Explorations with

The Winston™

Chlorophyll Solubility

Version 121422

Plant leaves contain several colored compounds, called pigments, involved in harvesting energy from light. In this hands-on experiment, you will extract pigments from spinach using water and isopropanol and observe the fluorescence of the two solutions. Can you explain the differences in solubility of the plant pigments using your knowledge of chemistry?

Laboratory Safety

1. Wear lab coats, gloves, and eye protection as required by district protocol.
2. Use caution with all electrical equipment such as PCR machines and electrophoresis units.
3. Wash your hands thoroughly after handling biological materials and chemicals.

Materials Required

Item	Quantity per group
Microcentrifuge tubes (1.5 mL or 2.0 mL)	4
Spinach Leaf	1 leaf
Isopropanol	500 µL
Water	500 µL
The Winston Fluorescence Reader with Photohood	1
Microcentrifuge tube rack	1
100 - 1000 µL adjustable volume micropipette with tips	1
Electronice balance (optional)	1

theminione.com (858) 684-3190 info@theminione.com

FastTaq, GreenGel, and PrepOne are trademarks of Embi Tec. GelGreen is a trademark of Biotium.MiniOne is a registered trademark of C.C. IMEX. Patents Pending.

Protocol

1. Label two microcentrifuge tubes "I" for isopropanol and label two microcentrifuge tubes "W" for water.

2. Tear up the spinach leaf into small pieces and put an equal amount (~0.1 gram) into one tube labelled "I" and one tube labelled "W". If you are using a microcentrifuge tube with graduations, you can fill the tube with spinach up to the 200 µL mark. **Do not pack** the leaves into the bottom of the tube.

3. Add 500 µL isopropanol to one tube and 500 µL water to the other using a new pipette tip.

4. Use a pipette tip to crush the leaves until the liquid changes color. Allow the leaf pieces to settle to the bottom of the tubes for about a minute.

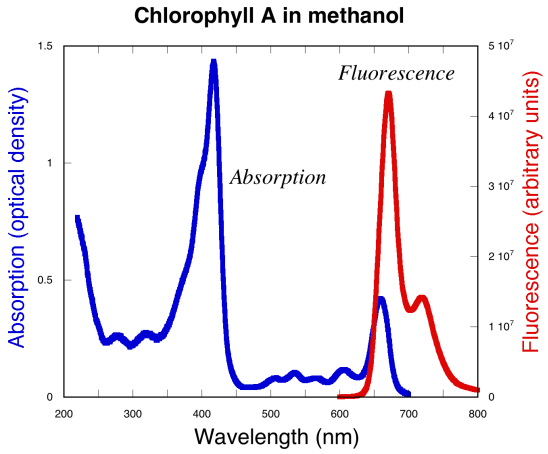
5. Use a micropipette to transfer the supernatant from each tube to the other clean, labeled microcentrifuge tube, being careful not to disturb the leaf pieces at the bottom.

6. Place the tubes in The Winston Fluorescence Reader and put the photo hood on top. Record an image of the tubes with your cell phone camera.

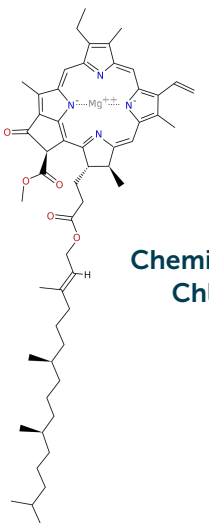
7. Look at the fluorescence excitation and emission spectrum of chlorophyll below. Which tube contains more chlorophyll? How do you know?

8. Look at the chemical structure of chlorophyll below. Why do you think it is more soluble in isopropanol?

9. What do you think is causing the green fluorescence? Do an internet search for other compounds from plants that might be contributing to fluorescence seen in this experiment.



Absorption (blue) and emission (red) spectra of Chlorophyll A



Chemical structure of Chlorophyll A