Discover Chlorophyll Variety in Different Plants Using Paper Chromatography

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Experimental Procedure

Note: To make sure you can compare your results, as many of your materials as possible should remain constant. This means that the temperature, brand of nail-polish remover, size of paper strips, where the plant extract is placed onto the stationary phase, etc., should remain the same throughout the experiment.

1. Grind up roughly equal samples of each of the different plant leaves and distribute them into test tubes. There should be at least three *labeled* test tubes for each type of plant (if using the Iceberg lettuce, "Iceberg 1," "Iceberg 2," and "Iceberg 3" are good names for the tubes).
2. Add enough acetone (nail-polish remover) to suspend the ground-up leaves.
3. Let the acetone/leaf mixture sit for 24 hours.
4. 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.
5. Use a pencil to lightly label which leaf extract will be spotted on each paper strip. Label three chromatography strips for each type of plant leave. *Tip:* do not use a pen for writing on the strips: the ink will run when the solvent passes through the strips.
6. Draw a pencil line 1 cm from the edge of each strip of paper, as shown in Figure 6 below.
    a. This will be the origin line.
    b. You will spot the plant extract for each strip right on this line, as shown in Figure 6.
7. Take a pipette and fill it with one of the plant leaf extracts.
8. Spot the sample in the middle of the origin (see Figure 6, below). You might want to practice a few times in order to get a nice round spot.

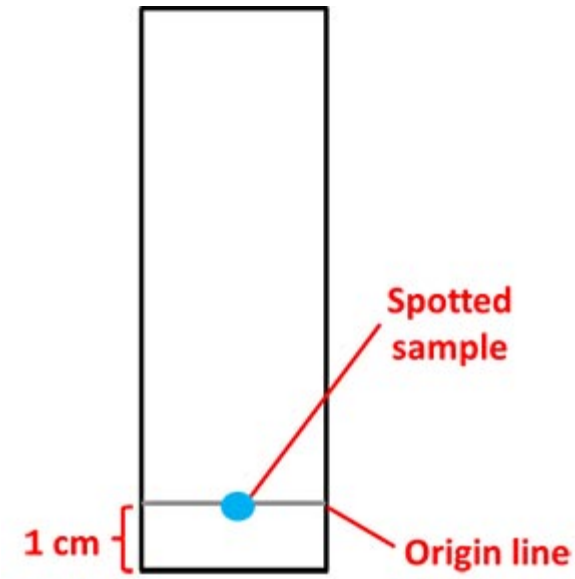

Figure 6. Each chromatography strip will have an origin line. The plant extract to be tested will be spotted in the middle of the origin line.

9. Repeat step 8 with the other plant leaf extracts you want to test. Use a new paper strip for each one.
10. Fill the 100 mL beaker to a depth of 1 cm with the acetone (nail-polish remover).
11. Clip two of the prepared chromatography strips to a wooden splint. Make sure the two strips do not touch each other or the beaker and that their bottoms are aligned. Rest the splint on top of the beaker so that the strips hang straight into the beaker.
12. If necessary, add more of the acetone. The goal is to have the end of the chromatography strip just touching the surface of the solvent solution, as shown in Figure 7 below.

Figure 7. Your setup should look similar to this example. The end of the chromatography strip should just touch the acetone. *Note*: This picture does not show chromatography strips with plant extract. The colors on your paper strips should look different.

13. Let the solvent rise up the strip (by capillary action) until it is about 0.5 cm from the top then remove the strip from the solvent. Keep a close eye on your chromatography strip and the solvent front — if you let it run too long the plant extract may run off the paper and become distorted.
14. Use a pencil to mark how far the solvent rose.
15. Allow the chromatography strip to dry, then measure (in centimeters) and calculate the R_f value for each plant pigment. Record your results in your lab notebook.
    a. *Tip:* Use Equation 1, which is given in the Introduction (#distance-equation1), for calculating the R_f value.
16. Repeat this experiment for each of your plant extracts three times.

Questions

- What types of pigments do you think are present in each type of leaf? How can you tell? (Consider color, R_f value, etc.)
- How were the R_f values different for each pigment and leaf? Why? (Make sure to consider molecular structure, polarity, etc.)
- How do the different pigments help the plant? Why do different plants have different amounts and different types of these pigments?