CSEO SCIENTIST IN THE CLASSROOM PARTNERSHIP

Chromatography

Last Updated: July 2022

GOAL: To demonstrate a technique or process for separating mixtures that is used by biologists, chemists, clinical scientists, and forensics scientists (detectives).

TN STATE STANDARDS: 5.PS1.4 Evaluate the results of an experiment to determine whether the mixing of two or more substances result in a change of properties

7.PS1.3 Classify matter as pure substances or mixtures based on composition

This lesson was adapted from Vanderbilt Student Volunteers for Science. Additional information about the lesson may be found at https://studentorg.vanderbilt.edu/vsvs/.

LESSON OUTLINE

I. Set Ups for Lesson: **Capillary Action and Separating Colors, p. 2**

Pour water into enough 16 oz cups to the marked level (about 30 mL.) for the whole class plus one. Also tear off enough sheets from the paper towel roll so each student has one.

II. Mixing Colors - Demonstration, p. 2

Demonstrate the mixing of yellow liquid with blue liquid to give green liquid. Mention that this lesson will demonstrate a process that separates the green color to give blue and yellow.

III. What is Chromatography?, p. 3

Explain to the students that chromatography is a process for separating mixtures. In today's lesson paper chromatography will be used to separate water-soluble inks into their different colors, starting with the separation of green into blue and yellow.

IV. Demonstration of Procedure for Chromatography, p. 3

Show students the proper procedure for setting up a chromatography experiment, using a 16 oz cup, a stick, and a paper strip.

V. Capillary Action and Separating Colors, p. 4

Give each student one of the instruction sheets. You will still need to give instructions, but the students can refer to the instruction sheet as they are doing the experiments. You will still need to guide them through the procedures, making sure they understand the instructions. Each student will prepare one chromatography strip by tracing the pencil line on the strip with a green felt tipped pen. This strip is taped to a stick and placed in a plastic cup containing 30 mL of water following the procedure in part IV. Students observe the separation of the green pen line into blue and yellow. By comparing their chromatography strips they can also see that the same pattern is present. This observation can be used as part of the discussion for the next part – the use of chromatography for analysis.

VI. Forensic Chromatography, p. 5

Each student does a chromatogram of one of the four pens to help determine which pen was used to write ransom note. After the chromatograms are finished, ask the students to compare their chromatograms with the chromatogram prepared from the guilty person's pen to determine who is the guilty person.

VII. Review of Chromatography, p. 8

Materials

- 3 plastic drink bottles filled with water (use to put water to the mark (about 30 mL) in 16-oz cups, and for Part II)
- 33 16 oz. clear plastic cups
- 1 #1 BAG containing: (For Section II - MIXING COLORS)
  - 1 bottle blue food coloring, 1 bottle yellow food coloring
  - 2 plastic spoons
  - 3 10 oz. clear plastic cups
- 1 #2 BAG containing: (For Section IV - DEMONSTRATION OF PROCEDURE)
  - 1 roll of clear tape
  - 1 wooden stick
  - 1 chromatography strip* (stored in plastic bag to keep it dry)
    - * horizontal pencil line is drawn 2 cm from the bottom of the 12 cm long strip
- 1 bag containing 32 green pens and 32 chromatography strips for Section V
- 1 bag containing 32 sticks
- 1 bag containing 32 rolls of Scotch Tape
- 1 large bag containing:
  - 4 bags – each with 8 of the same labeled suspect pen (PC, PS, JF, or MM)
  - 1 bag containing 32 chromatography paper strips
- 8 prepared laminated chromatograms from the "guilty" person's pen (PC)
- 32 Instruction Sheets in sheet protectors
- 33 sheets of paper towel

I. SET UPS FOR LESSON:

Set-up for Part V – SEPARATING COLORS - Add water to the mark in enough 16-oz cups for the class plus one for the demonstration in part IV.

While one team member starts Part II, another should write the following vocabulary words on the board.

chromatography chromatogram capillary action forensic chromatography

II. MIXING COLORS - Demonstration

Materials

- **1 bottle of water**
- **Bag #1 containing**
  - **1 bottle blue food coloring**
  - **1 bottle yellow food coloring**
  - **2 plastic spoons**
  - **3 10 oz. clear plastic cups**

- Fill 2 of the 10 oz. cups half full of water.
- Add 2 drops of yellow food coloring to the water in one cup and stir.
- Add 2 drops of blue food coloring to the water in the second cup and stir.
- Hold the cups up so the students can see them.

Ask the students: *What color do you get when you mix blue and yellow?*

- Tell students to watch as you hold the glass of yellow liquid directly behind and about 1/3 higher than the cup of blue liquid. (Students should be able to see all three colors.)
  Ask the students: *What color is the liquid in the middle?*
  It should appear that the liquid in the middle of the glass is green.

Ask: *"What will happen if you pour some of both liquids into a third glass?"*
Accept responses.

- Pour 1/3 of the blue liquid and 1/3 of the yellow liquid into the third cup and show students that the mixture of the blue and yellow liquids makes a green liquid.
- Tell students that mixing blue and yellow to make green is something easy to do; something they already know.
- Tell students that what we want to discover is a way to separate mixtures of colors and other mixtures of chemicals.

III. WHAT IS CHROMATOGRAPHY?

Explain to the students that chromatography is a process for separating mixtures. In today's lesson paper chromatography will be used to separate water-soluble inks into their different colors, starting with the separation of green into blue and yellow.

While the students are waiting on their chromatogram to develop in Part V, go over the explanation given in Part V about how chromatography works. Call attention to the vocabulary words on the board whenever you are discussing them in connection with the lesson.

IV. DEMONSTRATION OF PROCEDURE FOR CHROMATOGRAPHY

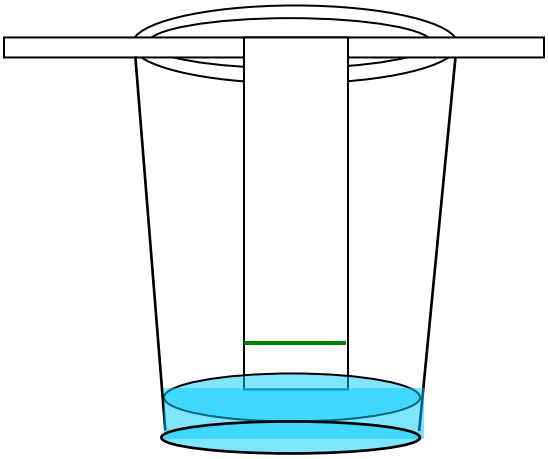
Materials

- **1 16 oz. clear plastic cup with 30 mL of water**
- **1 BAG # 2 containing**
  - **1 roll of Scotch tape, 1 wood stick**
  - **1 chromatography strip* (stored in plastic bag to keep it dry)**
    *** 12 cm long with horizontal pencil line is drawn 2 cm from the bottom**

Demonstrate the proper procedure for conducting the experiments.

SHOW STUDENTS HOW TO:

- Hold the paper strip so that the top edge of the paper is even with the top edge of the wooden stick.
- Tear a small piece of tape and tape the paper strip to the wooden stick so that the tape goes around the stick and is taped to both the front and the back of the paper strip.
- Take one of the 16 oz cups that contains 30 mL of water and gently place the stick across the 16 oz. cup so the stick and paper will not fall into the cup. The strip should hang free in the center of the cup without touching the sides.



As the water starts to go up the paper strip, show the strip to the students and tell them that this **capillary action** or wicking property of water will help separate the colors.

Explain that liquids can climb up paper, string, and other substances through the process of capillary action or wicking. The liquid moves upward through the small pores or capillaries that are found in paper towels, filter paper, chromatography paper, and other porous materials; this is what makes these materials absorbent. Scientists use this process to separate mixtures, including colors.

V. CAPILLARY ACTION AND SEPARATING COLORS

Materials

32 16 oz. clear plastic cups with water to the mark
1 bag containing 32 green pens and 32 chromatography strips for Section V
1 bag containing 32 sticks
1 bag containing 32 rolls of Scotch Tape
32 Instruction Sheets in sheet protectors
32 sheets of paper towel

Distribute the following materials to each student:

1 16-oz plastic cup with water
1 stick
1 roll of Scotch Tape
1 chromatography strip
1 green pen
1 sheet of paper towel

Give each student one of the instruction sheets. You will still need to give instructions, but the students can refer to the instruction sheet as they are doing the experiments. You will still need to guide them through the procedures, making sure they understand the instructions.

Have each student do the following:

- Take the paper strip and a wooden stick.
- Trace the pencil line with the green felt-tipped pen.
- Tear a small piece of tape and tape the paper strip to the wooden stick so that the tape goes around the stick and is taped to both the front and the back of the paper strip.
- Take the 16 oz cup that contains 30 mL of water and gently place the stick across the 16 oz. cup so the stick and paper will not fall into the cup. The strip should hang free in the center of the cup without touching the sides.

Note: The green line must not touch the water. The color will wash away if this happens, and the experiment will not work properly. Caution students to use care when lowering the strip into the water.

- While students are observing the experiment, share the following information with them.

EXPLANATION: The paper is the **support** in this experiment. The **solvent** used (water in this case) has different degrees of absorption to the support. The greater the porosity of the paper, the better the capillary action or wicking, and the faster the water will climb. As the water moves up the paper strip, it dissolves the water-soluble pigments of the green pen mark. Each pigment travels at a different speed depending on its solubility in water and its absorption on the paper. The color separation is called developing a **chromatogram** (a color pattern)**.** Chromatograms can be used to match and identify substances in biology, chemistry and forensic labs.

- To keep students on task, ask a student to describe what has happened at various intervals (every 30 seconds or every minute). Ask what is happening to the green line of color; and when the colors separate, ask which color is going farther up the strip.

Use the following point to finish the explanation of the color separation.
In this case the blue pigment travels faster than the yellow pigment. This means the blue pigment is more soluble in water and also less strongly absorbed by the paper. Since the yellow pigment is more strongly absorbed by the paper, it doesn't go very far up the paper.

- Have students lift stick out of the cup and remove the chromatogram from the stick by holding the paper near the taped end and sliding it off the stick.
- Have students place their chromatogram on a sheet of paper towel.
- Pick up the green felt-tipped pens when you distribute materials for Part VI.

VI. FORENSIC CHROMATOGRAPHY

Re-use 16 oz cups and sticks from Part IV (The same water can be used unless ink from the green felt pen colored the water – if this happened, dump the water out, rinse, and replace with 30 mL of fresh water.)

Materials

- **1 bag containing 4 small bags, each containing 8 of one of the labeled suspect pens (PC, PS, JF, MM)**
- **1 bag containing 32 chromatography paper strips**
- **8 prepared chromatograms from the "guilty" person's pen**

DISTRIBUTE materials **AFTER** sharing the following information with the students:

Students can see by comparing their chromatograms from Part IV that they look the same, showing an identical pattern of separation of the green ink into yellow and blue. This illustrates how scientists can use chromatography for analysis.

Forensic scientists or detectives can also use the process of chromatography in their work.

Chromatography is used in crime labs to separate components of "clue" substances such as blood, ink, or other mixtures found at the scene of the crime.

Sometimes chromatography is used to identify the pen that was used to write a ransom note.

Read or tell the following scenario to students and tell them they will use chromatography to determine "Whodunit"!

The police (represented by Sam Suede, a hard-boiled police detective) have been called to the scene of a crime. The scene is a chemistry laboratory, and a small vial of Solution X has been stolen. A ransom note has been received, written in black ink, demanding one million dollars for the return of Solution X. Through questioning, Sam Suede learns that rumors have been spreading that Solution X may be the long-awaited cure for the common cold!

Sam discovers that there are four prime suspects who all have a motive for committing this crime. They are as follows:

Pam Chromatogram (Pen PC) feels that she never gets credit for anything, even though she does most of the work. Instead the two heads of the lab, Drs. Tweedle and Deedle, win all the prizes and are interviewed in the newspapers. Pam thinks she is very underpaid for all the work she does.

John Fingerprint (Pen JF) once dreamed of winning the Nobel Prize. Now he seems resigned to washing bottles for the rest of his life. He resents most of the others at the lab, and Dr.Tweedle recently threatened to fire him if he does not keep his fingerprints off the lab glassware.

Mary Masonite (Pen MM), once a well-known trial lawyer, was hired by Drs. Tweedle and Deedle to arrange the patenting of Solution X after all tests are completed. She constantly suffers from colds, flus, and allergies and desperately consumes huge amounts of cold remedies, decongestants, vitamin C, and whatever else she can get her hands on.

Unbeknownst to Mary Masonite, her secretary, **Patrick Street (Pen PS)** has been embezzling large amounts of money from Mary's bank accounts. Mary hasn't realized it yet, but the next big check she writes for cold remedies could bounce.

Sam has obtained a pen of each of these suspects and has a chromatogram that was made from the ransom note. Sam needs your help in matching the suspect's pen to the ransom note.

<u>Pen PC belongs to Pam Chromatogram.</u>
<u>Pen JF belongs to John Fingerprint.</u>
<u>Pen MM belongs to Mary Masonite.</u>
<u>Pen PS belongs to Patrick Street.</u>

- Distribute the following new materials to each student:
    1 labeled suspect pen (PC,PS, JF, or MM)
    1 piece of chromatography paper

Distribute the suspect pens evenly so that approximately the same number of students have each type of pen.

Each student will follow the procedure in Part IV to obtain a chromatogram of one of the suspect's pens.

Have each of the students do the following:

- Trace the pencil line with their black pen.
- Tear a small piece of tape and tape the paper strip to the wooden stick so that the tape goes around the stick and is taped to both the front and the back of the paper strip.

- **Tell each student to mark the top of the chromatography paper near the stick with the initials on their pen (PC, PS, FF, or MM). Hold up a stick with a piece of chromatography paper taped to it to make sure they mark the top near the stick.**
- Take the 16 oz cup that contains 30 mL of water and gently place the stick across the 16 oz. cup so the stick and paper will not fall into the cup. The strip should hang free in the center of the cup without touching the sides.
- Wait about five or six minutes for development of the chromatogram.
- Lift stick out of the cup and remove the chromatogram from the stick by holding the paper near the taped end and sliding it off the stick.
- Place their chromatogram on a sheet of paper towel.

After the chromatograms are finished, distribute the 8 laminated chromatograms prepared from the guilty person (about 1 every 4 students) and ask them to compare their chromatograms with the chromatogram prepared from the guilty person's pen (made from the ransom note) and identify which pen matches the results from the ransom note. Ask, *Who is the guilty person?*

(PC - Pam Chromatogram)

VI. REVIEW OF CHROMATOGRAPHY

In most of the variations of chromatography, a substance (ink dot, candy coating, leaf extract) is placed onto a support (paper strip). A solvent (water, alcohol) is then added, which moves up the support because of capillary action. As the solvent moves through the test substance, some of the test substance is dissolved in the solvent and carried up the support. Different types of substances move different distances, which depend on their differences of solubility in the solvent and their absorption on the paper. As a result, separation occurs. This is always constant for a particular support and solvent. Chromatograms of these substances are then compared with known chromatograms to identify the substances.

REVIEW QUESTIONS

1. Why does water move up the paper strip?
   Answer: capillary action
2. In the separation of the green ink, the blue pigment moves higher (faster) than yellow pigment. Why?
   Answer: The speed of movement of a component of a mixture, in this case colors, depends on its relative solubility in the solvent (water) and its relative strength of attachment (absorption) to the paper. The blue pigment is more soluble (more attracted to water than to the paper) and less absorbent (less attracted to the paper) so it moves faster up the paper strip.

3. Why didn't the ink of one of the pens (FF, the Sharpie pen) separate into different colors?

Answer: The Sharpie is a permanent marker, which means its ink does not dissolve in water. Only water soluble inks will separate when water is the solvent.

References

1. M. Sarquis and J. Sarquis, Eds., ***Fun with Chemistry***, Vol. 2, pp. 3-8.
2. J. Barer, ***Crime Lab Chemistry***, Teacher's Guide: Lawrence Hall of Science, University of California, Berkeley, CA.
3. D. R. Kimbrough, ***J. Chem. Educ***., Vol. 69, p. 987, December, 1992.

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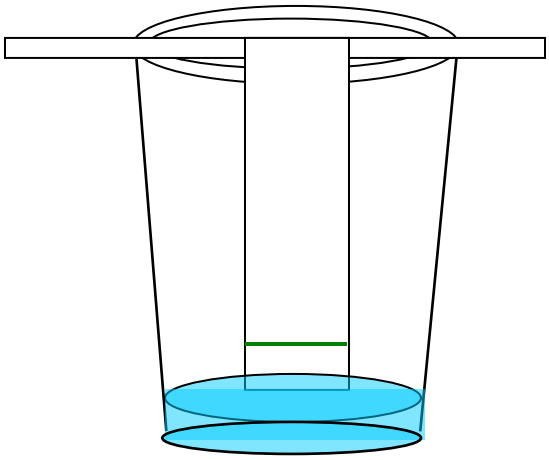
Instruction Sheet
Chromatography

I. Introduction
II. Mixing Colors – Demonstration
III. What is Chromatography?
IV. Demonstration of Procedure for Chromatography
V. Capillary Action and Separating Colors – look at drawing below

- Take the paper strip and a wooden stick.
- Trace the pencil line with the green felt-tipped pen.
- Tear a small piece of tape and tape the paper strip to the wooden stick so that the tape goes around the stick and is taped to both the front and the back of the paper strip.
- Take the 16 oz cup that contains 30 mL of water and gently place the stick across the 16 oz. cup so the stick and paper will not fall into the cup. The strip should hang free in the center of the cup without touching the sides.



Note: The green line must not touch the water. The color will wash away if this happens and the experiment will not work properly

- It will take about five minutes for the ink to separate. While you're waiting, we will explain capillary action.
- When the teacher team tells you it's time to take your chromatography paper strip out, lift the stick out of the cup and remove the chromatogram from the stick by holding the paper near the taped end and sliding it off the stick.
- Place your chromatogram on a sheet of paper towel.

VI. Forensic Chromatography

You will re-use your16 oz cup and stick from Part V (The same water can be used unless ink from the green felt pen colored the water – if this happened, ask your teacher to give you fresh water.)

- We will explain how chromatography will be used to determine which of four pens was used to write a ransom note.
- Trace the pencil line with your black pen.
- Tear a small piece of tape and tape the paper strip to the wooden stick so that the tape goes around the stick and is taped to both the front and the back of the paper strip.
- **Mark the top of the chromatography paper near the stick with the initials of your pen (PC, PS, JF, or MM).**
- Take the 16 oz cup that contains 30 mL of water and gently place the stick across the 16 oz. cup so the stick and paper will not fall into the cup. The strip should hang free in the center of the cup without touching the sides.
- Wait about five or six minutes for development of the chromatogram.
- Lift the stick out of the cup and remove the chromatogram from the stick by holding the paper near the taped end and sliding it off the stick.
- Place your chromatogram on a sheet of paper towel.

After the chromatograms are finished, your teacher will distribute some laminated chromatograms prepared from the guilty person's pen. Compare this with yours to see whether the pen you tested was the guilty person's pen.

VII. Review

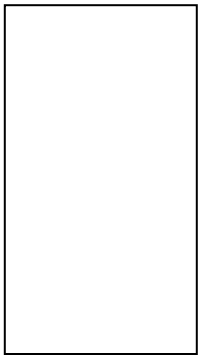
Name:_____ Class Period:_____ Date:_____

Chromatography Part I: Separation of Solutes

1. What is the difference between a compound and a mixture?

2. Is a solution a compound or a mixture?

3. Draw and label your results on the "chromatography paper" below.

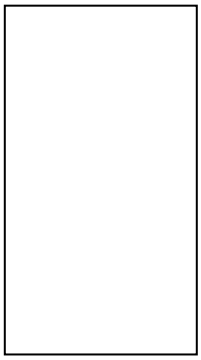


4. Which color traveled faster (higher) and why?

5. What can you conclude about the green ink? Is it soluble in water? How many dyes are used to give it its color?

Chromatography Part II: Forensics

1. Draw and label your results on the "chromatography paper" below. Include the initials of the suspect's pen on the diagram.



2. Describe in two or three sentences what you observed.

3. Do dyes of the same color always have the same chromatogram? Explain.

4. Whose pen was used to write the ransom note?