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Separation of a Dye Mixture Using Chromatography

AP* Chemistry Big Idea 2, Investigation 5

An Advanced Inquiry Lab

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, including their relative solubility or affinity for different solvents?

Concepts

- Chromatography
- Polarity
- Food chemistry
- R_f 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 became 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 to help ensure 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 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. Adsorbents 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.

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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.

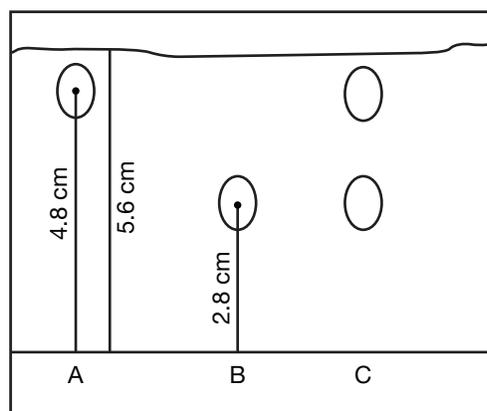
The choice of the eluent is the most difficult task in chromatography. Choosing the right polarity is critical because this determines the level of separation that will be achieved. Different samples will spend varying amounts of time interacting with the paper and the solvent. Through these different 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_f value or *rate of flow*.

Experiment Overview

The purpose of this advanced inquiry lab is to investigate the factors that influence the separation of food dyes using paper chromatography. The investigation begins with a baseline activity comparing the separation or resolution of three FD&C dyes, Red No. 40, Blue No. 1, and Yellow No. 5, using two solvents. Reviewing the evidence provided by the cooperative class data leads to the selection of a solvent for further study. In the guided-inquiry section of the lab, students will design an experiment to identify a solvent that will give maximum resolution of a mixture of dyes. The results may be applied to study the connection between structure and mobility of food dyes. An investigation into the composition of colored candy shells may be incorporated as an optional extension activity.

Pre-Lab Questions

- Figure 1 is a sample paper chromatogram for three samples: A, B and C. Label the drawing with the following items: the stationary phase, the mobile phase and the solvent front.
- Calculate the R_f value for the spot in sample B using sample A as an example.
- Sample C gave two spots on the paper chromatogram. What does this tell you about the composition of the sample?
- 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?



$$R_f \text{ for A} \\ R_f = \frac{4.8}{5.6} = 0.86$$

Figure 1.

Materials

FD&C food dye mixtures, 1 mL

Isopropyl alcohol solution, $\text{CH}_3\text{CHOHCH}_3$, 2%

Sodium chloride solution, NaCl, 2%

Unknown dye mixtures

Water, distilled or deionized

Beaker, 50-mL

Beakers, 100-mL, 2

Chromatography paper strips

Erlenmeyer flasks, 250-mL, 2

Graduated cylinder, 25-mL

Pencil

Ruler

Toothpicks

Wash bottle

Watch glasses, 2

Safety Precautions

Isopropyl alcohol is a flammable liquid and is slightly toxic by ingestion or inhalation. Use proper exhaust ventilation to keep airborne concentrations low. The FD&C dyes are slightly hazardous by ingestion, inhalation, and eye or skin contact. Red No. 40 may be absorbed through skin and 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.

Introductory Activity

1. 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.
2. 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.
3. 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.
4. Repeat steps 2 and 3 for a second paper strip.
5. Obtain the dye mixture.
6. Using a clean toothpick, spot the chromatography strip by placing a toothpick into the dye mixture solution and then touching the tip of the toothpick gently onto the designated pencil dot. Allow the sample to dry. 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.
7. Repeat step 6 for the second chromatography strip.
8. While the samples are drying, obtain two 250-mL Erlenmeyer flasks and watch glasses to cover the tops of the flasks.
9. Pour 20 mL of the assigned 2% chromatography solvent into each flask. Cover the flasks with the watch glasses.
10. Once the chromatography paper is dry, remove the watch glass from the top of the flask. Carefully hang the chromatography strip into the flask with the sample end down. Do not get any solvent on the upper portion of the strip. The sample spots must remain above the level of the solvent. If the solvent level is too high, the samples will dilute into the solvent!
11. Carefully place the watch glass back on the 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.
12. Repeat steps 10 and 11 using the other chromatography strip and flask.
13. When the chromatography solvent is within 1–2 cm of the fold in the chromatography strip, stop the run by removing the strip from the flask.
14. With a pencil, lightly draw a line to mark the distance the solvent traveled. This is called the solvent front.
15. 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.
16. 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.
17. 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.
18. Repeat steps 13–17 for the other chromatograms.

Analyze the Results

Compile the class data and calculate the average R_f value for each dye in both solvents. Compare general observations regarding the separation using the different solvents, including developing time, color spreading, and direction of travel.

Guided-Inquiry Design and Procedure

Form a working group with other students and discuss the following questions.

1. Examine the structures of the FD&C Red No. 40, Blue No. 1 and Yellow No. 5 dyes. What are the similarities and differences in the structures of the three dyes?
2. In the *Introductory Activity*, the developing solvents were 2% sodium chloride aqueous solution and 2% isopropyl alcohol aqueous solution. Draw separate molecular diagrams of how sodium chloride and isopropyl alcohol would interact in water. Identify the types of intermolecular attractions within each diagram.
3. Based on the diagrams and intermolecular attractions identified in Question 2, predict and compare the nature of intermolecular attractions experienced by the FD&C Red No. 40, Blue No. 1 and Yellow No. 5 dyes with the two solvents.
4. 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.
 - a. 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?
 - b. Explain the types of intermolecular interactions that would occur between the FD&C Red No. 40, Blue No. 1 and Yellow No. 5 food dyes and the paper.
5. Write a detailed step-by-step procedure using dilutions of more concentrated solvents to investigate the effect of concentration on dye separations in various unknown dye mixtures.
6. Include all materials, glassware and equipment that will be needed, safety precautions that must be followed, the concentrations of the solvents, etc.
7. Review additional variables that may affect the reproducibility or accuracy of the experiment and how these variables can be controlled.
8. Carry out the experiment and record the results in an appropriate data table.

Analyze the Results

Compile the data within your group. Identify the optimal solvent tested by your group. Propose an explanation for why the chosen solvent was best.

Opportunities for Inquiry

As noted in the *Background* section, FD&C food dyes are used in a wide range of food products, most notably the outer shells of candies. Candy may be placed in 5–6 drops of water. Stir the candy until the color dissolves. Repeat with two more candies. This is the color sample. Design an experiment to determine the composition of the dye mixture in the candy shell.

AP Chemistry Review Questions

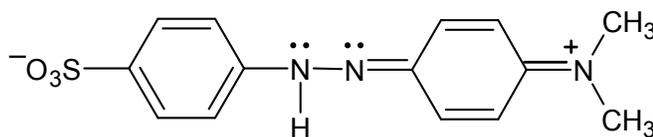
Integrating Content, Inquiry and Reasoning

1. Hydrocarbons are nonpolar compounds containing carbon and hydrogen atoms. The properties of three hydrocarbons are summarized below.

Methane	Octane	Eicosane
CH ₄	C ₈ H ₁₈	CH ₃ (CH ₂) ₁₈ CH ₃
Natural gas	Gasoline	Lubricant (grease)
Gas, bp -161 °C	Liquid, bp 126 °C	Solid, mp 37 °C

- a. How do the attractive forces between molecules change in the transition from the gas to the liquid to the solid state?
- b. Based on its properties, which compound has the strongest attractive forces? The weakest attractive forces?
- c. Write a general statement describing how the size of a molecule influences the strength of London dispersion forces between molecules.
2. Dyes are organic compounds that can be used to impart bright, permanent colors to fabrics. The affinity of a dye for a fabric depends on the chemical structures of the dye and fabric molecules and also on the interactions between them. Three common fabrics are wool, cotton and nylon. Wool is a protein, a naturally occurring polymer made up of amino acids with ionized (charged) side chains. Cotton is a naturally occurring polymer made up of glucose units with hydrophilic groups surrounding each glucose unit. Nylon is a synthetic polymer made of hydrocarbon repeating chains joined together by highly polar amide (–CONH–) functional groups.

- a. The chemical structure of methyl orange is drawn below. Identify the groups in the dye that will bind to ionic and polar sites in a fabric.



- b. Complete the following “If/then” hypothesis to explain how the structure of a fabric will influence the relative color intensity produced by methyl orange.

“If a fabric contains more ionic and polar groups in its structure, then the intensity of the dye color due to methyl orange should (increase/decrease), because _____.”

- c. Using this hypothesis, predict the relative color intensity that would be produced by methyl orange on cotton, nylon and wool. Rank the fabrics from 1 = lightest color to 3 = darkest color.