Plant Pigments and Paper Chromatography

Introduction

Chlorophyll, the green photosynthetic pigment present in plants, often hides the other pigments present in leaves. Paper chromatography is a useful technique for separating and identifying the pigments and other molecules from cell extracts that contain a complex mixture of the molecules. Chromatography means “color writing.” The separation takes places by absorption and capillarity. The paper holds the substances by absorption; capillarity pulls the substances up the paper at different rates. Pigments separated on the paper and show up as colored streaks. The pattern of separated components on the paper is called a chromatogram.

Materials

Various leaves
Spinach leaf
Dime
5 ml Acetone (in fume hood)
2-3 pieces Chromatography paper
Ruler
250 ml Beaker

Procedures: You are testing two types of leaves, one spinach, and one of your choice.

1. Take chromatography paper and fold so it will fit over the top of the beaker, but will still reach the bottom of the inside of the beaker. Draw a faint line with your pencil 2 cm from the bottom of the paper and 4 cm above that line. Do not touch the center of the paper with your fingers; it will cause error in your results. Put the leaf on top of the paper, with the ruler over top the leaf. Take your dime and rub the dime over the leaf, over your line. You should have a dark green line 2 cm above the bottom of your paper. Make sure it is a concentrated line. Do not let the pigment touch the solvent.

2. Place 5 ml of acetone in the beaker. This is your solvent. Pour the acetone in the fume hood only! Acetone is flammable, please use with caution.

3. Place the paper in the beaker.

4. Record your qualitative data.

5. When the liquid reaches your top line, or 15 minutes, whichever comes first, take the paper out of the acetone and put it on a paper towel to dry.

6. Repeat steps 1-6 for both samples.

7. Once the strip is dry, mark the bottom of each pigment band. Measure the distance each pigment migrated from the pigment origin to the bottom of the separated pigment band. In Table 1, record the distance that each front, including
the solvent front, moved. Depending on the species of plant used, you may be able to observe 3 or 4 pigment bands.

Ex. Chromatography Paper

![Distance Solvent moved](image)

<table>
<thead>
<tr>
<th>Solvent starts</th>
<th>Solvent stops</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solvent moved</td>
<td>2cm</td>
</tr>
</tbody>
</table>

Table 1. Distance moved by Pigment bands (cm) in Spinach

<table>
<thead>
<tr>
<th>Band Number (start at bottom)</th>
<th>Band Distance (cm)</th>
<th>Band Color</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
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<tr>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
<td></td>
<td></td>
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</tbody>
</table>

Table 2. Distance moved by Pigment bands (cm) in other leaf

<table>
<thead>
<tr>
<th>Band Number (start at bottom)</th>
<th>Band Distance (cm)</th>
<th>Band Color</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solvent</td>
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</tbody>
</table>

Observations: (Qualitative Data)

Analysis
The relationship of the distance moved by a pigment to the distance moved by the solvent is the constant called $R_f$. It can be calculated for each pigment in a mixture using the formula:

$$R_f = \frac{\text{distance pigment migrated}}{\text{Distance solvent migrated}}$$

Record the $R_f$ values in Table 3

Table 3. $R_f$ values for all pigments

<table>
<thead>
<tr>
<th>$R_f$ value</th>
<th>Color of band</th>
<th>Pigment name</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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Questions

1. Hypothesize reasons why some pigments moved farther up the paper than others.

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2. What do you think the function of the other leaf pigments might be?

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3. What do you think might happen to chlorophyll pigments in the fall when leaves change colors?

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4. What are some possible sources of error with this lab?

________________________________________________________
5. Pharmaceutical companies find this technique useful for separating out different chemicals found in plants. Why would they want to separate out chemicals in different plants?

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6. What are some possible problems the pharmaceutical companies might face when trying to isolate a single chemical from a plant using paper chromatography?

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