Reveal the Red: Exploring the Chemistry of Red Flower Pigments

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Experimental Procedure

To make sure you can compare your results, as many of your materials as possible should remain constant. This means that the temperature, type of water used, size of paper strips, where the ink is placed onto the paper, etc., should remain the same throughout the experiment.

Cutting and Marking the Paper Strips

1. Cut each chromatography paper in half (length-wise) to make approximately 2 centimeters (cm) wide by 7.5 cm long strips. You will need at least 9 chromatography strips.
2. In your lab notebook, assign a number to each different type of flower, starting with the number 1 and going up. Using a pencil, number three strips "1," three other strips "2," and three other strips "3" at the top of the strip. This is so that you can identify which flower was used with which strip later.
    a. If you are investigating more than three different types of flowers, similarly continue to number the test strips.
3. Draw a pencil line 1 cm from the edge of each strip of paper, as shown in Figure 3 below.
    a. This will be the origin line.
    b. You will spot the flower petal pigment for each strip right on this line, as shown in Figure 3.

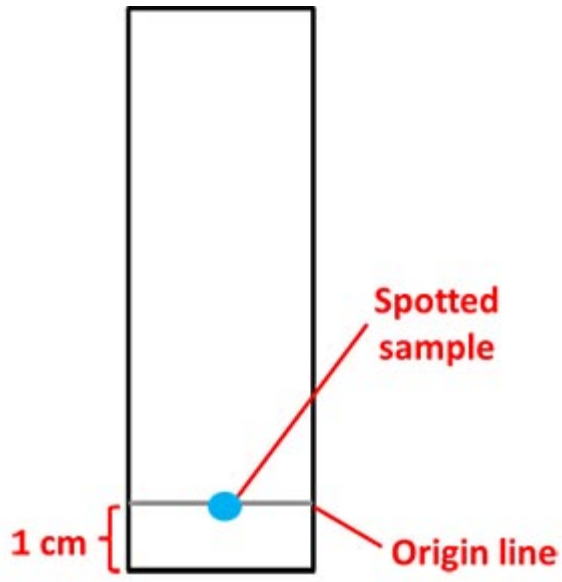

Figure 3. Each chromatography strip will have an origin line. The flower pigment to be tested will be spotted in the middle of the origin line.

Performing Paper Chromatography on the Paper Strips

1. In your lab notebook, make a data table that lists the kinds of flowers you will be testing. Also make four columns titled something similar to "Color of the Pigment Band," "Distance to the Solvent Front (in cm)," "Distance to the Top of the Band (in cm)," and "Retention Factor (R_f)."
2. In the jar, dilute the 90% isopropyl alcohol by mixing 75 milliliters (mL) of water with 30 mL of isopropyl alcohol. This water and isopropyl alcohol mixture is your solvent.
3. Pour a small amount of the solvent into your 100 mL beaker, about 1–2cm cm deep.
4. Next, transfer the pigments from one type of flower onto a strip of chromatography paper.

a. Pick a flower type you want to investigate.
b. Take one of the paper strips you prepared with this flower's number and place it on top of a piece of scratch paper on a hard surface. *Note:* Some pigments can stain so the paper strip should be prepared on a piece of scratch paper to protect the surface beneath it from getting stained.
c. Lay a petal from the selected flower on the paper strip, over the origin line.
d. Roll a coin, like a wheel, over the petal and across the origin line. Push down hard so that the petal is crushed and a strip of pigment is visibly transferred to the strip.
e. Repeat step d about three to four times (using a fresh, unused part of the petal each time) so that a thick line of pigment is transferred to the strip, on the origin line. Be careful to only transfer the pigment onto the origin line.
    i. If the origin line becomes a little wider with pigment, this is OK, but make a note of it in your lab notebook.
    ii. If you accidentally transfer pigment to an area that is away from the origin, prepare a new strip of paper as you did in the "Cutting and Making the Paper Strips" section and repeat steps 4b-e in the "Performing Paper Chromatography on the Paper Strips" section.
f. *Note* Only prepare one strip with sample at a time so that the sample does not dry out. Otherwise, the pigment separation will not work.

5. Clip the prepared chromatography strip to a wooden splint. Rest the splint on top of the beaker so that the strip hangs straight into the beaker. *Note:* The origin should not be immersed in the solvent.
6. If necessary, add more of the solvent. The goal is to have the end of the chromatography strip just touching the surface of the solvent solution, as shown in Figure 4 below.

Figure 4. Your setup should look similar to this example. The end of the chromatography strip should just touch the alcohol. *Note*: This picture does not show chromatography strips with flower pigments. The colors on your paper strips should look different.

7. Let the solvent rise up the strip (by capillary action) until the solvent front is about 2 cm from the top and then remove the strip from the solvent. Check on the strip and the solvent front every 5 to 10 minutes—if you let it run too long the pigments may run off the strip and become distorted.
    a. This may take about 20 to 60 minutes.

    b. If the solvent front has not reached 2 cm from the top of the strip after one hour, take out the strip anyway.
8. Use a pencil to mark the solvent front.
9. Allow the chromatography strip to dry. *Tip:* An easy way to do this is to tape the strip to the overhang of a counter or table so that the strip is dangling in the air.
10. After the strip has dried, measure the distance (in centimeters) from the origin to the solvent front and from the origin to the top of the pigment band that should be visible, as shown in Figure 5 below. Record the data in the data table you made in your lab notebook.
    a. Also record the color of the pigment band. For example, it may look "red," "purplish red," "pink," etc.
    b. *Note:* If you see more than one pigment band on the strip, record this band's color in your data table in a new column titled "Color of the Second Pigment Band." Also measure the distance from the origin to the top of this pigment band and record the data in your data table in a new column, titled "Distance to the Top of the Second Band (in cm)."
11. Repeat steps 4–10 two more times for the same type of flower.
12. Repeat steps 4–10 two more times, each time with a different type of flower, so that you have run at least three chromatography paper strips for each of the three different flower types you want to investigate.

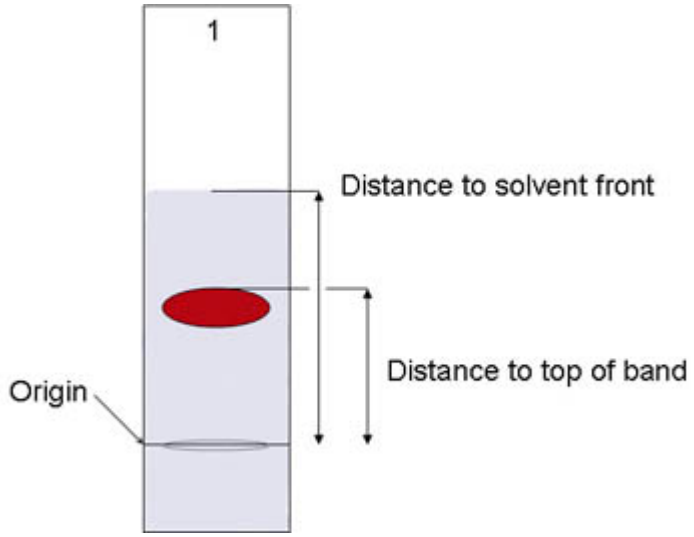

Figure 5. The pigment moves up the paper as the solvent front advances. The R_f value is the ratio of the distance to the top of the band, to the distance to the solvent front, measured from the origin.

Analyzing Your Data

1. Now, using Equation 1 from the Introduction (#distance-equation1), calculate the R_f value for each pigment for each strip. Record the values in the data table in your lab notebook.
    a. *Note:* If you saw more than one pigment band on a strip, calculate the R_f value for this band as well and record it in your data table in a new column titled "Retention Factor (R_f) of the Second Band."
2. Compare the R_f values and colors of the pigment bands for each different flower. To be the same pigment, the pigment bands should have similar R_f values and be a similar color. Do all of the different red flowers you investigated have the same pigments, as determined by paper chromatography? Or did the different flowers have different pigments? If they used different pigments, was there one pigment that was in most of the red flowers?
    a. If one pigment was used by multiple red flowers, which pigment do you think it might be? *Tip:* You may want to re-read the Introduction in the Background section, and do additional research on carotene and anthocyanin pigments.