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Fluorescent Dyes

A Colorful Demonstration

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" colors 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
Tonic water, 500 mL
Water, distilled or deionized, 1000 mL
Beakers, 600-mL, 4
Graduated cylinders, 10-mL, 3
Stirring rods, 3
Ultraviolet light source—black light

Safety Precautions

Ethyl alcohol is flammable and a dangerous fire risk. Addition of denaturant makes ethyl alcohol poisonous—it cannot be made nonpoisonous. Dye solutions will easily stain hands and clothing; avoid all contact with skin and clothing. 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.

Beaker 3: Add 5 mL of 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 1% rhodamine B solution to the fourth 600-mL beaker. 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 solution, eosin Y solution, and rhodamine B solution. 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 each of the solutions.
3. Turn off all the lights and completely darken the room. 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.

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

Discussion

The four solutions appear different colors under the normal classroom lights. The tonic water is clear, the fluorescein solution is yellow-green, the eosin Y solution is yellow-orange, and the rhodamine B solution is pinkish-red. Why? They are each composed of different molecules—molecules that absorb different wavelengths of light.

The fluorescein, eosin Y, and rhodamine B solutions appear colored to the human eye under the normal classroom lights. 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. When the classroom lights are turned off and the black light is shined on the solutions, they fluoresce. Under these conditions, the solutions are being hit with UVA light (320–400 nm). In each case, 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 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, the solution will appear to be two different colors under the two different light sources.

The tonic water, on the other hand, appears clear to the human eye under the normal classroom lights. Therefore, it must 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 in return. The human eye can see this visible photon, and therefore this solution appears to be 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, the interaction, and hence the resulting color, may also change.

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

Catalog No.	Description
E0034	Eosin Y Solution, 1%, 100 mL
E0009	Ethyl Alcohol Solution, 95%, 500 mL
F0043	Flourescein, 25 g
R0014	Rhodamine B Solution, 1%, 20 mL
AP9030	Ultraviolet Lamp, 18″
AP4848	Fluorescent Dye Kit

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

