**Introduction**

The entire palette of artificial food colors is derived from just seven dyes certified by the FDA for use in food, drugs, and cosmetics. How can these FD&C dyes be identified in a mixture? How do the molecular structures of the dye molecules influence their properties, relative solubility or affinity for different solvents?

**Concepts**

- Chromatography
- Polarity
- Food chemistry
- R<sub>y</sub> values
- Intermolecular forces

**Background**

The use of color additives increased dramatically in the United States in the second half of the nineteenth century. As the economy became more industrial, demographics shifted, fewer people lived on farms, and city populations grew. People were becoming more dependent on mass-produced foods.

Color additives were initially used to make food more visually appealing to the consumer and, in some cases, to mask poor-quality, inferior or imitation foods. For example, meat was colored to appear fresh long after it would have naturally turned brown. Jams and jellies were colored to give the impression of higher fruit content than they actually contained. Some food was colored to look like something else—imitation crab meat, for example. Many of the food colorings and additives were later discovered to be harmful or toxic.

In 1883, the United States Department of Agriculture (USDA) Bureau of Chemistry began regulating the food industry and thus ensuring a safe food supply. Food coloring regulation is just one example of the agency’s efforts. Food colorants were being added to food with little or no health testing. To propagate the food safety effort, in 1906 the USDA hired a consultant, Dr. Bernard Hesse, to determine colorants that would be safe to consume in food. In 1907, the number of synthetic food dyes approved for use in the United States was reduced from 695 to just seven. As additional data was collected through consumer reports and laboratory testing, more dyes were eliminated or restricted. Only two of the original dyes from 1907 are still accepted for use today. Five others were added between 1907 and 1971. In total, only seven dyes color all U.S. food today. All of the 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.

Chromatography is one of the most useful methods of separating organic compounds for identification or purification. There are many different types of chromatography but most work on the concept of adsorption. The two important components of chromatography are the adsorbent and the eluent. A good adsorbent is usually a solid material 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.

Paper chromatography is often used as a simple separation technique. In paper chromatography, the adsorbent is the paper itself, while the eluent can be any number of solvents. When the paper is placed in a chromatography chamber the eluent moves up the strip by capillary action. Organic molecules that are “spotted” onto the paper strip separate as they are carried with the eluent at different rates. Those molecules that have a polarity closest to the polarity of the eluent will move up the strip the fastest.

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<sub>y</sub> value or rate of flow.
**Pre-Lab Questions**

1. Figure 1 is a sample paper chromatogram for three samples A, B, C. Label the drawing with the following items: the stationary phase, the mobile phase, and the solvent front.

2. Calculate the $R_f$ value for the spot in sample B using sample A as an example.

3. Sample C gave two spots on the paper chromatogram. What does this tell you about the composition of the sample?

4. Based on the $R_f$ values of samples A and B, what can you conclude about the intermolecular attractions both samples have for the eluent and the paper?

![Figure 1](image)

Chromatography paper, and paper in general, is highly hydrophilic. Paper is made from a natural polymer called cellulose, which is a long chain of glucose molecules. Glucose is a cyclic structure with a number of –OH groups around the ring.

**Questions to answer**

5. Predict and explain the types of intermolecular forces that would occur between paper and water. How do these interactions account for the hydrophilic nature of paper?

6. Look at the structures of the different dyes on the back page of the lab handout. Remembering these are water-soluble dyes, the implication is that everywhere in the structure you see a Na, in reality the sodium ions have dissolved away and that is now an ionic site; the same holds true for any chloride ion. With that in mind, circle all the potential ion sites in each molecule. These sites can form interactions with the solvent (eluent).

7. Again, look at the structures, this time looking for any potential hydrogen bonding sites, such as –OH groups (or –NH) or simply oxygen or nitrogen atoms in the structures. Draw a square around each of these potential hydrogen-bonding sites. These sites can form interactions with both the solvent and the chromatography paper itself.

**Materials**

- FD&C food dye mixtures, 1 ml
- Sodium chloride solution, 0.1%
- Graduated cylinder, 100mL
- Chromatography paper strips, 3
- Erlenmeyer flasks, 250mL, 3
- toothpicks
- beakers, 50mL or 100mL, 3
- watch glasses, 3
- ruler
- pencil

**Safety precautions**

The FD&C dyes are slightly hazardous by ingestion, inhalation, and eye or skin contact. Red No. 40 may be absorbed through the skin and Yellow No. % may be a skin sensitizer. All dyes are irritating to the skin and eyes. Avoid contact with eyes, skin, and clothing. Wash hands thoroughly with soap and water before leaving the laboratory. Please follow all laboratory safety guidelines.

**Activity**

1. Position the chromatography paper strips so they are 152mm tall and 19mm wide. **Note:** handle the paper by the edges so the analysis area is not accidentally compacted or contaminated.
2. Using a ruler and a pencil, draw a faint line 15mm from the bottom of each paper strip across the width of the strip. Place a small mark in the middle of each pencil line. This is the starting point for the sample.

3. Using the sale ruler, measure 20mm from the top of each strip and fold across the length of the strips. This will allow the strips to hang on the lip of the flask.

4. Using a clean toothpick, spot the chromatography strip by placing a toothpick into the sample #1 dye mixture and then touching the tip of the toothpick gently onto the designated pencil mark for the starting point. Allow the sample to dry; this should only take 30 seconds or so. 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 dramatically increase.

5. Repeat step 5 using a new toothpick each time, using the sample dye mixture #2 on a second strip and the unknown dye mixture on the third strip.

6. While the samples are drying, measure out three 20mL samples of the 0.1% NaCl solution into three beakers. Return to your station and pour 20mL of the solution into each of your Erlenmeyer flasks. Cover the flasks with the watch glasses, with the curved side down.

7. Once the chromatography strips are dry, remove the watch glass from the top of the flask. Carefully hang one of the chromatography strips into the flask with the sample end down. Do not get any solvent on the upper portion of the strip. The paper should be freely hanging, with the bottom of the strip in the solution, but make sure the dye spot is above the solution in the bottom of the flask.

8. Carefully place the watch glass back on top of the flask. Allow the chromatogram to develop. Record observations of the dye sample as the solvent travels up the paper and the chromatogram develops.

9. Repeat steps 7 and 8 with the other two strips and flasks.

10. When the chromatography solvent reaches the neck of the flask, or is within 1 – 2 cm of the top of the flask, stop the run by removing the strip from the flask. (This should take about 20 – 25 minutes)

11. With a pencil, lightly draw a line to mark the distance the solvent traveled. This is called the solvent front.

12. 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.

13. Measure the distance from the pencil line at the bottom of the strip to the solvent front. Record this distance in millimeters in an appropriate data table.

14. Measure and record the distance in millimeters that each dye band or spot traveled. Measure from the line at the bottom of the strip the center of each band or spot.

15. Repeat steps 11 – 14 for each of the other two chromatograms.

**Analyze the results**

Calculate the $R_f$ value for each of the dyes from each of the knowns, 1 and 2, that were tested. Consider general observations regarding the separation of the different dyes in each known mixture including developing time, color spreading, and direction of travel.

Calculate the $R_f$ values for any dyes from the unknown sample. Consider general observations regarding the separation of the different dyes in each known mixture including developing time, color spreading, and direction of travel. Based on your observations and the calculated $R_f$ values from the two known samples tested, identify which dye(s) were present in the unknown sample.

Explain the types of intermolecular interactions that would occur between the dyes in known sample 1 (FD&C Red No. 40, Blue No. 1, and Yellow No. 5) and the paper. Explain why each of the dyes travelled different distances on the paper strip. Be specific; include a discussion of each of the dyes in your answer.
Supplementary Information

Figure 1. FD&C Blue No. 1

Figure 2. FD&C Blue No. 2

Figure 3. FD&C Green No. 3

Figure 4. FD&C Red No. 3

Figure 5. FD&C Red No. 40

Figure 6. FD&C Yellow No. 5

Figure 7. FD&C Yellow No. 6