Lab #4: Paper Chromatography to Separate Dye Mixtures

Objectives:

1. To understand the nature of chromatography.
2. To investigate different factors the can affect the separation of food dye mixtures using paper chromatography based on the seven FD&C (Federal Food, Drug and Cosmetics Act) dyes as references.
3. Design and evaluate an experiment to identify a solvent that will best result the proper resolution of different mixtures of dyes.
4. Using R_f values of reference food dyes to identify the components of food dye mixtures.
5. Apply the obtained results to recognize how the molecular structures of food dyes affect their mobility in a chromatography analysis.

Introduction of Chromatography:

Chromatography is a collective term that describes separation techniques that utilizes passing a mixture dissolve in a "mobile phase" through a stationary phase. Since chemicals in a mixture have small (or large) differences in polarity, they will spend different amount of time as they move with the "mobile phase" through a stationary phase. These differences in rates separate various components of a mixture.

A mobile phase is the phase that moves through in a definite direction. Chemicals that move with the mobile phase tend to have similar polarity with the eluent (the chemical chosen to be the mobile phase). The choice of eluent tends to be the most difficult task in any chromatography analysis. This is because the right polarity is very important as this decides the level of separation to be achieved. Changing the polarity of the eluent will change the solubility of the analyte (the substance that is separated out in a chromatography analysis) slightly, but it will greatly change the degree to which it is held by the absorbent. A general rule is that if the analyte is polar, then the eluent should be slightly less polar (if the analyte is non-polar, the eluent should be slightly more polar).

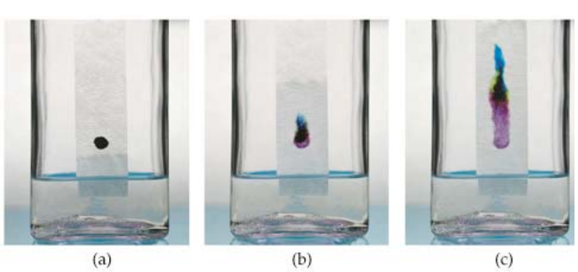
Common Eluents in Increasing Polarity:

Petroleum Ether or Hexanes	Cyclohexane	Toulene	Choloform	Ethyl Ether	Acetone	Ethanol	Methanol	Water

⟶ More Polar

A stationary phase is the phase that immobilizes the support particles (non-analyte chemical). It is commonly called an absorbent. A good absorbent is usually a solid material that will attract and absorb the materials to be separated. Paper, silica gel, or alumina are fairly good common absorbents.

Paper chromatography uses chromatography paper (can be filter paper or even coffee filter paper) as an absorbent. The paper is hanged into a jar or a container filled with a shallow layer of the solvent. The paper is made up of cellulose, which is a polar substance. The substance that travels the furthest up is the most non-polar solute. More polar substances form bonds with the paper, and therefore do not travel up as quickly.



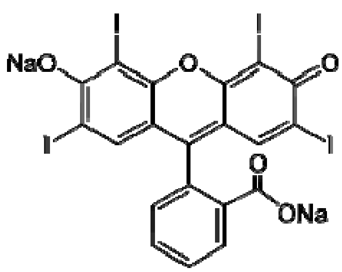
a) The mixture is placed at the end of the paper. b) The mixture begins to separate as the eluent travels up the paper. c) The most polar substance stays near the bottom of the paper and the most non-polar substance travels further up

The Rate of Flow (R_f) is the ratio of distance(s) the analyte spot(s) versus the overall distance of the eluent. By analyzing the R_f values of different known reference chemicals, we can quickly determine the composition of a sample analyte that contains a mixture of these chemicals.

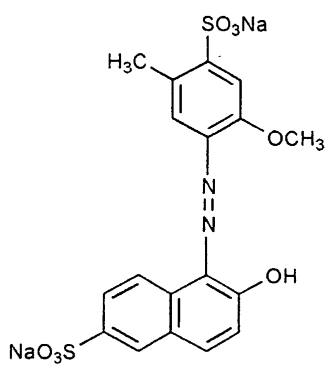
$$R_f = \frac{Distance\ of\ Analyte\ Front}{Distance\ of\ Eluent\ Front}$$

Background Information:

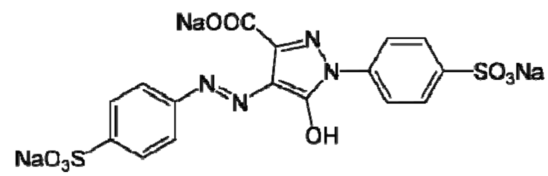
The US Department of Agriculture (USDA) is responsible to regulate the food industry to certify the safety of food product. Its Bureau of Chemistry began this task in 1883. One type of these chemicals under the purview of the bureau is food colour additives. Most processed food has food colour additives to make products more pleasing to look at and to induce buyers to think they contain more natural food content. Such common marketing practice is found from candies to frozen and fast food. Since early food colour additives are not regulated and are later determined to be toxic, USDA vigorously tested all food colour additives available and has currently only approved seven compounds. They are Red No. 3, Red No. 40, Yellow No. 5, Yellow No. 6, Green No. 3, Blue No. 1, and Blue No. 2. Their chemical structures are indicated below.



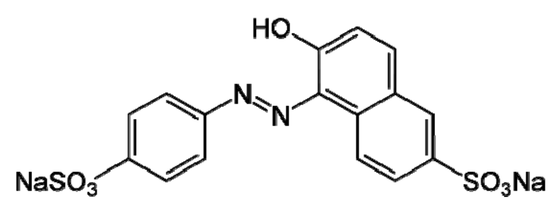
FD&C Red No. 3 (Erythrosine)



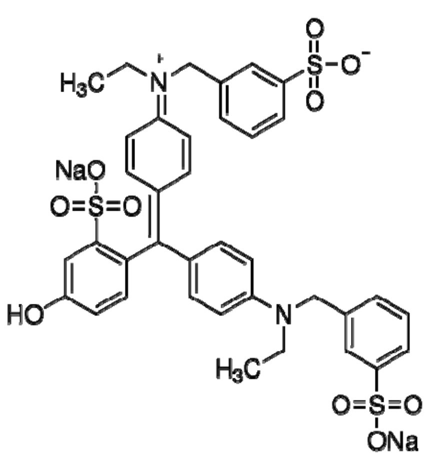
FD&C Red No. 40 (Allura Red AC)



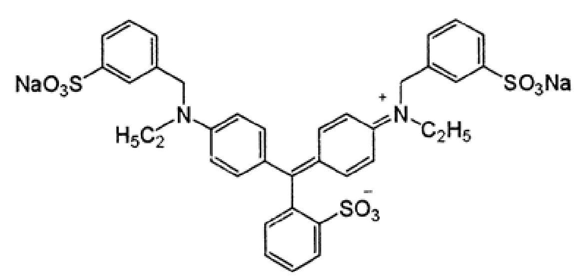
FD&C Yellow No. 5 (Tartrazine)



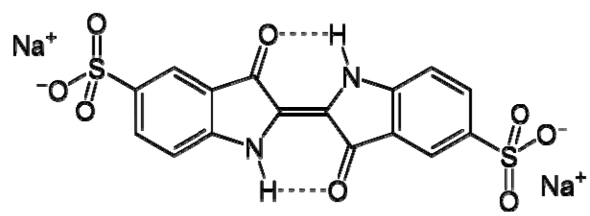
FD&C Yellow No. 6 (Sunset Yellow FCF)



FD&C Green No. 3 (Fast Green FCF)

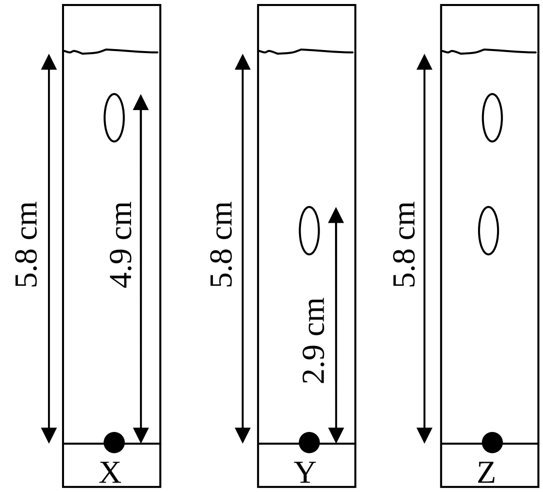


FD&C Blue No. 1 (Brilliant Blue)



FD&C Blue No. 2 (Indigo Carmine)

Pre-Lab Questions:



1. To the right are the paper chromatograms for three samples X, Y, and Z. Copy the drawing. Indicate the mobile phase, stationary phase, and the solvent front on the drawing.
2. Determine the R_f values for the spot in sample X and Y.
3. Sample Z consists of two spots on the paper chromatogram. What can you infer about its composition?
4. According the R_f values of samples X and Y, discuss the relationships between their structures and intermolecular forces with that of the eluent and the paper.

Materials:

13 Small Beakers (50 mL)
21 Erlenmeyer Flasks (250 mL)
21 Watch Glasses
2 Graduated Cylinders (50 mL)
Strips of Chromatography Paper
Rulers
Toothpicks
Pencils
FD&C Food Dyes and Mixtures
2% Isopropanol Solution
2% NaCl Solution

Initial Procedure:

1. Precut 14 strips of chromatography paper such that they have dimensions of 150 mm by 20 mm. You must handle these paper strips by the edges so the oil on your fingers does not contaminate analysis surface.
2. Draw a faint line around 15 mm from the bottom of paper strip using a pencil and a ruler. Place a dot on the line about 10 mm from the edge for the starting point of the reference food dye.
3. Measure about 22 mm from the top of the strip. Fold across it so that it can hang on the opening of the Erlenmeyer flask. You might want to test it on an empty flask so that it does not accidentally fall in to the flask during the chromatography run.
4. Repeat the above two steps for the remaining paper strips.
5. Take a reference food dye and using a clean toothpick, spot the chromatography paper strip by placing it into the food dye and then gently touch the pre-marked pencil dot. Let it dry on the paper and repeat this step a couple more times. You want to make the reference dot more concentrated but keep the relative size to stay the same. Label the food dye you use on the folded part of the paper strip.
6. Repeat step 5 for the rest of the 6 food dye references and let the strips dry. You should have 2 strips of each reference food dye.
7. Place 14 watch glasses on top of 14 Erlenmeyer flasks. Mark 7 of the flasks with the name of the one chromatography solvent use (see below) and the mark the other 7 flasks with the name of the other solvent.
8. Measure out 20 mL of each of the chromatography solvents given (2% isopropanol and 2% NaCl) with two different graduated cylinders and pour the measured solvent in each of the flask.
9. Remove the watch glass and place one chromatography paper strip in a flask carefully. Quickly place the watch glass over the flask. Repeat for the remaining reference strips. Each reference food dye should be in two flasks of two different solvents (isopropanol and NaCl).
10. When the solvent is within 1 to 2 cm from the top of the flask, remove the strip from the flask.
11. Using a pencil, draw a line to mark the where the solvent stops. Measure the distance from the bottom line to this mark in mm with the ruler for the solvent front and record it in your observation table.
12. Using a pencil, trace the shape of each dye band quickly as the spots might fade over time. Measure the distance from the bottom line to the front edge of the dye band in mm and record it in your observation table.
13. Repeat the last two steps with the remaining chromatograms.

Experimental Designs:

Discuss the following questions as a class.

1. Analysis the structure of the seven FD&C food dyes. Highlight their similarities and differences. Organize your finding in a table.
2. We used two different solvents in the initial procedure (isopropanol solution and NaCl solution). Draw the molecular diagrams of how the two solvents you used interact with water. Indicate the type of intermolecular forces in each diagram.
3. Using these diagrams in the previous questions and the analysis from question 1, hypothesize and compare the strengths of intermolecular interactions by these seven FD&C reference dyes with the two solvents used.
4. Paper is made up cellulose, which is a natural polymer. Glucose has a cyclic structures punctuated with many hydroxyl (-OH) groups.
   a. Define hydrophilic. Chromatography paper like many paper in general are very hydrophilic.
   b. Identify the type of intermolecular forces between paper and water, if any. Account for the hydrophilic tendency of paper.
   c. Describe the type of intermolecular forces between each of the food dyes tested and the paper.
5. If you were given food dye mixtures of different reference FD&C dyes, what solvent would you use to separate them? Explain your reasoning.
6. Based on the material list and the initial procedure, write a detailed procedure and list the material used in order for you to separate seven sample dye mixtures.
7. Discuss any possible sources of errors or variables that might affect the reliability and the accuracy of the experiment. How would these variables be controlled in your procedure?
8. Run the experiment and record the results in an appropriate observation table below the reference data table.
9. (Optional) Repeat your procedure for any available commercial candies with colour shells.

Analysis:

Calculate the R_f values for the components of the food dye mixtures and identify them. Show your calculations.

Evaluation:

1. Many hydrocarbons are nonpolar compounds. Methane is a major component of natural gas. Gasoline is mainly octane and grease (used as lubricant) is made up mostly of eicosane.
   a. What are the structural chemical formulas for methane, octane and eicosane? Research to find their melting and boiling points.
   b. What happens to their intermolecular forces when these nonpolar substances undergo phase changes (from solid to liquid and from liquid to gas)?
   c. Arrange these three compounds from the weakest to the strongest intermolecular force. Explain your reasoning.

2. Organic dyes are commonly used to give permanent colours to different fabrics. The types of dyes used depend on the chemical structures of the fabric as well as the dye. Cotton, wool and nylon are some common commercial fabrics. Cotton is a natural polymer with glucose units and hydrophilic groups surrounding each of these glucose units. Wool is a natural protein (amino acids) polymer with ionized or charged branched chains. On the other hand, nylon is a synthetic polymer with repeating monomers of hydrocarbons connected together by polar amide group (-CONH-) functional groups.
   a. Research and draw the structure of methyl orange. Identify and circle the groups in the methyl orange dye drawing the will connect to the ionic and polar sites of a fabric.
   b. Why do most fabrics have ionic sites? What would be the drawback and advantages of completely non-polar fabrics?

c. Complete the following hypothesis so it gives an explanation of how the colour intensity of methyl orange dye is affected by the fabric structure.
   "If a fabric structure consists of more ionic and polar groups, then the intensity of the methyl orange dye will ________________ (increase / decrease). This is because ______________________ ____."

d. Using the hypothesis you completed above, rank the relative colour intensity that would be produced on cotton, wool and nylon by the methyl orange dye.

Conclusion:

State what you have learned from this lab and whether you have met the objectives.