Fantastic Fluorescing Chlorophyll

Introduction

Watch as chlorophyll transforms from an intense green to a bright red colour as UV light is absorbed and photons are emitted from the solution.

Concepts

- Chlorophyll
- Fluorescence

Materials

Ethyl acetate, $CH_3COOC_2H_5$, 25 mL
Fresh spinach, 15–20 leaves
Centrifuge (recommended)
Centrifuge tubes, 2 (recommended)
Erlenmeyer flask, 50-mL (and cork or stopper to fit the Erlenmeyer flask)
Flashlight (optional)
Graduated cylinder, 25- or 50-mL
Mortar and pestle
Pipet, disposable
Ultraviolet lamp or black light

Safety Precautions

Ethyl acetate is a flammable liquid and a dangerous fire and explosion risk. Keep away from flames and other sources of ignition. It is slightly toxic by inhalation, ingestion, and skin absorption, and may be irritating to the skin and eyes. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Safety Data Sheets for additional safety, handling and disposal information.

Procedure

1. Mash 15–20 fresh spinach leaves using a mortar and pestle.
2. Add 25 mL of ethyl acetate to the mortar and continue to grind the mixture.
3. Decant off the liquid from the mixture into a centrifuge tube.
4. Centrifuge the solution for 5–10 minutes on the highest setting. *Note:* In order to balance the centrifuge, split the solution into two tubes of equal volume or place a tube containing an equal volume of water 180° from the tube containing chlorophyll.
5. Remove the liquid layer (this layer contains the chlorophyll) using a pipet. Discard the solid remaining in the bottom of the tube.
6. Place the chlorophyll solution into a small Erlenmeyer flask and stopper the flask.
7. Darken the room completely and observe the chlorophyll-containing solution under a UV light or black light. The solution should fluoresce a bright red colour.
8. *(Optional)* Try holding a flashlight at a 90-degree angle to the flask and make observations.

Disposal

It is recommended that you consult your local school board and/or municipal regulations for proper disposal methods that may apply before proceeding.

Publication No. 10599CA
022118

Tips

- Use caution while working with the chlorophyll solution. The solution will readily stain clothing and surfaces (unfortunately this was learned the hard way…).
- If a centrifuge is not available, the mixture may be filtered. Wet the filter paper with a little ethyl acetate and pour the mixture into a filtering apparatus.
- A few additional milliliters of ethyl acetate may be added to the solution to increase the volume for viewing purposes.
- The chlorophyll solution may be saved for a week, possibly longer. To store, stopper the flask and refrigerate.
- Dried spinach will also work, although the solution will not fluoresce quite as brightly as observed when prepared with fresh spinach. Frozen spinach does not work.
- Adding a few pinches of sand to the spinach during grinding will help "tear" the spinach apart and make extraction easier.
- Acetone or 95% ethyl alcohol also will work as a solvent, but will not fluoresce as brightly as solution prepared with ethyl acetate.

Discussion

Chlorophyll is the green pigment essential for photosynthesis. It is found in all plants and readily absorbs light in the range of 600–700 nm. Upon excitation by light, an electron in a chlorophyll molecule is moved to a higher energy level, called an excited state. The exciting source in this demonstration is the UV black light. In fluorescence, when a light source is shined on a material, a photon is absorbed. From this excited electronic state, the electron naturally wants to relax back down to the ground state. As it relaxes back down to the ground state, it emits a photon. If the emitted photon's wavelength is in the visible portion of the spectrum, we observe a colourful, glowing effect. Emission of this form is termed fluorescence. This process is practically instantaneous so the fluorescence is observed as soon as the exciting source is present, and it disappears as soon as the exciting source is removed. The fluorescent glow is brighter than the colour of the solution seen under normal visible light because light is being emitted from the solution, not just transmitted through it. When a flashlight is shined directly into the solution, a slight red fluorescence can be observed around the edges of the flask. This is due to a very small amount of light generated by the flashlight in a range which will excite the chlorophyll molecules.

Light is absorbed at specific wavelengths by the pigment molecules and is emitted at a different wavelength. In the case of chlorophyll, the light emitted is in the red region of the spectrum. In nature, excited electrons inside the chloroplast move to a protein in the thylakoid membrane as they are displaced by ions from water molecules. Separating these molecules from the membrane makes observing fluorescence caused by high energy electrons possible.

Materials for *Fantastic Fluorescing Chlorophyll* are available from Flinn Scientific Canada Inc.

Catalogue No.	Description
EJ0005	Ethyl Acetate, 500-mL
AP6066	Mortar and Pestle Set, Porcelain, Economy Choice, 65-mL

Consult www.flinnsci.ca or your *Flinn Scientific Canada Catalogue/Reference Manual* for current prices.