



Background: Chromatography is a method for analyzing mixtures by separating them into the chemicals from which they are made. It can be used to separate mixtures like ink, blood, gasoline, and lipstick. In ink chromatography, you are separating the colored pigments that make up the color of the pen. Even though a pen will only write in one color, the ink is actually made from a mixture of different colored pigments.

To perform ink chromatography, you put a small dot of ink to be separated at one end of a strip of paper. This end of the paper strip is then placed in a solvent. The solvent moves up the paper strip; and, as it travels upward it dissolves the mixture of chemicals and pulls them up the paper. The chemicals that dissolve best in the solvent will move up the paper strip further than chemicals that do not dissolve as well. What is produced from this method is a chromatogram.

Forensic scientists are able to use ink chromatography to solve crimes by matching documents or stains found at a crime scene to the marker or pen that belongs to a suspect. Forensic scientists analyze the unknown ink and compare it to writing utensils collected from possible suspects.

To prove that an ink sample is a certain brand scientists need to calculate the R_f (retention factor) values of the different colored chemicals present in the marker. R_f is a calculation that compares the distance the solvent traveled up the paper strip to the distance a pigment traveled up the same strip.

Pre-lab:

1. Define chromatography:
2. What are you separating in an ink chromatography lab?
3. Review: what is a solution and what is a solvent (use Biology text or do a search if needed)?
4. Which chemicals will move the farthest up the paper?
5. What is a chromatogram?
6. What does R_f stand for and what does it compare?

Materials:

Filter paper

3-4 different brands of black markers

Scissors

Small cups or beakers—one for each marker being tested

Toothpicks

Rulers

Calculators

Reference Table(s)

Water

Procedure:

1. Pour about 10 ml of water into a beaker or small cup.
2. Cut a strip of filter paper to form a point at one end.
3. Choose a marker to test. Record the brand of marker on your Reference Table. Use the marker to make a good-sized dot of color (like the size of a grain of rice) about 1.5 cm up from the pointed end of the paper. (Assign the marker a letter or code so you remember which marker is which.)
4. Use a pencil and make a mark on the paper strip beside the ink dot!
5. Lower the pointed end of the paper into the solvent BUT make sure the color dot stays above the solvent level. Carefully push a toothpick through the top of the paper to hold the strip at just the right level in the beaker or cup.
6. The solvent should immediately start moving up the paper strip carrying the ink pigments with it. While waiting for the solvent to rise toward the top of the paper, set up your other beakers and test the other markers. (It will take 3–5 minutes to complete a “run” as the water rises.
7. When the solvent has finished moving up the paper strip, you can remove the paper from the test tube and immediately mark with a pencil the highest point the solvent traveled up the paper strip.
8. Let the strip dry and tape it on your Reference Table under its correct brand name.
9. Continue testing all of the ink samples, including the unknown.

CALCULATING R_f VALUES AND DESCRIBING YOUR INK SAMPLES

1. You probably noticed that each marker brand uses a different combination of pigments to produce their black colors. Now test the unknown ink sample using chromatography and use your Reference Table to match color combinations and determine which marker was used to write the ransom note.
2. Look closely at each marker's chromatography strip. How many different colors are present in each ink sample? Record the Total Number of Colors present for each ink sample on your Reference Table.
3. Look closely at each marker's chromatography strip. Record the colors in the order that they appear. Each color represents a different pigment present in the ink. Record the colors you observe on your Reference Table (Colored Pigment #1 = pink color, colored Pigment #2 = orange color, etc.)
4. To prove that an ink sample is a certain brand you will also need to calculate the R_f (retention factor) values of the different colored chemicals present in the marker. First, look at a chromatography strip and measure the distance in millimeters from the original color dot to the final point the solvent traveled. The distance you just measured is the solvent distance measurement. Record it in the correct location on your Reference Table.
5. Next, measure in millimeters from the original color dot to the highest point the first colored pigment (Colored Pigment #1) traveled up the strip. This is the pigment distance measurement for Colored Pigment #1. Record this measurement in the correct location for Colored Pigment #1.
6. If there is a Colored Pigment #2 present on the strip, measure (in millimeters) from the original color dot to the highest point this second colored Pigment (Colored Pigment #2) traveled up the strip. This is the pigment distance measurement Colored Pigment #2. Record this information in the correct location for Colored Pigment #2.
7. If there is a Colored Pigment #3 present, repeat the Pigment distance measurement for Colored Pigment #3.
8. Calculate the R_f value for each colored pigment using the simple formula:
R_f Value = Distance traveled by solute (pigments in ink) / Distance traveled by solvent (water)
9. Record the R_f values for each colored pigment in the ink on your Reference Table.
10. Repeat these measurements and R_f calculations for each of the markers tested.
11. Keep your Reference Table so that you can use it to determine what kind of marker was used. This may be just the evidence you need to solve the case!

Conclusion questions:

1. In this experiment, what is the purpose of the water?
2. Do you think linking a brand of marker or pen to the crime is enough evidence to convict a suspect? Why or why not (use your forensic words)?
3. Did any of the marker samples have the same chromatography results? If so, which ones?
4. What are some other mixtures that you think can be separated by chromatography?

Known Marker Sample a-Tape here:	Known sample b-Tape here:	Known sample c-Tape here	Known sample d- Tape here
Total # of pigments:	Total # of pigments:	Total # of pigments:	Total # of pigments:
Total solvent distance traveled:	Total solvent distance traveled:	Total solvent distance traveled:	Total solvent distance traveled:
Colored Pigment #4: Color: Distance: Rf:	Colored Pigment #4: Color: Distance: Rf:	Colored Pigment #4: Color: Distance: Rf:	Colored Pigment #4: Color: Distance: Rf
Colored Pigment #3: Color: Distance: Rf:	Colored Pigment #3: Color: Distance: Rf:	Colored Pigment #3: Color: Distance: Rf:	Colored Pigment #3: Color: Distance: Rf:
Colored Pigment #2: Color: Distance: Rf:	Colored Pigment #2: Color: Distance: Rf:	Colored Pigment #2: Color: Distance: Rf:	Colored Pigment #2: Color: Distance: Rf:
Colored Pigment #1: Color: Distance: Rf:	Colored Pigment #1: Color: Distance: Rf:	Colored Pigment #1: Color: Distance: Rf:	Colored Pigment #1: Color: Distance: Rf: