Lab 3: Paper Chromatography of Food Dye and Spinach

PURPOSE: Complete this part in your lab notebook.

INTRODUCTION: When you look at a leaf, the green pigment chlorophyll is usually the only pigment that appears to be present. Actually, chlorophyll is only one of many types of pigments present in the leaf and one of several that are involved in the process of photosynthesis. Once removed from the leaf, the photosynthetic pigments can be separated from one another and identified using a process called chromatography.

Food color additives: The FDA separates color additives for foods into two categories: Certifiable or Exempt from Certification. Certifiable color additives are man-made. They must be tested for consumption safety and approved or certified by the FDA to be added to their list. There are nine certified color additives on the FDA approved list from which a multitude of colors can be mixed. Certified color additives are known as dyes or lakes. Dyes are polar and so are water-soluble and can be used in beverages, dry mixes, baked goods, confections, dairy products, pet foods, and other products. Lakes are nonpolar so will not
dissolve in water and are more stable than dyes. They are best-used in foods containing nonpolar fats and oils or those foods which do not contain a lot of moisture to dissolve dyes, such as tablets, cake mixes, hard candies, and chewing gum. Seven "certified" synthetic FD&C dyes can be added to food products. The average U.S. citizen consumes about 3 grams of these dyes per year. There are 2 more approved for food surfaces. Orange B for orange skins, and Citrus Red No. 2 for frankfurters and sausages casings.

Food coloring at home: The little 4 Pack you buy at the grocery store of commercial food coloring most used in contains vials composed of various combinations of Yellow No. 5, Red No. 40, Blue No. 1, and Red No. 3, from which you can create a rainbow of colors by mixing and diluting.

Black Ink Pigments: Colored and Black inks are mixtures of other colored pigments. Not all pens of the same color contain the same pigments. In forensic science (the use of science in legal proceedings) chemists could use a solvent to remove the black ink from a small portion of a piece of evidence. Then the ink could be analyzed by chromatography and compared to known inks.

Theory of Paper Chromatography: In paper chromatography the stationary phase is a sheet of absorbent paper, such as filter paper. A tiny drop of the mixture to be separated is placed on the paper near the bottom of the paper. A lightly drawn pencil line marks the location of the spot. This location is called the origin. The paper is suspended vertically in the mobile phase, a solvent or **eluent**. The eluent could be water or alcohol, or a solvent solution made form several reagents whose proportions are chosen to enhance their ability to "pull" along some substances in the mixture being separated better than others. We want each chemical in our mixture to have different attractions to the solvent so that they will travel at different speeds and be separated. The origin must be above the surface of the eluent. The eluent rises up the paper by capillary action. When the eluent reaches the origin, the components of the mixture rise at different rates. The container must be covered to prevent evaporation of eluent. The chromatogram must be removed from the eluent before the eluent reaches the top of the paper.

As the substances in the mixture rise up the paper, they spread out and the spots become larger. For this reason, the original spot should be as small as possible, less than 5 mm in diameter. If too much material is applied to the small spot, the spot may develop a long "tail." If too little material is applied to the spot, the color of the spot may be too faint to see as the spot enlarges while moving up the paper. Trial-and-error and experience help the experimenter obtain both a small spot and one with the proper amount of material. Substances can be identified by the heights they reach on the completed chromatogram by calculating Rf (rate of flow or retention factor) values. The Rf value is a constant for a given substance under the same experimental conditions. The Rf value may be calculated from the following equation:

$$Rf = \frac{x}{y}$$

x= distance that the pigment traveled
y= distance from the origin to the solvent front

MATERIALS

1. Chromatography jars or beakers (2)
2. Mortar & pestle
3. Spinach (a few pieces)
4. Various samples to test
5. Chromatography paper (5 pieces)
6. Chromatography solvent (90% Isopropyl Alcohol)
7. Ruler (with centimeter markings)
8. Graduated cylinder (small)
9. Capillary tube (7)
10. Wooden sticks or pencils (7)
11. Calculator
12. Scissors
13. Protective Eyewear

PROCEDURE

Here's the Plan…

- Prepare a spinach leaf pigment solution.
- Prepare paper chromatograms
- Separate pigments of spinach leaves, and then your choice of other samples by paper chromatography
- Calculate the R_f values for various pigments

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Prepare your data table in your lab notebook. It should look something like this:

Substance	Color of Spot(s) If there is more than one spot, list them	Distance(s) Traveled (in cm) If there is more than one spot, list each of them	Calculated R_f Values
1. Spinach			
2.			
3.			
4.			
5.			
6.			
7.			

Prepare the spinach solution

1. Place a large piece of spinach into your pestle and add approximately 5ml of 90% isopropyl alcohol.

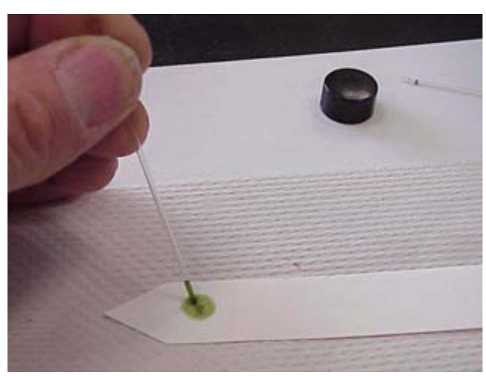
2. Thoroughly macerate the spinach/alcohol mixture to develop a thick liquid.

3. You will use this method to make a solution if you chose to try out any fruit, flower or veggie samples. If the sample is already in liquid form then you can skip to the chromatogram preparation.

Chromatogram Preparation

1. Cut the tip of a piece of chromatography paper such that it forms a triangle at the end.

2. Use the ruler to measure 1cm from the vertex of the triangle and mark it. Draw a line across width of the paper 1cm from the tip of the triangle. This is your "start line."

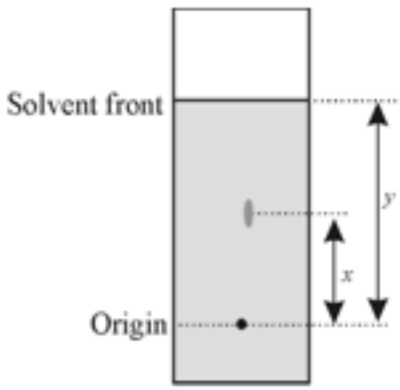


3. Using a capillary tube transfer a drop of the spinach pigment solution to the center of your start line.

4. Repeat steps 1-3 with two colors of food dye and two types of other liquids. Take note in your data table about what you used and label each paper strip with the corresponding number in pencil (pencil is made of graphite which won't interfere with the process of chromatography).

5. Pour the chromatography solvent into the chromatography jar until it fills it a little less than 1cm from the bottom.

6. Measure the length of the jar with the chromatography paper so that the tip of the triangle just touches the solvent. Attach the top of the paper to the wooden stick with tape and hang it over the jar (you may need two jars to fit all the papers). Do not submerge dots of sample below the solvent level otherwise it will be lost in the solvent and the chromatography won't work.



7. Watch as the solvent gradually rises up the chromatography paper and makes a "solvent line" which moves up towards the top of the paper. You will know when the process is almost complete when the solvent line nears the top of the paper. This will be about 15mins.

8. When the solvent line is about 1cm from the top of your papers, remove them and mark the farthest point of the solvent's progress with your pencil before this line evaporates.

9. Place each piece of filter paper on a paper towel and allow them to dry. Then use a pencil to outline the spots on the chromatography paper and label the colors. Tape them into your lab notebook and write any additional observations next to the paper.

10. Measure the distance from the start point to the front line and each of the pigment lines. Record these measurements in the data table in your lab notebook.

CALCULATIONS:
Calculate the R_f values for each pigment according to the formula given in the introduction. Show all your work in your lab notebook. Label the heading, "calculations."
Note: You don't have to test all the solutions we have for you to sample. If you are really interested in this, work with another lab group so you can make sure they test the ones you don't have time for.

Use this information to complete your analysis of the spinach
For the spinach solution, some possible colors and the pigments they represent are:
- Faint yellow - carotenes
- Yellow - xanthophylls
- Bright green - chlorophyll a
- Yellow-green - chlorophyll b
- Red - anthocyanin

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LAB GRADING (40points)
This lab will be graded based on the completion of the components in your lab notebook (30points), and the lab questions (10points). Your lab notebook will be graded based on the following components: a) at least five total substances were tested (including spinach) b) all chromatography strips are taped in the notebook and properly labeled. c) all calculations of the Rf values are clearly recorded. d) Data table is completed in entirety. e) the purpose of the lab is written out in your own words.

LAB QUESTIONS
Complete these on the computer or on a separate sheet of paper. You can email this to malika@santacruzlearningcenter.com or share your google doc with santacruzlearningcenter@gmail.com

1. How many pigments were found in the spinach leaf and what is your hypothesis of which pigments you were able to isolate with the paper chromatography?
2. Which pigments did you find in the dyes that you tested?
3. Which of the pigments in the spinach would you consider to be the most polar? Which one is the least polar? How do you know?
4. What do you think would have happened if we used water for the solvent instead of the isopropyl alcohol?

RESOURCES:
Bloomfield; Laboratory Experiments for Chemistry & the Living Organism; 1996
Home cooking: the color of food; http://homecooking.about.com
U. S. Food and Drug Administration: Center for Food Safety and Applied Nutrition; Office of Cosmetics and Colors Fact Sheet; July 30, 2001
http://www.cfsan.fda.gov/~dms/cos221.html
http://homecooking.about.com/library/weekly/aa020298.htmhttp://www.cfsan.fda.gov/%7Edms/cos-221.htmlhttp://www.cfsan.fda.gov/%7Edms/opa-col2.html - table1B