Paper Chromatography: A Sticky Question
How Do You Separate Molecules That Are Attracted to One Another?

Central Challenge:

The central challenge in this lab investigation is to develop a method to separate three similar molecules.

Context for This Investigation:

You are working for a crime lab and a chemical residue has been turned in for analysis. To identify the chemicals in the residue, you will need to separate them from the mixture and identify them individually. Another lab technologist has made an attempt to separate the molecules but was not as successful as the boss woman would like. There was only one molecule separated from the mixture, but your boss suspects that there are at least three different molecules. Science is often a process, where a method is tried and then modified for a second attempt. Your job will be to propose a modification and attempt to improve the separation attained.

Pre-Lab Questions:

1. In your kitchen, there is a mixture that is usually listed as a single ingredient in recipes: baking powder. Baking powder is actually a mixture of sodium bicarbonate (baking soda), cream of tartar (sodium bitartate), and cornstarch. Look at these three molecules below in Figure 1 and describe how they are similar and how are they different in terms of size, bonds present, polarity, and types of intermolecular forces they can participate in, etc.

2. In the space around Figure 1, show how water molecules interact with the components of baking powder by drawing H_2O molecules in the correct position and orientation around each. Use a Lewis dot structure to represent water.

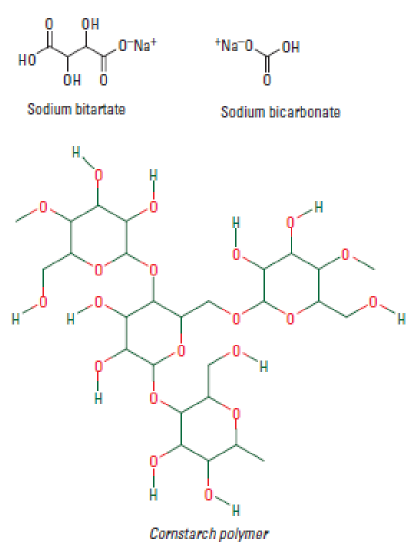

Figure 1. Components of baking powder

3. Use the video (https://www.youtube.com/watch?v=7UShRCcxw9Q) to help you answer the following questions:
   a. How can molecules attract each other when they are in a mixture? Predict how ethanol (C_2H_5OH) would interact with water molecules.

      Draw a picture illustrating the interactions between water and ethanol.

   b. What does the R_f value describe on a microscopic level? Why is this important?

   c. If the molecule had a very high affinity for the stationary phase, how would this affect the R_f value? Explain.

   d. What role does the mobile phase play in the distance a molecule travels in chromatography? What does the mobile phase describe?

   e. If you combined a polar solvent with a molecule that has a carbonyl group (carbon with a double-bonded oxygen), would it have a high or low R_f value? Justify your answer with what you understand of intermolecular forces.

Further Explanation to Strengthen Your Understanding:

Chromatography is a very useful technique that is applied in many situations in the real world. It is used to separate compounds and analyze subtle differences, like the ones between different food dyes. Liquid chromatography is used to test water samples for pollution. Gas chromatography is used to detect bombs in airports, identify drugs, and in forensics for fiber analysis. Thin layer chromatography can be used for detecting pesticide. Paper chromatography is used in separating amino acids, DNA fingerprinting, and separation of antibiotics.

There are two phases in paper chromatography, a stationary phase (the paper) and a mobile phase (the solvent). A molecule can have a greater affinity for either the paper or for the solvent. The filter paper is made of cellulose, a polymer. Cellulose will attract water molecules to its exposed hydroxyl (OH) groups along the polymer. This interaction makes a thin layer of water on the paper that competes for the attraction of the molecules being separated. Alternately, the molecule can be attracted to the solvent and travel with the solvent up the paper. When doing chromatography, a small amount of solvent is placed in a sealed container. The mixture being separated is applied to a piece of filter/chromatography paper, the starting point is marked and the paper is put into the solvent. The container must be sealed so the solvent saturates the paper and does not evaporate first. The level of separation is measured by a ratio that compares the distance that the molecule travels to the distance the solvent travels. This ratio is called the R_f value. To calculate the R_f value, you must identify, mark and measure the distance that the solvent traveled on the paper. Second, you must measure the distance that the molecule traveled. It is best to run the test more than once to reach the most accurate average R_f values possible. The R_f value is a ratio of the distance the molecule traveled divided by the distance solvent traveled. The greater the distance the molecule travels, the greater its affinity for the solvent and the larger the R_f value.

In this experiment, you will have a choice of different solvents to use. In modern chemistry, chemists use principles of green chemistry to evaluate the solvents that are used in a chemical process for their level of toxicity to humans and the environment. Solvents are also evaluated in terms of their life cycle or how long the molecule remains in the environment and if the molecule breaks down to become more benign or more toxic. The overall focus of green chemistry is to be more efficient in chemical production, producing less waste, using fewer toxic molecules, and producing waste that biodegrades and does not pose a risk to the environment.

The food dyes that are in the mixture have their own green chemistry issues. For example, the molecules used may have a life cycle that is longer than previously anticipated and possibly increased toxicity. When scientists evaluate the toxicity of molecules based on the experimental data, efforts to understand the origins of toxicity often look at the molecular structure of the substance. A key strategy for looking at molecular structure is to identify functional groups that are present. There are specific functional groups that are known to create toxic by-products when they are metabolized in the human body, such as acetaminophen that can be converted to N-acetyl-p-benzoquinone imine (organic chemistry – barf). All three food dyes used in this lab are azo dyes, which means that they contain a double-bonded nitrogen connecting multiple aromatic carbons. While the molecules resemble one another, only Red Dye #40 has been linked to allergic reactions in some people, but the FDA has not found conclusive evidence that such a dye is unsafe. In Europe, these food dyes are not used and natural pigments are used instead.

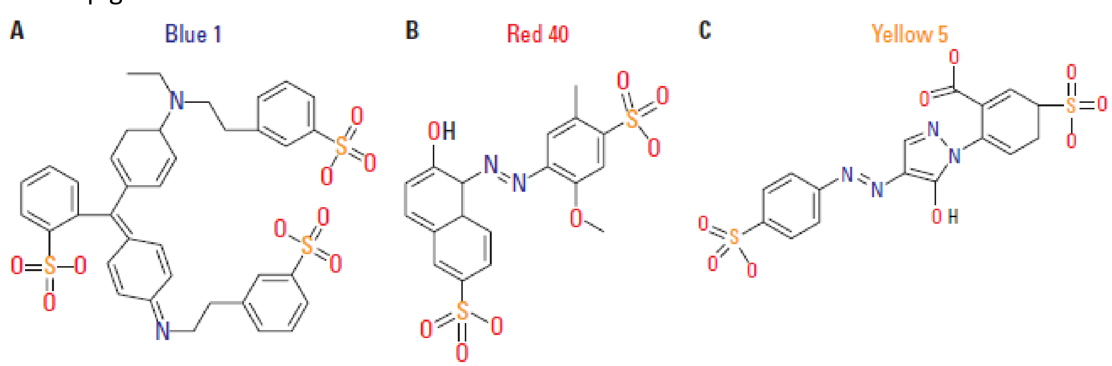

Figure 2. Molecular structure of food dyes

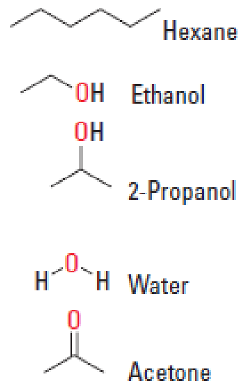

Figure 3. Molecular structure of typical solvents

Beginning question:

1. You will design an experiment that tests the solvents (from Figure 3 above) that you believe will provide the best separation of the food dyes (from Figure 2) in the mixture of food dyes.
2. Once the best solvent has been determined, separate the dyes using that solvent (time allowing).

Materials:

Sample mixture to separate	Metric rulers accurate to mm	Tape for labeling
Pencils	100 mL graduated cylinders	Distilled water (solvent)
2-propanol (isopropanol) (solvent)	Ethanol (solvent)	Acetone (solvent)
Chromatography solvent (petroleum ether and acetone mixture)	Chromatography Chambers: Small (25 - 50 mL) containers with lids/stoppers	Filter paper or chromatography paper (cut to fit the diameter of the chamber but shorter than its height)

Safety Considerations and Waste Disposal:

The mixture of food dyes is safe to dispose of down the sink, as well as the distilled water. The other solvents – acetone, 2-propanol, ethanol and chromatography solvent – should be collected and disposed of in designated waste containers. Normal laboratory precautions should be taken, including wearing splash-proof goggles at all times. If solutions are spilled on skin, wash with copious amounts of water. Whenever chemists work with a new or unfamiliar reagent, they consult its Material Safety Data Sheet (MSDS) to inform themselves of safety considerations and proper disposal methods. An MSDS (or SDS – Safety Data Sheet) should be included by the manufacturer for each reagent shipped out and facilities usually keep a record of them for emergency situations. Additionally, general MSDS information can be obtained from online databases such as https://www.flinnsci.com/sds/ or https://www.osha.gov/chemicaldata/.

Look up at least 3 safety considerations that are pertinent to this lab (hazards, first aid, disposal etc) for the solvents you will be using in this investigation:

- 2-propanol (AKA: isopropyl alcohol, isopropanol, rubbing alcohol)
- acetone
- ethanol (AKA: ethyl alcohol)
- chromatography solvent (AKA: petroleum ether and acetone mixture)

Include these considerations in the safety section of your lab report.

Procedure:

You will need to write a step-by-step procedure and design a data table to record data. The procedure needs to be clear enough that someone else could repeat the experiment and get the same results. The materials listed above are available – consult with your teacher if you would like to use any additional equipment.

Data:

NEATLY draw your data table in your lab report. Include both your individual data and a summary of the class data.

Your claims and evidence and reasoning sections should address the following questions:

1. Which solvent was the best for separating the three molecules?

2. What explanations can you provide for your separation of the three molecules? How was the choice of the solvent connected to the separation process?

3. What part of the chromatography setup did the molecules interact with, stationary or mobile phase? Explain this interaction using intermolecular forces.

4. Draw a picture of how the chromatography worked. Explain your picture using the following terms: stationary phase, mobile phase, and intermolecular forces.

5. Calculate the R_f values for each chromatography trial that you completed and include it in your data table. Show your work in the calculation section.