

PAPER CHROMATOGRAPHY

Chloroplast & Pigment Analysis

Name: _____

Date: _____

Period: _____

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.

Theory of paper chromatography

A small sample of a mixture is placed on porous paper which is in contact with a solvent. The solvent moves through the paper due to capillary action and dissolves the mixture spot. The components of the sample start to move along the paper at the same rate as the solvent.

Components of the mixture with a stronger attraction to the paper (stationary phase) than to the solvent will move more slowly than the components with a strong attraction to the solvent (mobile phase). The difference in the rates with which the components travel along the paper, over time, leads to their separation.

Particular mixtures will have chromatographic patterns that are consistent and reproducible as long as the paper, solvent, and time are constant. This makes paper chromatography a qualitative method for identifying some of the components in a mixture.

Objectives

- Prepare a spinach leaf pigment solution.
- Prepare a paper chromatogram.
- Separate pigments of spinach leaves by paper chromatography
- Calculate the R_f values for various photosynthetic pigments

Materials

1. Chromatography Jar
2. Mortar & Pestle
3. Spinach
4. Chromatography paper
5. Chromatography solvent (90% Isopropyl Alcohol)
6. Ruler
7. Capillary tube
8. Calculator

Solution Preparation:

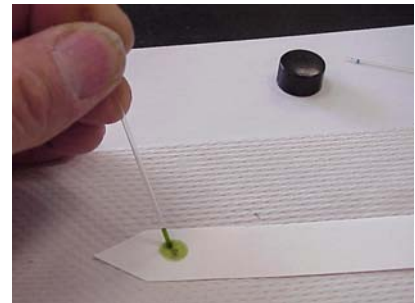
1. Place a large piece of spinach into your pestle and add approximately 5ml of 90% isopropyl alcohol.
2. Thoroughly macerate the spinach/alcohol mixture to develop a thick liquid,

Chromatogram Preparation:

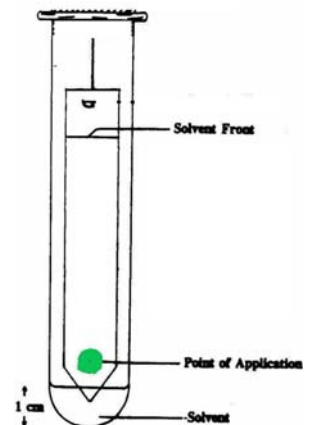
1. Obtain a chromatography jar, a piece of fresh spinach, and a length of chromatography paper (just long enough to fit from top to bottom of the jar.).
2. Cut the tip of the paper such that it forms a point of a triangle.



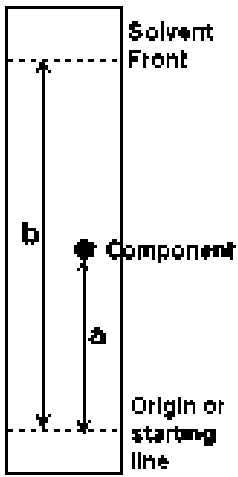
3. Draw a line across the paper 1 cm up from the triangle. This is your "start line".
4. Using a capillary tube transfer a drop of the green pigment solution to the center of your start line.
5. Pour approx. 1 cm of chromatography solvent into the chromatography jar. .



6. Open chromatography jars and hang the papers into the jar so the tip of the triangle dips into the solvent. **Do not submerge pigment lines below the solvent level.** Recap the jars immediately.
7. Allow the solvent to rise for about 15 minutes or until the solvent line nears the top of your papers.
8. When the solvent line is about 1 cm from the top of your paper. Remove the papers and **mark the farthest point of the solvent's progress before this line evaporates.**



9. Allow the filter papers to dry, and then make a sketch of the chromatogram. Some possible colors and the pigments they represent are:
- Faint yellow - carotenes
 - Yellow - xanthophylls
 - Bright green - chlorophyll a
 - Yellow-green - chlorophyll b
 - Red - anthocyanin
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. Calculate the R_f values for each pigment according to the following formula:

Calculation of R_f	
$R_f =$	<u>distance the pigment travels from the original spot of solvent</u> distance to the solvent front
<p>Paper Chromatography</p>  <p style="text-align: center;">$R_f = a / b$</p>	

Observations/Data

Spinach Leaf

Line	Color	Probable Pigment	Distance Traveled from start	R _f Value
1				
2				
3				
4				
5 (Front)				

Analysis & Conclusions

1. How many pigments were found in the spinach leaf?
2. Which of these pigments would be considered the most polar
3. Which one the least??

Critical Thinking and Application

4. Many trees have leaves that are green in the summer and red, yellow, or orange in autumn. Where were these colors during the summer? How can they suddenly appear in autumn?
5. In addition to separating plant pigments, what are some other possible applications for paper chromatography