Fluorescence

Introduction

Fluorescent dyes absorb light energy and emit visible light of longer wavelengths (lower energy) than the absorbed radiation. In many cases, the absorbed radiation is in the ultraviolet range, so that a fluorescent solution will appear one color in transmitted light and a different color when viewed with a black light. Probably the most common fluorescent dye is fluorescein, which is added to commercial antifreeze to make it less likely that animals will accidentally drink it. Other examples of fluorescent dyes and pigments are rhodamine B and chlorophyll.

Concepts

- Fluorescence
- Ground state
- Light absorption and emission
- Excited electron energy levels

Materials

Antifreeze solution, commercial, 250 mL

Chlorophyll extract (see *Tips*) or chlorophyllin solution (optional)

Rhodamine B solution, 1%, 1 mL

Water, distilled or deionized

Beakers, 400-mL, 3

Construction paper, black, 2 sheets

Overhead projector

Scissors

Tape

UV light source, or light box with a blacklight bulb (optional)

Safety Precautions

Antifreeze (ethylene glycol) is toxic by ingestion, inhalation, and skin absorption. The ingestion of even small quantities of ethylene glycol can be fatal. Rhodamine B solution is slightly toxic by ingestion. Wear chemical splash goggles and chemical-resistant gloves and apron. Wash hands thoroughly with soap and water after using any chemicals and before leaving the lab. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Procedure

1. Pour about 250 mL of commercial antifreeze into a 400-mL beaker.
2. In a second beaker, add 1 mL of rhodamine B solution to 250 mL of distilled or deionized water.
3. (Optional) Prepare a chlorophyll extract from spinach leaves (see the *Tips* section) or obtain a solution of chlorophyllin, the water soluble extract of chlorophyll.
4. Tape two sheets of black construction paper together to get a sheet large enough to cover the overhead projector stage. Trace the bottom of a 400-mL beaker in the middle of the large black sheet and cut out the circle. Tape the paper with the cut-out circle in the middle to the overhead projector stage. The black paper will block all light from the sides of the beaker and give clearer images for comparing the colors of transmitted versus emitted light.
5. Place the beaker with the antifreeze solution on the overhead projector stage and turn on the light. Focus the image of the beaker on a screen or classroom wall.
6. Turn off the classroom lights and observe the yellow color of transmitted light on the projected image of the antifreeze solution.
7. After viewing the projected image, look directly at the beaker, not at the image, and observe the color of the emitted light. The solution is bright fluorescent green!
8. Turn on the classroom lights, remove the antifreeze beaker from the overhead projector stage, and replace with the rhodamine B solution.
9. Observe the pink color of transmitted light on the projected image of the rhodamine B solution.

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10. Turn off the classroom lights and look directly at the beaker to view the color of the emitted light from the rhodamine B solution—it is orange.
11. (Optional) Turn on a blacklight or UV lamp and turn off the overhead projector light. Observe the enhanced, bright pink fluorescence of the rhodamine B solution.
12. (Optional) Repeat steps 8–11 using the chlorophyll extract solution. The chlorophyll solution appears dark green due to transmitted light, but exhibits a dark red fluorescence.
13. Turn on the classroom lights and discuss the color wheel of complementary colors (see the *Discussion* section). Point out that the color of transmitted light from a transparent solution is complementary to the color or wavelength of light absorbed. Identify the color of absorbed light for the fluorescein and rhodamine solutions and compare with the color of emitted light (fluorescence). Note that the emitted color is always longer wavelength and therefore lower energy than the color of absorbed light. Point out that for most fluorescent dyes, such as rhodamine, for example, fluorescence is actually due to absorption of ultraviolet light and emission of visible light.

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. Save the antifreeze solution for repeat use. Store in a properly labeled bottle using the Flinn Compatible Chemical Family Storage Guide for ethylene glycol (Storage Code O2). The rhodamine solution may be disposed of down the drain with excess water according to Flinn Suggested Disposal Method #26b.

Tips

- A common test performed as part of a standard eye exam involves the use of fluorescein dye to observe the cornea for possible damage and also to detect foreign bodies in the eye. A few drops of the dye solution are placed in the eye, which is then observed using a blacklight.
- To prepare a chlorophyll extract, mash or grind 15–20 fresh spinach leaves using a mortar and pestle. Add 25 mL of anhydrous ethyl alcohol and continue to grind the mixture. Decant the liquid into a centrifuge tube and centrifuge for about 10 minutes on the highest setting. Remove the liquid layer and discard the solid remaining in the bottom of the tube. Chlorophyll is easily degraded by light, heat, oxygen, and acids or bases. Store in a dark bottle in the refrigerator.
- Other dyes that exhibit fluorescence include eosin and quinine, which is found in tonic water. A quinine solution is interesting because it is colorless in visible light but exhibits bright blue fluorescence in ultraviolet light.
- Light emission without heat is an interesting phenomenon and students are intrigued by any process that spontaneously produces light. Students may associate all light production with the same mechanism. Take advantage of the natural interest in these phenomena to explain the difference between chemiluminescence, which is observed in lightsticks, phos phorescence, which occurs in all “glow-in-the-dark” toys, and fluorescence, which can be observed with minerals, stamps, antique glassware, paper currency, etc.

Discussion

The color wheel, shown below, can be used to illustrate the difference between the colors of absorbed and transmitted light. Colors opposite each other on the color wheel are called complementary colors. A transparent object or solution appears a specific color in visible light because it transmits the wavelengths of light corresponding to that color and absorbs the wavelengths of light corresponding to the complementary color. In order of increasing energy, the colors of visible light are red, orange, yellow, green, blue, purple. See Figure 1.

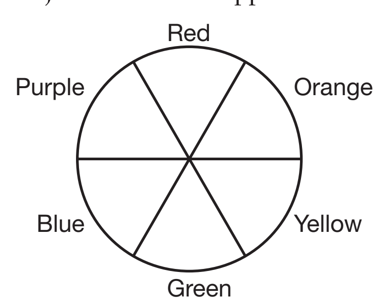

Figure 1.

The yellow fluorescein solution in commercial antifreeze transmits yellow light and absorbs mostly violet light. The color of emitted light from the fluorescence of fluorescein is green, which is lower energy than the absorbed light. The energy of the emitted light will always be lower than the energy of the absorbed light due to vibrational relaxation of the electron in the excited energy level before it falls back down to the ground state. See Figure 2.

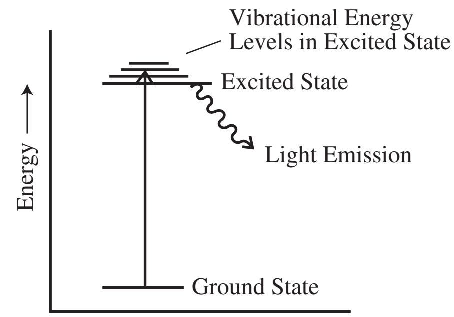

Figure 2.

Connecting to the National Standards

This laboratory activity relates to the following National Science Education Standards (1996):

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Constancy, change, and measurement
Content Standards: Grades 9–12
Content Standard A: Science as Inquiry
Content Standard B: Physical Science, structure and properties of matter, interactions of energy and matter

Flinn Scientific—Teaching Chemistry™ eLearning Video Series

A video of the *Fluorescence* activity, presented by Annis Hapkiewicz, is available in *Fluorescence*, part of the Flinn Scientific—Teaching Chemistry eLearning Video Series.

Materials for *Fluorescence* are available from Flinn Scientific, Inc.

Materials required to perform this activity are available in the *Fluorescent Dyes—Chemical Demonstration Kit* available from Flinn Scientific. Materials may also be purchased separately.

Catalog No.	Description
AP4848	Fluorescent Dyes—Chemical Demonstration Kit
F0043	Fluorescein, 25 g
R0014	Rhodamine B Solution, 1%, 20 mL
GP1025	Beaker, Borosilicate Glass, 400-mL
AP1901	Ultraviolet Lamp, Hand-Held

Consult your *Flinn Scientific Catalog/Reference Manual* for current prices.