Chromatography: Candy Coating and Marker Colors
Teacher Advanced Version

In this lab you will separate a mixture of unknown composition using several common household items. You will then perform a more specific separation, thin layer chromatography, in which you separate the dyes in Skittles and M&M's, and the differing colors used in making black ink.

California Science Content Standards:

- **2. Chemical Bonds: Biological, chemical, and physical properties of matter result from the ability of atoms to form bonds from electro-static forces between electrons and protons and between atoms and molecules.**
- 2a. Students know atoms combine to form molecules by sharing electrons to form covalent or metallic bonds or by exchanging electrons to form ionic bonds.
- 2d. Students know the atoms and molecules in liquids move in a random pattern relative to one another because the intermolecular forces are too weak to hold the atoms or molecules in a solid form.
- **2g. Students know how electro-negativity and ionization energy relate to bond formation.
- **6. Solutions: Solutions are homogeneous mixtures of two or more substances.**
- 6a. Students know the definitions of solute and solvent.
- **6f. Students know how molecules in a solution are separated or purified by the methods of chromatography and distillation.

Key Concepts:

- In chemistry and engineering, a **separation** refers to a process in which components of a mixture are separated from one another.
- A **basis of separation** is a property which differs among the components of a mixture that we take advantage of in order to separate those components.
- **Chromatography** is a general type of separation method in which a mixture of compounds passes through a **stationary phase**. Different compounds have different relative affinities for the mixture traveling along the stationary phase, or the **mobile phase**, and the stationary phase, causing these compounds to separate from one another.
- **Thin layer chromatography (TLC)** is a chromatography technique that separates components of liquid mixtures based on the polarity of the individual components.
- **Polarity** refers to the separation of charge in a molecule – in other words, how unequally the electrons in a molecule are shared among the different atoms.
- **Capillary action** occurs when liquids are able to move uphill against the force of gravity due to interactions between the stationary and mobile phases.

Prerequisites:

There are no math prerequisites for this lab. The basic version progresses through the separations puzzle more slowly and with more teacher guidance.

Complete List of Materials:

- Part 1: The more of these items you can have for each group, the better, though it is fine to have groups working at different tables share some of the larger items.
  - Ping pong or golf balls (at least three per group of 3-4 students)
  - Tongs
  - Red beans (preferably 1 cup per group); a few pinto beans optional
  - Colander (with holes large enough to let grape nuts through but not beans)
  - Tweezers
  - Salt (1 tsp. per group)
  - Fine strainer
  - Steel scrub brush
  - Scissors
  - Magnet (any old classroom magnet should do the trick)
  - Napkins or paper towels
  - Grape nuts cereal (3 tbsp. per group)
  - Flat toothpicks (at least four per group)
  - Water in a cup or bottle (at least 20 oz. per group) and access to a water source
  - Spoon
  - Three large bowls
- Part 2: For every group of 2-4 students, you will need the following:
  - M&M's and Skittles
  - Coffee filter paper
  - Water
  - Rubbing Alcohol
  - Pencil
  - Scissors
  - Ruler
  - Toothpicks
  - Aluminum foil
  - 2 clear drinking glass
  - Three (or more) different black markers (ie: Sharpie, Expo, Crayola, Vis-à-vis)

Optional Materials for Part 1:

- The more of these items you can find scattered around your room, the better. Strictly speaking, students shouldn't need to use these, but they will serve as "decoys" and force the students to identify the best tools for the separation they're attempting.
  - Magnifying glass
  - Tape
  - Toothbrush and/or paint brush
  - Ruler
  - Fork
  - Coffee filters
  - Ziploc bag
  - Cotton balls
  - Anything else you may have lying around the classroom that could conceivably be used as a tool for this separations puzzle

Introductory Mini-Lecture for Part 1:

In this lab we will learn about some ways in which different substances are separated. In chemistry and engineering, a **separation** refers to a process that we use to separate components of a mixture from one another. This may sound fancy, but you see and perform separations in everyday life all the time.

For example, you use a strainer to separate water from pasta after you are done cooking your favorite spaghetti dish. In this case, the strainer acts as a separation device that takes in a mixture of water, cooked pasta, and perhaps a bit of salt and separates them into two distinct components: the water and dissolved salt which flow through the strainer, and the cooked pasta which cannot fit through the holes in the strainer and thus stays behind.

Now, we must consider the something called the basis of separation. The **basis of separation** is a property which differs among the components of the mixture that we take advantage of in order to separate those components. In the pasta example, the basis of separation was size; the salt water solution is a liquid and thus flows through the holes in the strainer without a problem, while the spaghetti strands are much too large to fit through the strainer holes. Can you think of some other properties we could use as a basis of separation? (Examples include appearance, density, solubility, charge, magnetism, and boiling temperature.)

When scientists and engineers are given a mixture and asked to isolate the product they're interested in – for example, chemists who have made a promising new medicine and need to separate that molecule from all the other side products – they think about the properties of the different components of the mixture to see if any of these properties can be used as a basis of separation. Today, you will be given a mixture of items and asked to separate them into seven individual components using any number of a wide variety of tools at your disposal. You'll recognize some of the components of the mixture with your naked eye, while some of the others will seem indistinguishable. Just remember to consider which basis of separation your tools can take advantage of and work from there!

Part 1 – Separations Puzzle

In this first part, you will separate a mixture of unidentified items into seven individual components using the tools provided.

Teacher Set-Up Instructions:

Note: You will want to have this puzzle set up ahead of time for each group so that they won't know what the individual components of the mixture are before they start.

1) Measure out the following substances for each group (all quantities/amounts approximate): 3 ping pong or golf balls, 1 cup of red beans (optional: mix a few pinto beans in with the pinto beans), 3 tbsp. of grape nuts, and 1 tsp. salt.

2) For each group, chop up enough flat toothpicks into small pieces (each approximately the size of the tip of a sharpened wooden pencil) to be able to give each group ¼ tsp.

3) Chop up a steel scrub brush into enough small iron filings (each approximately the size of the tip of a sharpened wooden pencil) to be able to give each group a pinch.

4) Mix the substances from steps 1-3 in a large cereal bowl for each group.

5) Lay out the following essential tools on the table on which the students will be working: tongs, a colander, tweezers, a fine strainer, a magnet, napkins or paper towels, ~20 oz. of water in a bottle, a spoon, and at least three more large bowls.

6) Mix in as many of the "decoy" tools with the essential tools as possible; see the materials section for ideas.

While there are many possible separation sequences that will result in students isolating all the items without touching them, here is one recommended path for you to keep in mind as you work to help the students discover a solution:

1) Use the tongs to mechanically remove the ping pong balls from the rest of the mixture.

2) Run the rest of the mixture through the colander. The beans should be retained, while the rest of the components should go straight through the gaps.

3) If you included two colors of beans, use the tweezers to separate out the pinto beans from the red beans.

4) Run the remaining mixture through a fine strainer so that only the salt will go through.

5) Use the magnet to remove the iron filings from the remaining mixture.

6) ** Trickiest step: Add water to the remaining mixture in a sufficiently large bowl. The flat toothpick parts should float, while the grape nuts will sink to the bottom of the bowl.

7) Use the spoon to carefully collect the toothpick pieces from the surface of the water.

8) Carefully pour out the water, leaving (soggy) grape nuts as the last item remaining.

1. Take a few minutes with your group members to examine the mixture of unknown composition which you have been provided. **For the purposes of this lab, consider all the substances in this mixture radioactive, meaning that you cannot touch any of them with your hands.**

Q1. Do you recognize any of the components of the mixture? If so, which ones?

Students will likely be able to pick out the ping pong balls, the red and pinto beans, and perhaps the salt and the grape nuts.

Q2. How many distinct items can you see in the mixture? If you are unsure what some of the items are, describe them briefly.

Student answers will vary. However, the students shouldn't be able to identify more than seven distinct components (or six if you're only using one type of bean).

2. It is typically a good idea to separate out the components which seem most unlike the others first. **Among your group, determine which item seems the most different from the others.** Devise a strategy for separating this item from the rest.

Q3. Which item seems most different from the others? What will be the basis of separation that you will use to remove this item from the mixture?

Most students will likely choose to remove the ping pong balls or the beans first. In this case, the basis of separation is size. Some students might also suspect that the small black specks are metallic and thus try to remove them first; the basis of separation is then magnetism.

3. Go ahead and try your strategy for removing the first component of the mixture.

Q4. Which tool did you use to try to separate your first item of choice? Why did you choose that item? Were you successful?

Student answers will vary.

4. Among your group, agree on a strategy for isolating another compound from the mixture. If your first plan resulted in a mixture of two items that is separate from the rest, you may also choose to separate those two items from one another.

5. Continue separating items by finding relevant bases of separation and using appropriate tools to take advantage of those properties that differ among the items. **Try not to ask your teacher for hints unless your group is truly stumped!**

Concept Questions

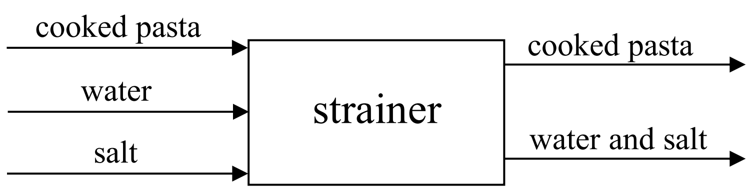
Q5. Fill in the following table with all the items you separated from the mixture, in order from the first item you separated to the last. In the columns on the right, note the basis of separation for each item and the tool you used to take advantage of that basis(es) of separation.

Note: The below table is filled out based on the recommended separation sequence.

Order	Item	Basis of Separation	Tool
1	*ping pong balls*	*size, appearance*	*tongs*
2	*pinto/navy beans*	*size*	*colander*
3	*navy beans*	*appearance*	*tweezers*
4	*salt*	*size*	*fine strainer*
5	*iron filings*	*magnetism*	*magnet*
6	*toothpick pieces*	*density*	*water, filter paper*
7	*grape nuts*	*density*	*water*

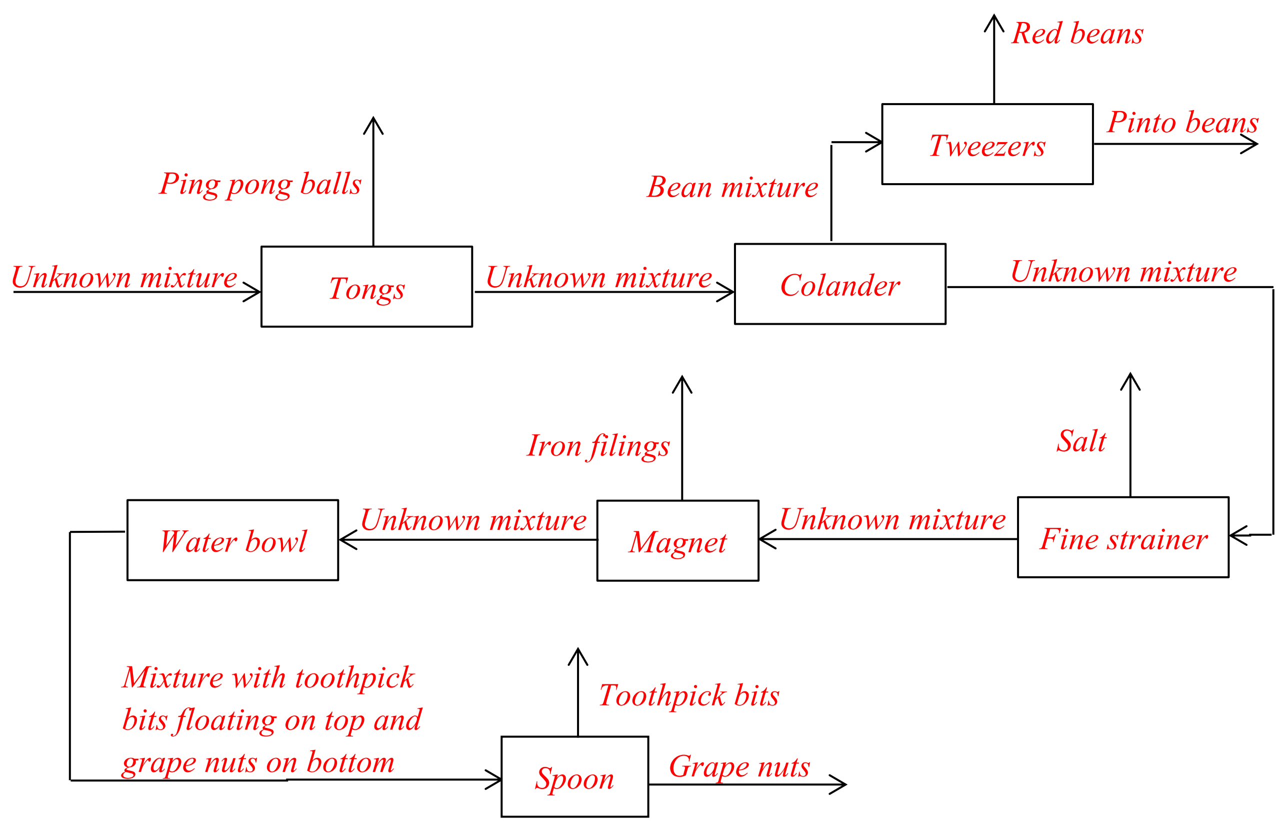
A ***process flow diagram*** is a tool commonly used by engineers to keep track of the general flow of a process and the equipment used in that process. We can use this type of diagram to track a mixture as it is being separated into its various components. Below is an example of a process flow diagram for using a strainer to separate cooked pasta from salt and water:

Any equipment we use to separate the mixture is shown in a box, while the components coming into and out of that equipment are represented by labeled arrows. **We only show components as separate arrows if they are truly separate.** Thus, it would be incorrect to represent the pasta straining process as shown below, because the cooked pasta, water, and salt enter the strainer as one mixture, not as three separate parts:



Q6. With this background, work as a group to construct a process flow diagram for the separation puzzle that you just completed.

Student answers will vary depending on the separation strategy they used. Below is a process flow diagram for the recommended path given in the notes at the beginning of Part 1.



Q7. Now that you are able to visualize your separation strategy using a process flow diagram, can you suggest any ways in which you could have separated the mixture more efficiently?

Student answers will vary.

Q8. Let's say that you're given a mixture of salt dissolved in water. Can you think of any basis of separation you could exploit to separate the salt from the water?

Students might recognize that if we bring the solution to a boil, the water will evaporate, leaving salt at the bottom of the container. The basis of separation is the boiling point.

Q9. Name two real-life examples of separation processes and the basis of separation for each.

Possible answers include recycling and/or mail centers, filtering dirt out of water, sorting candies by color, evaporating water out of a salt solution to form rock crystals, and using chromatography to separate ink into its various dyes.

Introductory Mini-Lecture for Part 2:

Now that we have had some practice taking advantage of various bases of separation to separate a mixture into its individual components, we are going to learn how to use a powerful separation technique called chromatography which can separate compounds that are quite similar to one another. **Chromatography** is a general type of separation in which a mixture of compounds passes through a **stationary phase**. Different compounds have different relative affinities for the mixture traveling along the stationary phase, or the **mobile phase**, and the stationary phase, causing these compounds to separate from one another.

More specifically, we're going to use a technique called **thin layer chromatography (TLC)**, which separates components of liquid mixtures based on polarity. **Polarity** refers to the separation of charge in a molecule – in other words, how unequally the electrons in a molecule are shared among the different atoms. For this experiment, the stationary phase will be a coffee filter, while the mobile phase will be rubbing alcohol. The dyes from the coatings of M&M's and Skittles will be carried varying distances along the coffee filter by the rubbing alcohol, allowing us to visualize the many different dyes that are used on the surface of each piece of candy. For this TLC experiment, the more polar the compound, the greater attraction it will have for the mobile phase and the farther up it will be carried by the mobile phase. Conversely, the more nonpolar the compound, the weaker the attraction it will have with the rubbing alcohol.

[For teacher's information only: this separation of positive and negative charge is dependent on the electronegativities of the atoms within a molecule. An atom is comprised of a nucleus containing positive charges and neutral charges and outer rings of negatively charged electrons. The outermost shell of electrons is called the valence shell. The electronegativity of an atom is the tendency of an atom to attract electrons to itself based upon properties such as the distance the valence electrons of an atom are away from the positive charges in the nucleus.]

Before we start, we should talk about what makes the mobile phase move in the first place. In TLC, the mobile phase moves uphill due to a phenomenon called capillary action, which we see in everyday life when a paper towel absorbs a liquid. **Capillary action** occurs when a liquid moves uphill against gravity due to interactions between the stationary and mobile phases.

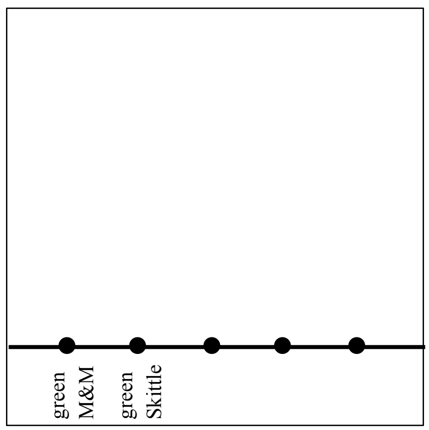
Part 2 –Chromatography

In this part of the lab, you will use thin layer chromatography (TLC) to separate the individual dyes from M&M's and/or Skittles, as well as observing the different colors that make up black ink. In this experiment, coffee filter paper will be the stationary phase, while a solution of rubbing alcohol will serve as the mobile phase.

1. Cut the coffee filter paper into two 3 inch x 3 inch squares, one for candy and one for ink.

2. For each square, lightly draw a pencil line ½ inch from the edge of one side of the paper.

3. For the candy filter square, make a pencil dot for each color to be used along the line about ¼ inch apart and label each dot (see diagram below).



4. Remove the color (dyes) off the candies you choose to analyze by placing each candy in a piece of foil and adding 6 drops of water. Try to select pairs of candies of the same color (e.g., green M&M's and green Skittles, orange M&Ms and orange Skittles, etc.)

5. While you are waiting for the candy dye to dissolve, prepare your ink filter square. Make a small dot (approx. 2mm diameter) with each marker along the pencil line about ½ inch apart and label each dot.

6. For the candy filter square, dip a toothpick into each colored mixture left behind in the foil and dab the color on the corresponding pencil dot on the filter paper. Allow the filter paper to dry and add more color to each dot. Repeat so that you have added color to the dot three times.

7. When the papers are dry, fold in half so that they stands up on their own. **Make sure that the fold is vertical, leaving the line with the dots near the bottom edge of the paper.**

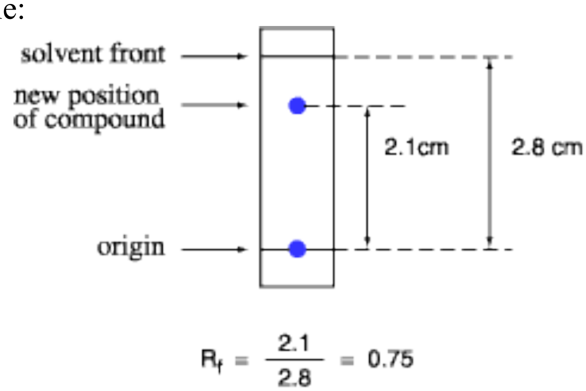
8. For each filter, pour rubbing alcohol (isopropyl alcohol) into a clean tall glass to a liquid level of 1/4 in. Then stand the filter paper inside the glass with the sample side down and the edge of paper wetted by the developing solution. **Make sure that the alcohol level is below the line of the samples.**

9. Observe as the rubbing alcohol progresses up the paper by capillary action. When the rubbing alcohol is ¼ inch from the top edge of the paper, remove the paper from the glass and let dry.

The retardation factor (R_f) is the distance traveled by the compound divided by the distance traveled by the solvent front:

$$R_f = \frac{distance\ traveled\ by\ the\ compound}{distance\ traveled\ by\ the\ solvent\ front}$$

See the following example:



If you could repeat this experiment under exactly the same conditions, the R_f values for each sample would always be the same!

Concept Questions

Q10. Which are the stationary and mobile phases in this experiment?

The coffee filter paper is the stationary phase, while the rubbing alcohol is the mobile phase.

Q11. Why did the dyes travel different distances?

The dyes travel different distances because they all have different polarities – that is, the atoms in some dye molecules share their electrons more unequally than in others. The more polar dyes are more attracted to the stationary phase and thus aren't carried as far by the mobile phase, while the more nonpolar dyes have less affinity for the coffee filter paper and are carried further by the rubbing alcohol.

Q12. Why did we use rubbing alcohol as the mobile phase?

Rubbing alcohol has the correct polarity relative to the stationary phase and the sample dyes. It is more polar than the stationary phase, allowing it to carry some of the dyes along the coffee filter. However, it is not so polar that it carries all the dye molecules all the way up the filter paper, so it allows us to distinguish the various dyes.

Q13. What does coffee filter paper consist of and why would it be used as the stationary phase?

Coffee filter paper consists mainly of cellulose, a compound that is considerably less polar than rubbing alcohol. Thus, the filter paper interacts strongly with molecules that aren't nearly as polar as the rubbing alcohol, allowing us to differentiate the various dyes.

Q14. What do you think would happen if we varied the solvent (for example, if we used water instead)? Would the dyes travel the same distances as when we used rubbing alcohol?

Water has a different polarity than rubbing alcohol, so if we were to use water instead of rubbing alcohol, the dyes would have different relative affinity for the mobile phase, which would affect how far those components would travel along the filter paper.

Q15. Why might we want to cover the glass in which TLC is taking place?

Covering the glass keeps most of our rubbing alcohol from dissolving. If all of the rubbing alcohol were to dissolve, there would be no mobile phase to carry our dye samples.

SKITTLES: Yellow 6 Lake, Red 40 Lake, Yellow 5 Lake, Blue 2 Lake, **Yellow 5, Red 40**, **Yellow 6**, Blue 1 Lake, **Blue 1**
M&Ms: Blue 1 Lake, Blue 2 Lake, Red 40 Lake, Yellow 5 Lake, Yellow 6 Lake, **Blue 1, Blue 2, Red 40, Yellow 5, Yellow 6**