Contributors

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Intended Audience

<table>
<thead>
<tr>
<th>Age Range</th>
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<tr>
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<td>5-8</td>
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<tr>
<td>9-12</td>
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Activity Characteristics

<table>
<thead>
<tr>
<th>Activity Characteristic</th>
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<tbody>
<tr>
<td>Classroom Setting</td>
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<td></td>
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<tr>
<td>Requires special equipment</td>
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<td></td>
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<tr>
<td>Uses hands-on manipulatives</td>
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<td></td>
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<tr>
<td>Requires mathematical skills</td>
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<td></td>
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<tr>
<td>Can be performed individually</td>
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<tr>
<td>Requires group work</td>
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<tr>
<td>Requires more than one (45 min class) period</td>
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<tr>
<td>Appropriate for special needs student</td>
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Introduction

Description

Students separate photosynthetic pigments using paper chromatography to explore how photosynthesis works.

Abstract

Students place extracted plant pigments onto a strip of chromatography paper, the stationary phase, and submerge one end of the paper in a solvent, the mobile phase. The distance that each plant pigment moves upward with the solvent front is due to its affinity to the stationary or mobile phase.

Core Themes Addressed

<table>
<thead>
<tr>
<th>Key Themes</th>
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<tbody>
<tr>
<td>Microbial Cell Biology</td>
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<td>Microbial Genetics</td>
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<td>Microorganisms and Humans</td>
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<td>Microorganisms and the Environment</td>
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<td>Microbial Evolution and Diversity</td>
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<td>Other -Chromotography</td>
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Keywords

Stationary phase, mobile phase, affinity

Learning Objectives

At completion of this activity, learner will

1. Explain how chromatography works
2. List some real-world applications of chromatography
3. Describe the function of photosynthetic pigments and explain why each has a different color.

National Science Education Standards Addressed

Standard A: Science as Inquiry

- Abilities necessary to do scientific inquiry

Standard C: Life Science

- Matter, energy, and organization in living systems
**Student Prior Knowledge**

Chromatography is a term for a family of laboratory techniques that physically separate a mixture into its individual components. Chromatography separates the components of a mixture by their distinctive attraction to the mobile phase and the stationary phase. The separation of mixtures into their components are essential for identifying the components which are present and quantifying the amount of each component. Chromatography can also be used to purify substances, which has many applications such as drug development.

**Teacher Background Information**

The plant pigments can be extracted from spinach leaves by boiling shredded leaves in concentrated ethanol. Students can apply the pigments onto the chromatography paper using capillary tubes or micropipette tips. The solvent can be prepared using acetone and petroleum ether.

**Class Time**

This activity will require a minimum of one 45 minute class period.

1. It will take students 15-20 minutes to prepare the chromatography paper strip and apply the plant pigments to it.
2. It will take 20-25 minutes for the pigments to separate.

**Teacher Preparation Time**

This lesson will require approximately 10 minutes of preparation time.

1. Divide supplies so that each group will have each necessary component.

**Materials and Equipment**

1. One 50ml plastic tube
2. One pencil
3. One strip of chromatography paper
4. 2ml chromatography solvent
5. One pair of scissors
6. One ruler
7. One plastic Pasteur pipette (dropper)
Methods

1. Obtain a strip of chromatography paper and one 50ml plastic tube.
2. Using a plastic Pasteur pipette, add 1 ml of chromatography solvent to the bottom of the 50ml plastic tube. Keep the tube closed until you are ready to perform the experiment to ensure that the atmosphere within the tube is saturated with solvent vapors (equilibration).
3. With a pencil (NOT pen), gently draw a line about 1.5 cm from the bottom of the strip.
4. Use a pair of scissors to cut two small pieces below the pencil line to form a pointed end and also cut away 4.5 cm of the chromatography paper at the top of the strip.
5. Obtain a fresh piece of spinach and place it on the pencil line on the chromatography strip. Rub the ribbed edge of a coin (dime or quarter) over the spinach leaf to extract the pigments. Repeat 10 to 15 times with different portions of the spinach leaf, making sure you are rubbing the coin on the pencil line.
6. Remove the cap from your chromatography chamber. Using forceps, carefully place the chromatography strip into the tube so that the pointed end is barely immersed in the solvent. Do not immerse the pigments in the solvent!
7. Cap the tube and leave it undisturbed. Watch as the solvent is drawn up the chromatography strip by capillary action. It is possible to observe the different plant pigments separating along the strip, as each one has a distinct color.
8. When the solvent front is approximately 1 cm from the top of the chromatography strip, remove the cap from the tube. Using forceps, remove the strip from the tube. Immediately mark the location of the solvent front, as the solvent evaporates quickly.

Tips/Suggestions

1. To get better results, prepare the plant pigments extract by boiling a handful of spinach leaves in approximately 25 ml of 70% rubbing alcohol for 5 – 10 minutes or until the solution is a deep green color. Do this in a well-ventilated area.
2. Have the students applied the plant pigments extract onto the chromatography paper using capillary tubes or micropipette tips.

References

This activity was modified from WARD’S Photosynthesis and Cell Respiration Lab Activity http://www.hsu.edu/pictures.aspx?id=1653
Answers to Student Handouts

1. What factors are involved in the separation of the pigments?

The factors that are involved in the separation of the pigments are the differential affinity of each pigment to either the mobile phase (the solvent) or the stationary phase (the chromatography paper).

2. Which pigment migrated the farthest? Why?

The pigment that travelled the farthest is carotene because it has the highest affinity to the mobile phase (the solvent).

3. During summer, leaves are generally bright green. What would you hypothesize that this indicates about the role of different light wavelengths, chlorophyll and the photosynthetic process?

During summer the leaves have an abundance of chlorophylls, which are the pigments active in the photosynthetic process. Chlorophylls absorb light at different wavelengths but reflect green light.
Introduction

All living organisms require energy for their metabolic processes. The ultimate source of this energy is the sun. Photosynthetic organisms such as plants convert light energy into the chemical energy of sugars, which can be used to power metabolism. During photosynthesis, molecules referred to as pigments (due to the wavelength, thus color, they reflect) are used to capture light energy.

Four primary pigments of green plants can easily be separated and identified using a technique called paper chromatography. These pigments include two greenish pigments called chlorophylls and two yellowish pigments called carotenoids. Pigments are separated according to differences in their relative solubilities.

Vocabulary

Chromatography: a family of laboratory techniques that physically separate a mixture into its individual components

Stationary phase: the component in which the mixture is applied; the paper is the stationary phase in paper chromatography

Mobile phase: the solvent in which the mixture is dissolved

Affinity: the attraction between compounds or molecules

Materials Checklist

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<tbody>
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<td>1 50ml plastic tube</td>
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**Procedure**

1. Obtain a strip of chromatography paper and one 50ml plastic tube.

2. Using a plastic Pasteur pipette, add 1 ml of chromatography solvent to the bottom of the 50ml plastic tube. Keep the tube closed until you are ready to perform the experiment to ensure that the atmosphere within the tube is saturated with solvent vapors (equilibration).

2. With a pencil (NOT pen), gently draw a line about 1.5 cm from the bottom of the strip.

3. Use a pair of scissors to cut two small pieces below the pencil line to form a pointed end (Figure 1) and also cut away 4.5 cm of the chromatography paper at the top of the strip.

4. Obtain a fresh piece of spinach and place it on the pencil line on the chromatography strip. Rub the ribbed edge of a coin (dime or quarter) over the spinach leaf to extract the pigments. Repeat 10 to 15 times with different portions of the spinach leaf, making sure you are rubbing the coin on the pencil line (Figure 2).

5. Remove the cap from your chromatography chamber. Using forceps, carefully place the chromatography strip into the tube so that the pointed end is barely immersed in the solvent. Do not immerse the pigments in the solvent!

6. Cap the tube and leave it undisturbed. Watch as the solvent is drawn up the chromatography strip by capillary action. It is possible to observe the different plant pigments separating along the strip, as each one has a distinct color.

7. When the solvent front is approximately 1 cm from the top of the chromatography strip, remove the cap from the tube. Using forceps, remove the strip from the tube. Immediately mark the location of the solvent front, as the solvent evaporates quickly.
Student Worksheet

Chromatography

Name:___________________________________Block:_________________

*Answer all questions in complete sentences

1. What factors are involved in the separation of the pigments?

2. Which pigment migrated the farthest? Why?

3. During summer, leaves are generally bright green. What would you hypothesize that this indicates about the role of different light wavelengths, chlorophyll and the photosynthetic process?