Paper Chromatography of Food Dyes and Colors

Cautions

Food, Drug and Cosmetic (FD&C) dyes will stain your clothes and skin so avoid contact.

Purpose

To identify the food drug and cosmetic (FD&C) dyes found in Kool-Aid™ and other mixtures by determining the R_f values for the seven accepted FD&C dyes.

Introduction

Historically, chromatography means "graph of colors" and was used to separate the various colors from natural substances like dyes and plant pigments. Chromatography employs two distinct phases, mobile and stationary phases, to accomplish the separation of the components from the mixture. The mobile phase consists of the components from the mixture and the solvent; it is generally a mixture of solvents having different polarities. The stationary phase is an adsorbent material that has an intermolecular affinity for the solvent and the components of the mixture. Development of the chromatogram will occur when the mobile phase passes over the stationary phase. The different components of the mobile phase have different affinities for the stationary phase; those components with the strongest affinity for the stationary phase will move the shortest distance up the chromatogram while those components with the weakest interactions with the stationary phase will move the greatest distance. When the solvent front, the leading edge of the mobile phase, reaches the edge of the chromatogram paper development is complete. The development process results in separation or resolution of an unknown mixture's components. A successful chromatography experiment with good resolution enables identification of the components found within the mixture when compared to chromatograms of known solutions.

In this lab, chromatographic paper (similar to filter paper) will be used which is made up of polar cellulose paper as the stationary phase. The mobile phase will be an aqueous sodium chloride solution. The cellulose paper contains a large number of hydroxyl groups that have the ability to form hydrogen bonds with water. Typically, chromatography paper is "spotted" with individual solutions of each of the food dyes along with the mixtures and then dried. Then the spotted chromatography paper is placed in contact with the mobile phase, and the dye molecules are transported through the paper by the mobile phase during development.

After development each individual dye molecule may be characterized by its retention factor (R_f). The R_f is a unitless mathematical expression for the distance traveled by the dye molecules divided by the distance traveled by the solvent front. The R_f factor will be constant for a given dye provided all conditions in the experiment are kept constant. The R_f factor is affected by variations within the stationary phase and changes in temperature.

$$R_f = \frac{D_{Dye}}{D_{Solvent}} = \frac{\text{Distance Traveled by Dye}}{\text{Distance Traveled by Solvent Front}}$$

The point where the dye molecules were spotted is known as the origin of the chromatogram. After the chromatogram is dried the distance that the individual dyes traveled can be identified. The distance from the center of the dye spot to the origin line is the distance traveled by the dye. The solvent front distance is the distance between the origin and the highest point the solvent front reached. The best solvent system for a given series of molecules will significantly separate each component from each other resulting in very different R_f values; the mixture components are well *resolved* on the chromatogram. For example a chromatogram of a sample with two components that have R_f values equal to 0.5 and 0.6 is not well resolved; the components were not separated very well and thus has very similar retention factors. If the R_f values for these two components

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were 0.1 and 0.8, the chromatogram is considered to be better resolved or have higher resolution because the components were separated more than in the first example.

The FDA (Food and Drug Administration) is responsible for approving the dyes that can be used in food products for consumption by humans. Currently, there are seven approved dyes for use in food products. These are red 3, red 40, blue 1, blue 2, yellow 5, yellow 6, and green 3, shown in Figure 1. Due to allergic reactions that may occur in humans, certain food dyes (yellow 5, yellow 6, blue 1, red 3, and red 40) must be clearly indicated on all food labels. All seven of these food dyes are rather large compounds that contain a conjugated ring system with alternating single and double carbon to carbon bonds. These types of compounds result in colored molecules and produce a colored solution.

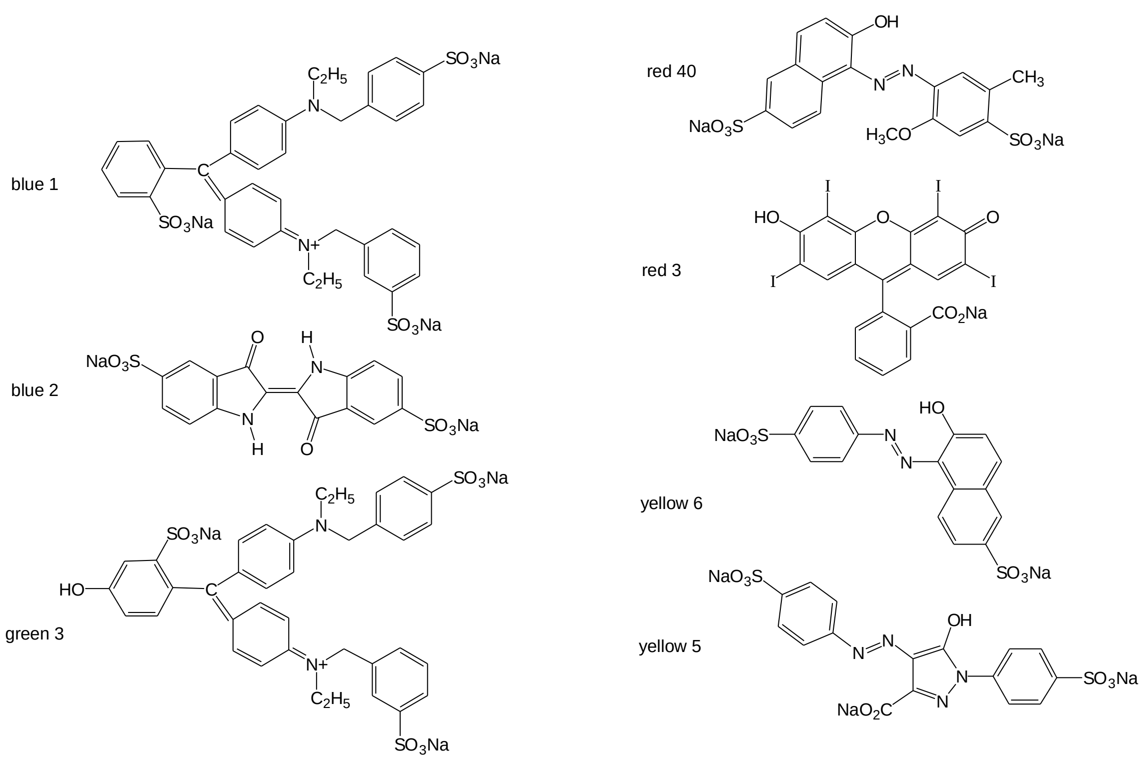

Figure 1: Seven FD&C Food Dyes

In this experiment, a chromatogram for each of the pure dye molecules will be developed and R_f values of each dye calculated. The components of 5 unknown mixtures of dyes, 4 commercial food colors and 3 samples of Kool-Aid™ drink mix will then be identified.

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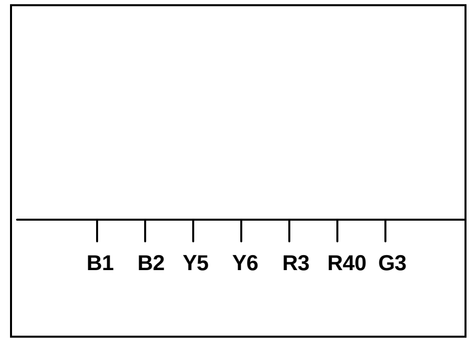
Procedure

Part A. Equipment and Set Up



A1. Obtain 3 pieces of 11 by 18 cm Whatman # 1 Chromatography paper.

A2. Using a pencil, draw an origin line that is 2 cm from the paper edge along the long side. See diagram at right.

A3. Beginning 3 cm from the left, place small marks on origin line every 1.5 cm.

A4. Label the marks on origin line of the 1st piece with the names of the known FD&C dyes: B1 B2 Y5 Y6 R3 R40 G3.

A5. Label the origin line marks on the 2nd piece with your choice of 3 of the unknown mixtures.

A6. Label the origin line marks on the 3rd piece with the four commercial food colors and three Kool-Aid™ mixes you choose.

A7. Obtain three clean beakers (600 mL or larger). Place 25 mL of 0.10% NaCl solution in each beaker and cover each beaker with a piece of Saran Wrap.

A8. Using a spot plate get ONE drop of each of the FD&C food dyes, 3 unknown dye mixtures, 4 commercial food colors, and 3 Kool Aid™ samples of your choice.

Part B. Spotting

B1. Use a separate wooden toothpick for each solution. Using the thinnest end slowly dip into the solution and then spot the paper keeping the toothpick vertical.

B2. Using the handheld dryer slowly dry the spot. You may opt to dry the chromatogram after applying all the spots sequentially.

B3. Repeat steps **B1** and **B2**:

a. Two more times (total of 3 spots directly on top of each other) for the FD&C dye.

b. Two more times (total of 3 spots directly on top of each other) for the unknown mixtures

c. Two more times (total of 3 spots directly on top of each other) for the commercial food colors.

d. Nine more times (total of 10 spots directly on top of each other) for the Kool-Aid™ samples.

B4. After the spots are dry, slowly and carefully roll the paper into a cylinder and staple edges together. ***Note:*** *the edges should be parallel to each other and not overlap or touch. There should be a gap between the two edges*

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Part C. Development of Chromatograms

C1. Remove the Saran Wrap and place the paper cylinder into the beaker developing chamber.

  a. The Origin Line must be at the bottom with the spots should be above the developing solvent.

  b. Paper must not touch the sides of the beaker.

C2. Replace the Saran wrap.

C3. Remove the paper from the beaker when the solvent front is 2 cm from the top of the paper, lay it flat on a paper towel.

C4. With a pencil, mark the top point on the chromatogram where the developing solution reached. All spots can be marked either immediately upon removal of the Chromatogram *or* after it has been allowed to sit for one minute.

C5. Using the handheld dryer slowly dry the Chromatogram.

Part D. Data & Calculations

D1. Measure and record the distances traveled by the solvent front from the origin line on all chromatograms.

D2. Measure and record the distances from the origin line traveled by all samples.

D3. Record the flavors of Kool-Aid™ samples and describe colors of observed spots in the appropriate place in the attached data sheets. Note: Only record spots you actually see, some parts of the tables may be left blank.

D4. Calculate the R_f factor for all spots; record each value in the appropriate table.

D5. Identify the FD&C food dyes in the unknown mixtures, commercial food colors and Kool Aid™ samples.

ASK YOUR INSTRUCTOR BEFORE DISCARDING YOUR CHROMATOGRAMS.

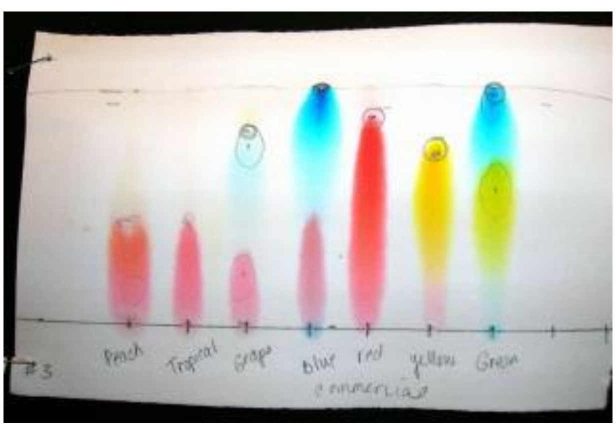
Waste Disposal

Pour all solutions down the sink with an excess of water.

Clean-Up

Wash all glassware with soap then rinse 3 times with tap water, and once with deionized water.

Return all borrowed items to their original locations.



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Data Sheet

Name: ____ **Lab Partner**__________________________________

Standard FD&C Dyes

	Spot Color	Distance Solvent Traveled	Distance Spot Traveled	R_f
Red 3				
Red 40				
Blue 1				
Blue 2				
Yellow 5				
Yellow 6				
Green 3				

Unknown FD&C Mixtures

Unknown ID#:

	Spot color	Distance Solvent Traveled	Distance Spot Traveled	R_f	Identity
Spot 1					
Spot 2					

Unknown ID#:

	Spot color	Distance Solvent traveled	Distance Spot Traveled	R_f	Identity
Spot 1					
Spot 2					

Unknown ID#:

	Spot color	Distance Solvent traveled	Distance Spot Traveled	R_f	Identity
Spot 1					
Spot 2					

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Data Sheet

Name: ____ **Lab Partner**__________________________________

Food Dyes

Blue Food Color					
	Spot color	**Distance Solvent traveled**	**Distance Spot Traveled**	**R_f**	**Identity**
Spot 1					
Spot 2					

Red Food Color					
	Spot color	**Distance Solvent traveled**	**Distance Spot Traveled**	**R_f**	**Identity**
Spot 1					
Spot 2					

Yellow Food Color					
	Spot color	**Distance Solvent traveled**	**Distance Spot Traveled**	**R_f**	**Identity**
Spot 1					
Spot 2					

Green Food Color					
	Spot color	**Distance Solvent traveled**	**Distance Spot Traveled**	**R_f**	**Identity**
Spot 1					
Spot 2					

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Data Sheet

Name: ____ **Lab Partner**__________________________________

Kool Aid™ Drink Mix

Flavor:					
	Spot color	**Distance Solvent traveled**	**Distance Spot Traveled**	**R_f**	**Identity**
Spot 1					
Spot 2					
Spot 3					
Spot 4					

Flavor:					
	Spot color	**Distance Solvent traveled**	**Distance Spot Traveled**	**R_f**	**Identity**
Spot 1					
Spot 2					
Spot 3					
Spot 4					

Flavor:					
	Spot color	**Distance Solvent traveled**	**Distance Spot Traveled**	**R_f**	**Identity**
Spot 1					
Spot 2					
Spot 3					
Spot 4					

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Post-lab Assignment

Name: ______________________________________

1. Explain how you determined the FD&C food dyes present in the unknown mixtures. How did you decide which components were in each mixture?

2. Knowing that water is a very polar solvent, what do the separations observed in this experiment suggest about the polarity of different food dyes?

3. Explain why it is important for the chromatogram to be removed from the developing solution before the solvent front reaches the top of the chromatogram. What problem does this avoid?

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Pre-lab Assignment

Name: ______________________________________

1. Define the following in your own words:

   a. mobile phase –

   b. stationary phase –

2. Consider the following chromatography data obtained from a 2% NaCl solution in water.

Food Dye	Distance Traveled (cm)
Yellow-5	1.75
Blue-1	6.0
Red-3	0.25
Green-3	5.75
Solvent Front	6.63

   a. Calculate the R_f values for each dye.

   b. Which 2 dyes may be difficult to resolve using this solvent system for a mixture? Why?