

8. Measurements of Glucose Synthesis during Photosynthesis



Figure 1

Introduction

Photosynthesis is the fundamental process whereby carbohydrates are produced from inorganic materials: carbon dioxide and water. Light, absorbed by pigments of



photosynthetic organisms (for example, chlorophyll in green plants) is the energy source for this process.


Glucose synthesis depends upon abiotic factors such as light intensity and temperature, upon levels of chlorophyll in the plant's leaves and on surface area of the plant exposed to light.

In this experiment, we follow levels of glucose synthesized in terrestrial plants using a Colorimeter.

Equipment

- Nova5000
- Colorimeter
- A plant with at least 20 - 40 leaves (Coleus, Pelargonium or Jasminum fruticans are good choices)
- 150 W reflector lamp
- A rack with tubes
- A mortar
- 1% glucose solution in water
- Acetone
- n-Hexane
- 40% Potassium Sodium Tartrate solution
- 1% DNS solution:
 - a. 10 g DNS (Dinitrosalicylic acid)
 - b. 2 g Phenol (optional, it intensifies the color received)
 - c. 0.5 g Sodium Sulfite
 - d. 10 g NaOH
 - e. Add water to 1 liter
- 20 g per liter Phenol (optional)

Equipment Setup Procedure

1. Launch MultiLab.
2. Connect the Colorimeter to Input 1 (I/O-1) of the Nova5000.
3. Assemble the equipment as illustrated in Figure 1.
4. Click **Setup**  on the main toolbar and set the data logger up according to the setup specified below. The first setup is for calibration and the second is for taking measurements.

Data Logger Setup

Sensors:

Input 1: Colorimeter

Rate:

Every second

Samples:

100 samples

Use the **green** filter for chlorophyll extracts and the **red** filter for glucose determination.

For the measurements of color with the Colorimeter, the setup is:

Sensors:

Input 1: Colorimeter

Rate:

Manual

Samples:

20 samples



Experimental Procedure

1. Illuminate the plant you choose or place it outside in full day light.
2. Over intervals of one hour take off 8 leaves.
3. Preparation of chlorophyll and glucose extracts:
 - a. Weigh the leaves.
 - b. Crush the leaves in a mortar.
 - c. Add 10 ml acetone and continue to crush.
 - d. Filter the extract through a gauge and collect the fluid in a tube.
 - e. Add 10 ml n-Hexane. Mix well.
 - f. Add 10 ml tap water. Mix well. The n-Hexane is lighter than water and does not dissolve in water. A green separate layer of n-Hexane with the chlorophyll extract is formed above the water.
 - g. Collect the upper layer into a tube, mark it – one hour.
 - h. Collect 3 ml from the tap water layer and add them to a tube for glucose determination. Mark it: Glucose one hour.
 - i. Repeat stages a-h after two, three and four hours.



Measure chlorophyll concentration in your samples

Dilute the samples in tap water (the extent of dilution depends on color intensity, but should be at least 1:3 to enable color reading in the colorimeter).



Note: You can omit the measurement of chlorophyll concentration and rely on your weighing data alone.

Calibrate the Colorimeter

1. Use the green filter.
2. n-Hexane diluted 1:3 in tap water will serve as a blank solution.
3. Pour the blank into a cuvette and insert it into the Colorimeter. Cover the cuvette to avoid n-Hexane evaporation.
4. Close the Colorimeter cover tightly

5. Click **Run**  on the upper toolbar to begin recording data.
6. Turn the knob on the Colorimeter until you receive 100% transmission.
7. Click **Stop**  on the upper toolbar to stop collecting data.


Measure the colour in each sample

1. Pour each sample into a cuvette and insert it into the Colorimeter.
2. Close the Colorimeter cover tightly.
3. Click **Run**  on the upper toolbar to begin recording data.
4. The setup is *manual*; therefore you have to press **Run** for each sample.
5. Click **Stop**  on the upper toolbar to stop collecting data.


Measure glucose levels in your samples

1. Prepare a standard curve: five tubes with 3 ml of the following glucose concentration: 1%, 0.5%, 0.1%, 0.05%, 0 (blank solution).
2. Add 3 ml of 1% DNS solution to 3 ml samples of glucose solutions.
3. Cover the test tubes with glass caps or other cover to avoid evaporation.
4. Heat the mixture to 90 °C for 10 minutes until a red-brown color is obtained.
5. Add 1 ml of 40% Potassium Sodium Tartrate solution. It stabilizes the color.
6. Cool to room temperature in cold water. After cooling you can add 1 ml of Phenol solution to intensify the color.



Calibrate the Colorimeter

1. Use the red filter.
2. Pour the blank solution into a cuvette and insert it into the Colorimeter.
3. Close the Colorimeter cover tightly.
4. Click **Run**  on the upper toolbar to begin recording data.
5. Turn the knob on the Colorimeter until you receive 100% transmission.



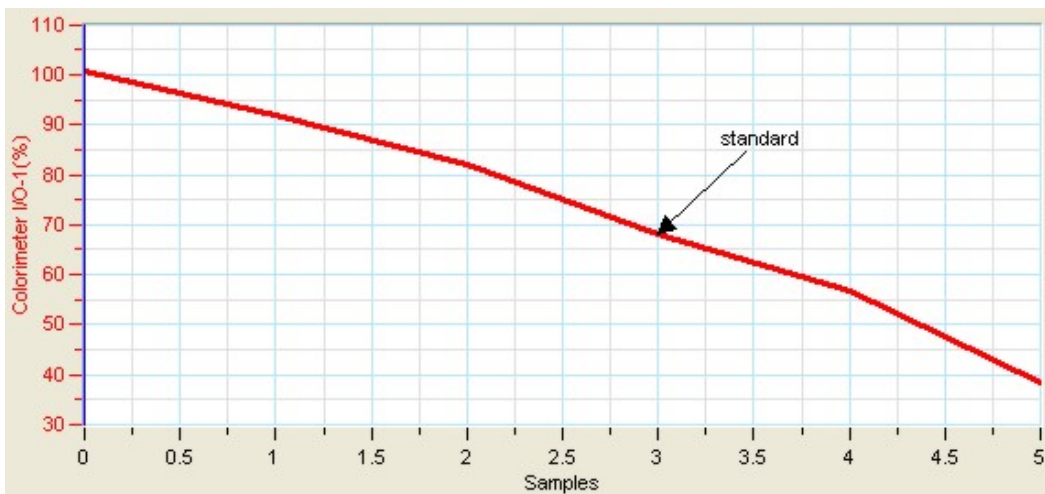
6. Click **Stop**  on the upper toolbar to stop collecting data.


Measure the color in each sample

1. Pour each sample into a cuvette and insert it into the Colorimeter.
2. Close the Colorimeter cover tightly.
3. Click **Run**  on the upper toolbar to begin recording data.
4. The setup is *manual*; therefore you have to press **Run** for each sample.
5. Click **Stop**  on the upper toolbar to stop collecting data.

Data Analysis

1. Prepare a standard curve of glucose: light transmittance versus glucose concentration.
2. An example of the graph, obtained in this experiment, is shown below:



3. Use the First cursor  to read from the graph the % Transmittance for each glucose level at each time point of your samples, and prepare a table as shown below:

Time (Hours)	% Transmittance	Glucose Concentration	Glucose Concentration per g Leaf Weight (%/g)

- Use PlanMaker to prepare a graph presenting the changes in glucose concentration per g leaf with time.

Questions

- Describe the graph showing glucose synthesis with time. Is the graph linear?
- Why is it important to calculate glucose concentration per gram leaf weight or chlorophyll concentration?
- Which of the parameters is more accurate: leaf weight or chlorophyll concentration? Explain.
- What are the relationships between glucose synthesis and O_2 released during photosynthesis?
- Assume what is the effect of temperature on glucose synthesis rate in photosynthesis. Design an experiment to examine your assumption.

Further Suggestions

- Keep plants in different light intensities and measure the effect on glucose synthesis.
- Keep the plants in closed transparent chambers: In one add KOH grains (KOH reacts with CO_2 and omits it from the free air). Compare glucose synthesis in the chambers.
- Compare glucose synthesis in different plants.