

9. PLANT PIGMENTS

Background

Most plants have green leaves, a color that results from the presence of chlorophyll in the leaves. Leaves may also contain accessory pigments that may not be visible to the naked eye but play an important role in acquiring energy for photosynthesis. Paper chromatography is a simple process that can be used to determine the pigments present in leaves.

Chromatography separates the components of a mixture using a solvent as a moving phase. The solvent moves up the paper and as it travels past the sample on the paper, it attracts the particles of the mixture. Differences in the chemical properties of these particles make some more attracted and others less attracted to the solvent and to the paper. Differences in attractions to the moving and stationary phases cause pigments in a mixture to travel at different rates up the chromatography paper, resulting in the separation of the pigments.

In the cells of plant leaves, chloroplasts carry out photosynthesis. Sunlight provides the energy needed for this process. Sunlight is composed of different colors (red, orange, yellow, green, blue, and violet)—each color corresponds to a narrow range of wavelengths. The structure of a pigment molecule allows it to absorb some wavelengths and reflect others. A colorimeter or spectrometer can be used to determine which wavelengths are absorbed.

A colorimeter shines six colors of light at specific wavelengths through a sample: red (650 nm), orange (600 nm), yellow (570 nm), green (550 nm), blue (500 nm), and violet (450 nm). A sensor on the opposite side of the light source detects how much light of each color gets absorbed by or transmitted through the sample. A spectrometer is similar in function to a colorimeter. However, the spectrometer can determine the absorbance or transmittance for all variations of the colors (wavelengths) in the visible light spectrum.

Absorption of light excites electrons in pigment molecules. These excited electrons, and the electron transport systems within chloroplasts, are vital to the photosynthesis process. As in cellular respiration, certain molecules act as *electron acceptors* during the process. In this investigation, a colored compound, DPIP, acts as an electron acceptor to allow you to observe the effect of light on chloroplasts and photosynthesis.

Driving Questions

What pigments are present in spinach leaves and what colors of light are absorbed by these pigments?

What role do pigments play in the light-dependent reactions of photosynthesis?

Materials and Equipment

Use the following materials to complete the initial investigation. For conducting an experiment of your own design, check with your teacher to see what materials and equipment are available.

PART 1

- Data collection system
- Colorimeter
- PASCO Wireless Spectrometer and Spectrometry software
- Colorimeter or spectrometer cuvettes (2)
- Plastic pipets (3), 1-mL
- Graduated cylinder, 10-mL (graduated to 0.2 mL)
- Capillary tube or eye dropper without a bulb
- Chromatography chamber with solvent
- Chromatography paper
- Ethanol, 10 mL
- Pigment extract, 10 mL, or if necessary to prepare Spinach leaves (3)
- Ethanol, 5–10 mL
- Beaker, small
- Mortar and pestle
- Cheesecloth or coffee filter
- Scissors
- Small stapler or paper clips
- Ruler
- Pencil
- Kimwipes®

PART 2

- Data collection system
- Colorimeter
- Colorimeter or spectrometer cuvettes (3)
- Plastic pipets (2), 1-mL
- Graduated cylinder, 10-mL (graduated to 0.2 mL)
- DPIP (2,6-dichlorophenolindophenol) solution, 2 mL
- Chloroplast suspension, 9 drops, stored on ice
- Buffer solution A, 3 mL
- Buffer solution B, 5 mL
- Rinse bottle with distilled water
- Lamp with a compact fluorescent (CFL) light bulb
- Aluminum foil, to cover a cuvette
- Kimwipes®

Safety

Follow these important safety precautions in addition to your regular classroom procedures:

- Wear safety goggles at all times.
- Never use ethanol near a flame; it is highly flammable.
- Work in a well-ventilated area, ideally a fume hood, when carrying out the extraction and chromatography procedures that use organic solvents like acetone and ethanol.

Initial Investigation

Complete the following investigation before designing and conducting your own experiment. Record all observations, data, explanations, and answers in your lab notebook.

Part 1 — Pigment Chromatography and Absorbance of Light

1. Put on your safety goggles.
2. If your teacher has prepared the pigment extract using ethanol, continue to the next step. Otherwise, follow the procedure below for preparing this extract yourself.
 - a. Cut three large spinach leaves into small pieces. Discard the stem of the leaves. Place the leaves into a mortar and add approximately 5–10 mL of ethanol to soak the leaves.
 - b. Use a pestle to grind the spinach to help the ethanol dissolve the pigments in the leaves. Continue grinding for 3–5 minutes.
 - c. Place a piece of cheesecloth or a coffee filter over the top of a beaker. Filter the spinach mixture from the mortar, taking care to prevent the cheesecloth or filter paper from falling into the beaker.

NOTE: You can squeeze the cheesecloth to help the liquid pass through.
3. Use a pencil to draw a line across a square of chromatography paper 2 cm above the bottom edge of the paper.
4. Make a green line of pigment extract on or slightly above the pencil line as follows:
 - a. Place a capillary tube or the tip of an eye dropper into the pigment extract. Place your finger over the top of the tube or dropper to prevent the liquid from falling out when you remove it from the beaker.
 - b. Place the capillary tube or pipet tip at the edge of the chromatography paper. Slightly release your finger from the top of the tube, allowing a small amount of the green extract to absorb into the paper while moving the capillary tube or dropper across the paper. Collect more extract in the capillary tube or pipet tip as needed. Keep the extract at or above the line.
 - c. Let the extract dry on the paper and then repeat the process once.

5. Roll the paper into a cylinder and staple or paper clip the paper where the edges meet to prevent it from unrolling. Place the paper into a chromatography chamber that contains a small volume of solvent at the bottom as shown in Figure 1. It is important that the pencil line be above the solvent.
6. Seal the chromatography chamber and observe the movement of the solvent up the paper. Leave the chamber undisturbed until the solvent moves most of the way up the paper. While you wait, continue with the questions and procedures that follow.

NOTE: Do not allow the solvent to reach the top of the paper.

7. For some chromatography investigations, water is used as the solvent. In this investigation, the solvent is a solution that contains acetone (C_3H_6O). Why is an organic solvent used in this chromatography experiment rather than water?

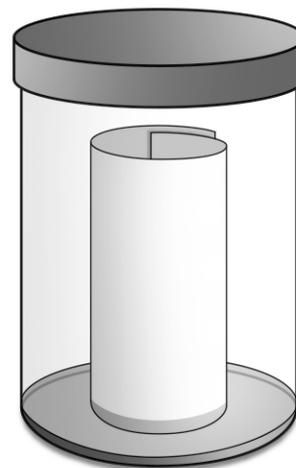


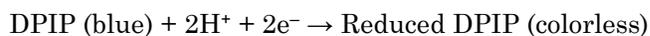
Figure 1: Chromatography setup

8. Describe the location of pigments within plant leaf cells. Be specific.
9. Open the 9 ABI Plant Pigments lab file. Connect the colorimeter to your device.
- NOTE: If the lab file is not available, you can build one after calibration is complete (explained in the next step). Create a page with 2 displays: a bar meter display and a table display. Change the sampling mode from Periodic to Manual. For the bar meter display, add Absorbance for all six colors. For the table, create a user-entered data set for Time in minutes for the first column. Add the values 0, 5, 10 and 15 to the Time column. Set the second column to Red (650 nm) Absorbance.*
10. Calibrate the colorimeter with a blank that is filled three-quarters full with ethanol.
- NOTE: Always wipe cuvettes with a lint- and scratch-free wipe before placing into either the colorimeter or spectrometer. Orient the cuvette in the colorimeter or spectrometer so the light path is not blocked by labels or cuvette ridges. Never use an acetone-based solvent with the plastic cuvettes.*
11. Fill a clean cuvette about half full with ethanol. Add 15 drops of pigment extract to the ethanol, cover, and invert the cuvette to mix. The mixture should appear light green and transparent. Wipe the cuvette and place it into the calibrated colorimeter.
12. Start collecting data. Record the absorbance of light for each of the six colors the colorimeter detects, then stop collecting data.
- NOTE: If the absorbance reading for any color is greater than 2.0, pour some solution out of the cuvette and add more ethanol to dilute the solution.*
13. For the pigment extract, which two colors of light have the greatest absorbance? Which two colors of light have the lowest absorbance? Provide an explanation for these results.
14. When the paper chromatography is complete, remove the paper from the chromatography chamber and sketch the results in your lab notebook. Lay the paper flat to dry.
15. How many different types of pigments are present in spinach leaves? Provide evidence to support your claim.
16. The absorbance of green light is low for a sample extracted from green leaves. However, the absorbance is not zero. Which pigments absorb green light in the spinach leaves?
17. Use a PASCO Wireless Spectrometer and Spectrometry software to observe the full absorbance spectrum for the pigment extract:
- Connect the spectrometer to the Spectrometry application using the Bluetooth® or USB connection. Conduct the light calibration using the same cuvette blank that contains ethanol.
 - Use the same diluted extract as before (ethanol plus pigment extract). Start recording data, and draw a sketch or print a record of the spectrometer data.
 - Stop recording data.

Part 2 — Measuring Photosynthetic Activity with DPIP

NOTE: Use the spinach chloroplasts your teacher prepared by blending spinach with an ice-cold sucrose solution. Keep the chloroplast suspension on ice until you use it to prepare cuvettes.

18. DPIP (2, 6-dichlorophenolindophenol) is a blue-colored compound. When reduced, it turns colorless.



In the following procedure, DPIP acts as an electron acceptor for excited electrons.

- a. Given what you know about the light-dependent reactions of photosynthesis, describe how electrons in chloroplasts become excited.
 - b. In this investigation, excited electrons will be transferred to DPIP. In a typical photosynthesis reaction, what happens to these excited electrons?
19. Return to SPARKvue on your device to measure the change in DPIP color under different conditions with the colorimeter.

NOTE: Whether you record transmittance or absorbance, and whether you collect data for more than one color, are decisions left up to your group. Modify the table display in the lab file as desired. Create a data table to organize the results.

20. Rinse the graduated cylinder thoroughly with distilled water.
21. Label three colorimeter cuvette caps “1,” “2,” and “3.” Use the graduated cylinder to add 3 mL of buffer solution A to cuvette 1, then add 2.4 mL of buffer solution B to cuvettes 2 and 3 as specified in Table 1.

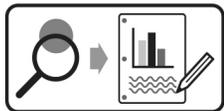
Table 1: Setup for the DPIP photosynthesis experiment

Cuvette Contents	Cuvette 1 (blank)	Cuvette 2 (placed in light)	Cuvette 3 (placed in the dark)
Buffer solution A	3 mL	None	None
Buffer solution B	None	2.4 mL	2.4 mL
Chloroplast suspension	3 drops	3 drops	3 drops
DPIP	None	13 drops	13 drops

22. Cuvette 1 is a blank, a cuvette that contains the same contents as the other cuvettes but lacks the colored DPIP. Stir the chloroplast suspension and use a pipet to add 3 drops to Cuvette 1. Close the cuvette and invert it a few times to mix the contents.
23. Calibrate the colorimeter with Cuvette 1, then start recording data.
24. Stir the chloroplast suspension Add 3 drops to Cuvette 2 and 3 drops to Cuvette 3.
25. Use a clean pipet to add 13 drops of DPIP to Cuvette 2. Close and invert the cuvette to mix the contents. Place Cuvette 2 into the colorimeter. Record the initial transmittance or absorbance (time = 0 minutes) for the suspension. Repeat the process for Cuvette 3.
26. Place Cuvette 2 near a bright light source. Wrap Cuvette 3 completely in foil and place it next to Cuvette 2.
27. After 5 minutes, 10 minutes, and 15 minutes, record the transmittance or absorbance of light for Cuvettes 2 and 3. Be sure to invert the cuvettes to mix the contents well before recording measurements. Also, unwrap Cuvette 3 each time, make the measurement, and then re-wrap it.
- NOTE: It is a good science practice to place the blank in the colorimeter each time to ensure the transmittance of the blank continues to read 100% (or the absorbance reads 0.00).*
28. Stop recording data. Create an appropriately labeled graph to illustrate the results of the DPIP investigation.
29. Explain any difference in results for Cuvettes 2 and 3.

Design and Conduct an Experiment

Use one of the tools or techniques from the Initial Investigation to explore a question of your own related to plant leaves and pigments, or to photosynthesis.



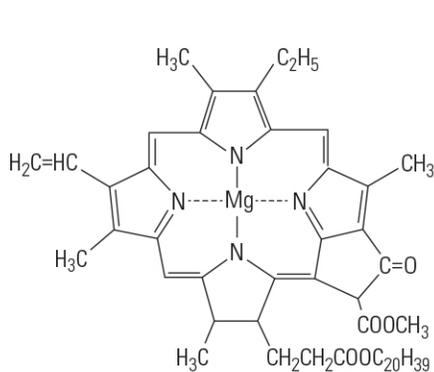
Design and carry out your experiment using either the Design and Conduct an Experiment Worksheet or the Experiment Design Plan. Then complete the Data Analysis and Synthesis Questions.

Design and Conduct an Experiment: Data Analysis

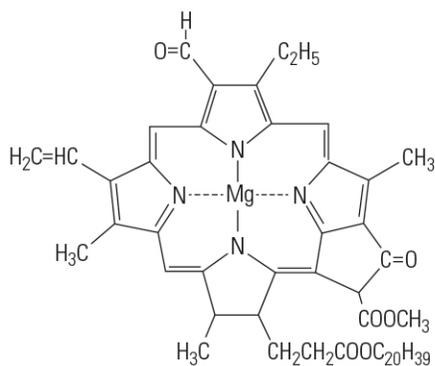
1. From your observations and your data:
 - a. Describe how the independent variable you manipulated affected the rate of respiration. Does the data support your hypothesis? Justify your claim with evidence from your experiment.
 - b. Based on the evidence you collected, explain why the results occurred.
2. Is there any evidence in your data or from your observations that experimental error or other uncontrolled variables affected your results? If yes, is the data reliable enough to determine if your hypothesis was supported?
3. Identify any new questions that have arisen as a result of your research.

Synthesis Questions

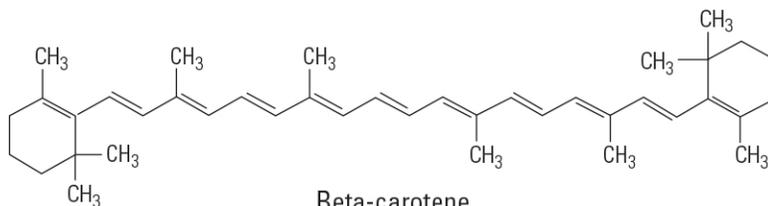
1. Refer to the diagrams below illustrating the structures of four common plant pigments.



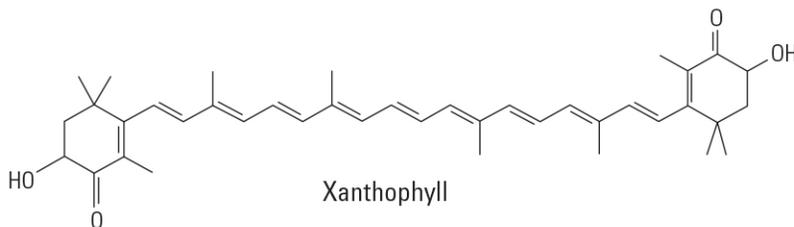
Chlorophyll a



Chlorophyll b



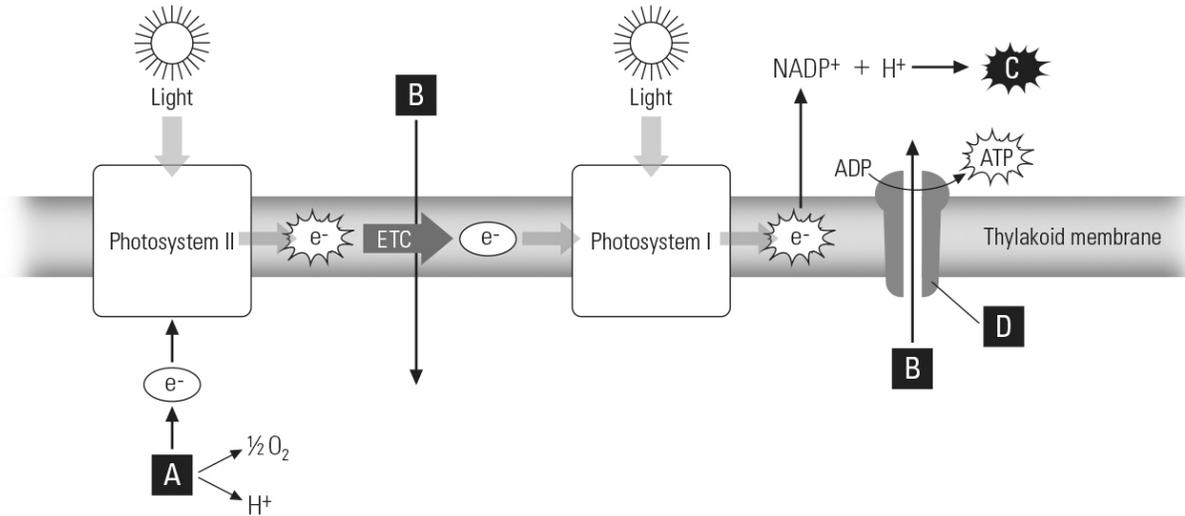
Beta-carotene



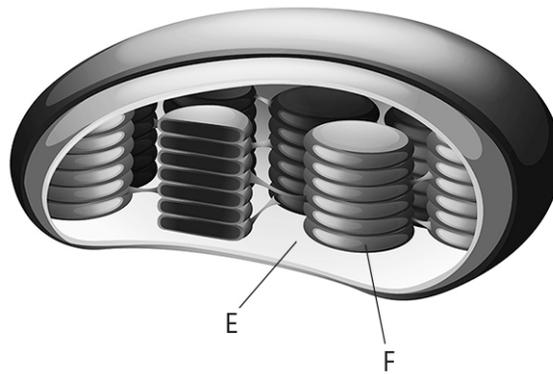
Xanthophyll

- Which pigment is more polar, chlorophyll *a* or chlorophyll *b*? Justify your answer by describing aspects of the pigment structure that relate to its polarity.
- Which pigment is least polar, beta carotene or xanthophyll? Explain your reasoning for your choice.
- How do the structures of these pigments relate to the process of paper chromatography?

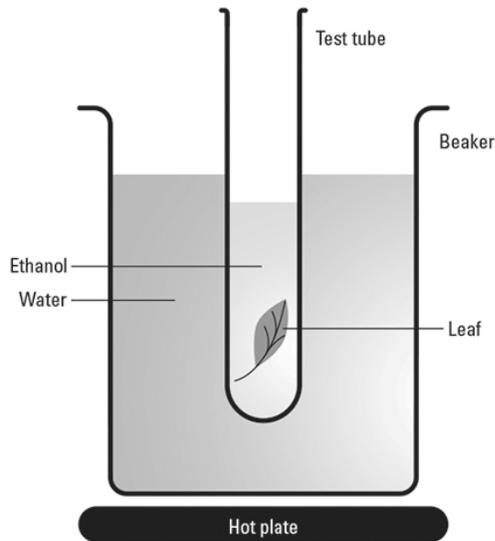
2. The diagram below shows events of the light reactions of photosynthesis.



- Identify the items labeled A, B, C, and D.
- Of what importance is B to the function of D?
- Of what importance are C and D to the reactions of the Calvin cycle—the series of reactions that follow the light reactions?
- Identify labels E and F on the diagram of a chloroplast. Describe the processes that occur in each area.



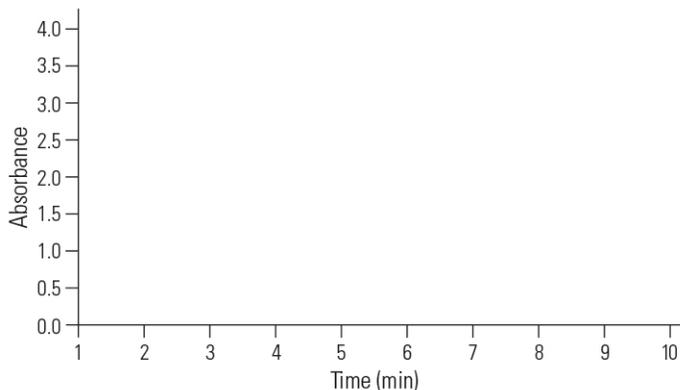
3. A student researches various methods of extracting chlorophyll from leaves. One method describes boiling leaves in ethanol, as illustrated:



The boiling process causes the ethanol to turn green and “bleaches” the leaf.

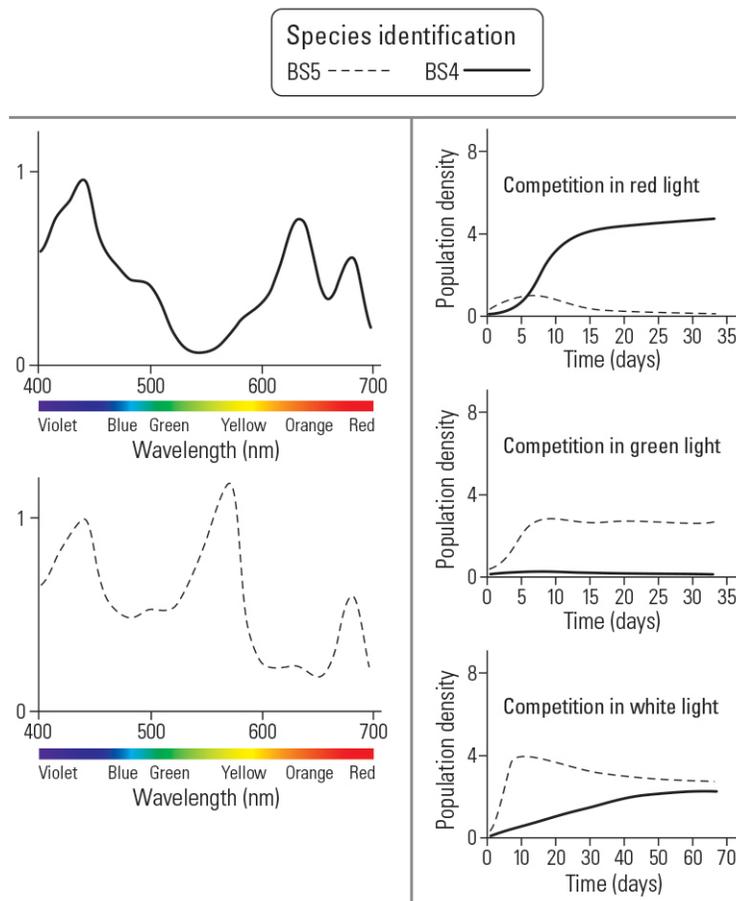
The student reads about this method in an investigation that tests leaves for the presence of starch and wonders whether this method might be used for a DPIP investigation similar to the Initial Investigation. He sets up a cuvette with the following contents: phosphate buffer, distilled water, DPIP, and 3 drops of the green-colored ethanol obtained after boiling the leaf in ethanol in a test tube, as described in the investigation. The cuvette is placed under a bright light source and the student measures light absorbance in the solution at 1 minute intervals for 10 minutes.

Copy the graph below and sketch a line on the graph to illustrate what you predict will happen to the absorbance of the solution in the cuvette the student set up. Explain the reasoning for your prediction.



4. In the Baltic Sea, two similar species of photosynthetic picocyanobacteria were found to occupy water at a similar depth. One species, BS4, is blue-green in color and the other species, BS5, is red. Both species contain chlorophyll *a* but differ in the presence of certain accessory pigments: phycocyanin is found only in BS4 and phycoerythrin is found only in BS5. The absorbance

spectra for the pigments in these species are shown below. Also shown are the results of experiments in which the species were grown together under different light conditions.¹

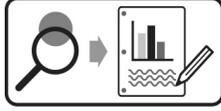


- Explain why the picocyanobacterium BS5 is red in color.
- Describe evidence from the absorbance spectra that both of the species contain chlorophyll *a*.
- Describe the results of growing BS4 and BS5 together in green light and provide a biological explanation for the results.
- Describe the results of growing BS4 and BS5 together in white light and provide a biological explanation for the results.
- If paper chromatography was performed using pigments extracted from BS5, what color or colors would you expect to see on the paper after chromatography is complete? Explain the reasoning for your choice(s).

¹ Stomp, M. et al. Adaptive divergence in pigment composition promotes phytoplankton biodiversity. *Nature* (Impact Factor: 38.6). 12/2004; 432(7013):104–7. DOI: 10.1038/nature03044

Design and Conduct an Experiment Worksheet

Use one of the tools or techniques from the Initial Investigation to explore a question of your own related to plant leaves and pigments, or to photosynthesis.



Develop and conduct your experiment using the following guide.

1. Create a driving question: choose one of the factors you've identified that can be controlled in the lab and develop a testable question for your experiment.

2. What is the justification for your question? That is, why is it biologically significant, relevant, or interesting?

3. What will be the independent variable of the experiment? Describe how this variable will be manipulated in your experiment.

4. What is the dependent variable of the experiment? Describe how the data will be collected and processed in the experiment.

5. Write a testable hypothesis (If...then...).

6. What conditions will need to be held constant in the experiment? Quantify these values where possible.

7. How many trials will be run for each experimental group? Justify your choice.

8. What will you compare or calculate? What analysis will you perform to evaluate your results and hypothesis?

9. Describe at least 3 potential sources of error that could affect the accuracy or reliability of data.

10. Use the space below to create an outline of the experiment. In your lab notebook, write the steps for the procedure of the lab. (Another student or group should be able to repeat the procedure and obtain similar results.)

11. Have your teacher approve your answers to these questions and your plan before beginning the experiment.
