow the chromatograms to develop. Record observations of the samples as the solvent travels up the papers and the chromatograms develop.
12. When the chromatography solvent is within 1–2 cm of the fold in the chromatography strips, stop the runs by removing the strips from the flasks.
13. With a pencil, lightly draw a line to mark the distance the solvent traveled. This is called the solvent front.
14. Measure the distance from the pencil line at the bottom of the strip to the solvent front.
15. With a pencil, trace the shape of each dye band or spot to mark its location on the chromatography strip. This should be done immediately because the color and brightness of some spots may fade over time.
16. Measure and record the distance in millimeters that each dye band or spot traveled. Measure from the line at the bottom of the paper to the center of each band or spot.