Fluorescent Dyes

Fluorescence

Introduction

Color is a result of the interaction of light with matter. The color that a solution appears to the human eye can change depending on the nature of the light source used to illuminate it. In this demonstration, four solutions that appear one color under visible light will "change" color when exposed to an ultraviolet "black" light.

Concepts

- Fluorescence
- Transmittance
- Absorbance
- Emission

Materials

Eosin Y solution, 1%, 5 mL
Ethyl alcohol, 95%, 500 mL
Fluorescein solution, 1%, 15 mL
Rhodamine B solution, 1%, 1 mL
Water, distilled or deionized, 1000 mL
Water, tonic, 500 mL
Beakers, 600-mL, 4
Graduated cylinders, 10-mL, 3
Stirring rods, 3
Ultraviolet light source—black light
Visible light source—classroom lights work well

Safety Precautions

Ethyl alcohol is flammable and a dangerous fire risk—avoid flames, heat, and other potential sources of ignition. Addition of denaturant makes ethyl alcohol poisonous; it cannot be made nonpoisonous. Dye solutions will easily stain hands and clothing; avoid contact of all chemicals with eyes and skin. Do not look directly at the black light; its high-energy output can be damaging to eyes. Wear chemical splash goggles, chemical-resistant gloves, and a chemical-resistant apron. Please review current Material Safety Data Sheets for additional safety, handling, and disposal information.

Preparation

Beaker 1: Pour approximately 500 mL of tonic water into one of the 600-mL beakers.

Beaker 2: Add 15 mL of 1% fluorescein solution to the second 600-mL beaker. Dilute the fluorescein solution by adding enough distilled or deionized water to reach the 500-mL mark on the beaker. Stir. *Note:* Prepare 1% fluorescein solution by dissolving 1 g of fluorescein in 100-mL distilled water.

Beaker 3: Add 5 mL of the 1% eosin Y solution to the third 600-mL beaker. Dilute the eosin Y solution by adding enough ethyl alcohol to reach the 500-mL mark on the beaker. Stir. *Note:* Eosin Y is soluble in water, but the fluorescence is not nearly as strong in water as it is in ethyl alcohol.

Beaker 4: Add 1 mL of the 1% rhodamine B solution to the fourth 600-mL beaker. Use a graduated cylinder or add 15 drops from the dropping bottle. Dilute the rhodamine B solution by adding enough distilled or deionized water to reach the 500-mL mark on the beaker. Stir.

Procedure

1. Place the four beakers in a row on the demonstration table in the following order: tonic water, fluorescein, eosin Y, and rhodamine B. Set the beakers on a light box or place a white background both below and behind the beakers. This will make it easier to clearly see the colors of each of the solutions.
2. Turn on the light box or just use the classroom lights to observe the colors of the solutions.

3. Turn off all of the lights and completely darken the room. The demonstration is most dramatic in a completely dark room.
4. Turn on the black light and place it on the demonstration table in front of the row of beakers. Do not look directly at the black light. Observe the fluorescence from each beaker. Note that the fluorescent color of each solution is different than the color observed under the normal classroom lights!

Disposal

Please consult your current *Flinn Scientific Catalog/Reference Manual* for general guidelines and specific procedures governing the disposal of laboratory waste. Flush all solutions down the drain according to Flinn Suggested Disposal Method #26b.

Tips

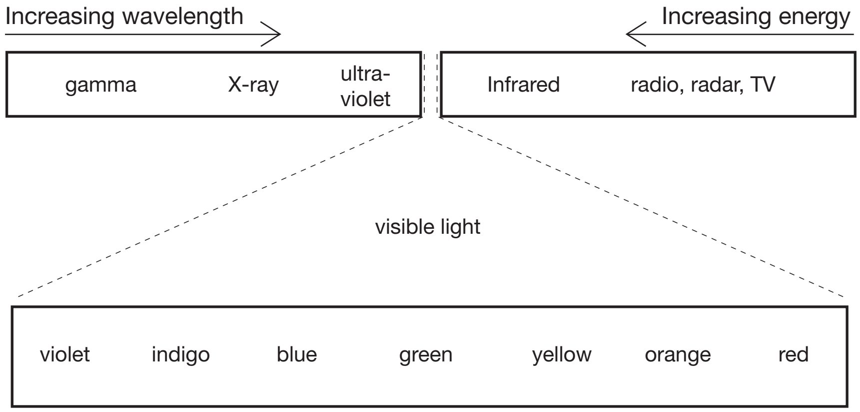
- The difference in the colors of the solutions under the classroom lights compared to the black light is most obvious in a completely darkened room. Try to extinguish all light sources.
- The solutions will last all day, even a whole week; however, some evaporation will occur. The solutions will keep for an extended period of time if the beakers are covered with Parafilm.®
- The tonic water does not have to be carbonated for fluorescence to be observed—the solution will still fluoresce even if it goes flat.
- The prescribed dilutions listed above are not strict. The same effects are noticeable over a range of dilutions; however, fluorescence is most easily observed in dilute solutions. For optimum viewing, solutions more concentrated than those suggested above are not recommended.

Discussion

The Electromagnetic Spectrum

Visible light is a form of *electromagnetic radiation*. All forms of electromagnetic radiation consist of oscillating electric and magnetic fields travelling at a constant speed, the speed of light, 2.998×10^8 m/s. Other familiar forms of electromagnetic radiation include microwave radiation from a microwave oven, X-rays, the infrared radiation in heat from a fire, and radio waves. Together, all forms of electromagnetic radiation make up the electromagnetic spectrum.

Electromagnetic Spectrum



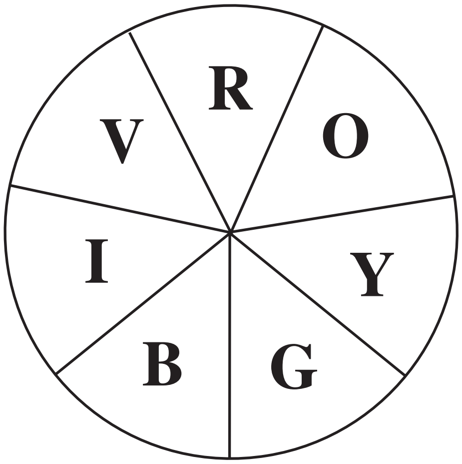
The visible portion of the electromagnetic spectrum is only a small part of the entire spectrum. It spans the wavelength region from about 400 to 700 nm. We see light of 400 nm as violet and 700 nm as red. Because wavelength is inversely proportional to energy according to the equation $E = hc/\lambda$, violet light is higher energy light than red light. The color of light we see with the human eye varies from red to violet (low to high energy) according to the familiar phrase ROY G BIV: red, orange, yellow, green, blue, indigo, violet. As the color of the light changes, so does the amount of energy it possesses. White light, like that from normal classroom lights, contains all of the colors in the visible spectrum.

A typical black light gives off UVA light. UVA is ultraviolet light in the wavelength range from approximately 320 to 400 nm;

therefore, it is higher energy light than visible light. The human eye cannot see ultraviolet light. A substance that gives off ultraviolet light (and does not also give off visible light) will appear colorless. Because the light from a black light can be seen with the human eye, it clearly must give off some wavelengths of visible light in addition to the UVA wavelengths. These additional wavelengths are in the low 400's, so the black light appears purple to the human eye.

The four solutions appear different colors under normal classroom lights. The tonic water is colorless, the fluorescein solution is yellow-green, the eosin Y solution is yellow-orange, and the rhodamine B solution is pinkish-red. Each of these dyes absorb different wavelengths of light. In general, a green solution looks green to the human eye because it is transmitting green light. When white light is shined through this solution, the molecules in the solution absorb some of the wavelengths of the light and transmit others. All non-green wavelengths of light will be absorbed by a green solution to some extent, although red light will be absorbed the most. The red photons hit the solution and are absorbed by the molecules in the solution. They do not make it through the solution, and hence, we do not see a red color from this solution. In contrast, green photons are not absorbed by the molecules in the green solution. So, they pass right through the solution, and we see a green color.

How do we know that the green solution absorbs the red wavelengths of light? Red and green are complementary colors—they are across from each other on the color wheel.



In general, colors opposite each other on the color wheel are complementary colors. For example, by looking at the wheel, it can be seen that violet and yellow are complementary colors. Therefore, it can be assumed that a yellow solution absorbs violet light and transmits yellow light. The color wheel and the idea of complementary colors can be used as a first estimation of the wavelengths that are absorbed by a substance based on its transmitted color.

The following table lists the wavelengths associated with each of the colors in the visible spectrum, as well as their complements. The representative wavelength can be used as a benchmark for each color. For example, instead of referring to green as light in the wavelength range 500–560 nm, one could simply say that green light is 520 nm.

Representative Wavelength, nm	Wavelength Region, nm	Color	Complementary Color
410	400–425	Violet	Yellow-green
470	425–480	Blue	Yellow
490	480–500	Blue-green	Red
520	500–560	Green	Reddish-violet
565	560–580	Yellow-green	Violet
580	580–585	Yellow	Blue
600	585–650	Orange	Green-blue
650	650–700	Red	Blue-green

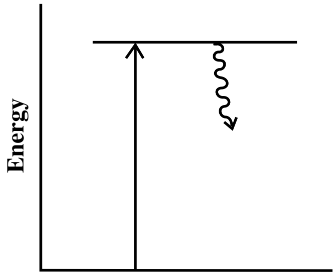
Fluorescence

Luminescence is the emission of radiation (light) by a substance as a result of absorption of energy from photons, charged particles, or chemical change. It is a general term that includes fluorescence, phosphorescence, and chemiluminescence. Fluorescence is different from other types of luminescence in that is it restricted to phenomena in which the time interval between absorption and emission of energy is extremely short. Therefore, fluorescence only occurs in the presence of the exciting source. This is different from light emission due to phosphorescence, which continues after the exciting source has been removed. In this demonstration, the exciting source is the UV black light.

In fluorescence, when a light source is shined on a material, a photon is absorbed. The energy from the photon is transferred to an electron that makes a transition to an excited electronic state. From this excited electronic state, the electron naturally wants to relax back down to the ground state. When it relaxes back down to the ground state, it emits a photon (symbolized by the squiggly arrow in the diagram to the right). This relaxation may occur in a single step or in a series of steps. If it occurs in a single step, the emitted photon will be the same wavelength as the exciting photon. If the relaxation occurs in a series of steps emitting a photon along the way, the emitted photon will have a greater wavelength (lower energy) than the exciting photon.

If the emitted photon's wavelength is in the visible portion of the spectrum, we observe a colorful, 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 color of the solution seen under normal classroom lights because light is being emitted from the solution, not just transmitted through it.

Energy Level Diagram



Fluorescence

Absorption Curves and Color

Information about the absorption and emission curves of each of the solutions in this demonstration can be inferred from the observations made during the demonstration—that is, what wavelengths of light these solutions absorb and emit.

First consider the fluorescein, eosin Y, and rhodamine B solutions

- These solutions appear colored to the human eye under the normal classroom lights. Recall that normal classroom light is composed of all the visible wavelengths of light. Therefore, these solutions must absorb some wavelengths of visible light while transmitting others—the color of the solution is the transmitted color in each case. Each of these colored solutions has an absorption (and transmission) peak in the visible region of the electromagnetic spectrum (400–700 nm).
- When the normal classroom lights are turned off and the black light is shined on the solutions, they fluoresce. Under these conditions, the solutions are not being hit with visible light, but instead are being hit with UVA light (320–400 nm). In each case, when a molecule in the solution is hit with ultraviolet photons, the molecule absorbs an ultraviolet photon and promotes an electron up to an excited state. This electron then relaxes back down to the ground state in a series of steps emitting a visible photon along the way. It is evident that the photon is in the visible region of the spectrum because the fluorescence can be seen with the human eye. Therefore, the molecules in each of the solutions must have an absorption peak in the UVA portion of the electromagnetic spectrum with a corresponding emission peak in the visible portion of the spectrum.

Clearly, each of these dye solutions has two absorption peaks—one in the visible and another in the UVA portion of the spectrum. If the transmitted wavelength of visible light is not the same wavelength as the emitted photon during fluorescence, then the solution will appear to be two different colors under the two different light sources.

Now consider the tonic water solution. Tonic water appears colorless to the human eye, meaning it does not absorb any wavelengths of visible light. Consequently, in contrast to the three solutions discussed above, it does not have an absorption peak in the visible region of the spectrum. But, under the UVA black light, it is blue! When hit with ultraviolet light, one of the ingredients in tonic water, quinine, absorbs an ultraviolet photon and emits a visible photon. Therefore, the solution appears colored when viewed under the black light.

It is evident from these examples that color is not an inherent quality of a substance, but instead, a result of the interaction of light with matter. If the wavelength of the light changes, then the interaction, and hence the resulting color, may also change.

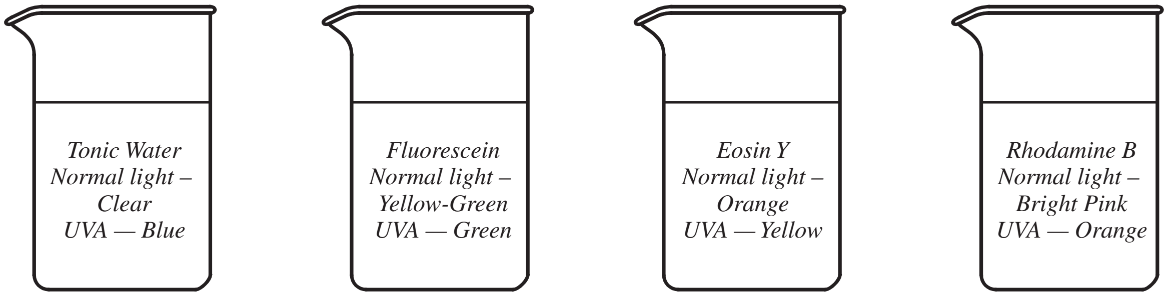
Connecting to the National Standards

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

Unifying Concepts and Processes: Grades K–12
Evidence, models, and explanation
Content Standards: Grades 5–8
Content Standard B: Physical Science, properties and changes of properties in matter, transfer of energy
Content Standards: Grades 9–12
Content Standard B: Physical Science, structure and properties of matter, interactions of energy and matter

Answers to Worksheet Questions

1. Draw the four beakers. Label each one with its contents, the color of the solution under normal, white light and the color of the solution under a black light.



2. The visible wavelengths of light for the human eye ranges from about 400 to 700 nm. UVA light (black light) transmits in a range from about 320 to 400 nm. Explain why we cannot see the light from a black light like we can light from a normal light.

Black lights transmit higher energy light than the light that is within our visible range, therefore we cannot see that particular kind of light. But often black lights will transmit wavelengths in the low 400s. These wavelengths appear violet to the human eye, giving black light its purple glow.

3. Fluorescence occurs when a substance absorbs a photon from a light source. The energy from that photon causes an electron to move to an “excited” state (higher energy level). If that electron returns to its ground state in a series of steps, rather than just one, it will release a photon with a different wavelength than the original. Explain how this relates to the “colorful glow” you see when a substance fluoresces.

The glow is caused by the energy that is released by the electron relaxing from a high energy level to a low energy level. If the photon that is released at this time has a wavelength that is within the visible spectrum, then we can see the colorful glow it causes.

Flinn Scientific—Teaching Chemistry™ eLearning Video Series

A video of the *Fluorescent Dyes* activity, presented by Irene Cesa, is available in *Fluorescence*, part of the Flinn Scientific—Teaching Chemistry eLearning Video Series.

Materials for *Fluorescent Dyes* are available from Flinn Scientific, Inc.

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

Catalog No.	Description
AP4848	Fluorescent Dye Kit—Chemical Demonstration Kit
AP9030	Ultraviolet Lamp, 18"
E0034	Eosin Y Solution, 1%, 100 mL
E0009	Ethyl Alcohol, 95%, 500 mL
F0043	Fluorescein, Reagent, 25 g
R0008	Rhodamine B, 25 g

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

Fluorescent Dyes Worksheet

Discussion Questions

1. Draw the four beakers. Label each one with its contents, the color of the solution under normal white light, and the color of the solution under a black light.

2. The visible spectra of wavelengths for the human eye ranges from about 400 to 700 nm. UVA light (black light) transmits in a range from about 320 to 400 nm. Explain why we cannot see the light from a black light like we can light from a normal light.

3. Fluorescence occurs when a substance absorbs a photon from a light source. The energy from that photon causes an electron to move to an "excited" state (higher energy level). If that electron returns to its ground state in a series of steps, rather than just one, it will release a photon with a different wavelength than the original. Explain how this relates to the "colorful glow" you see when a substance fluoresces.