Subject Areas: Chemistry, Physical Science, Numbers & Operations, Problem Solving

Associated Unit: Serial Dilution – the Solution to Pollution?

Activity Title: ACTIVITY II: Serial Dilution of a Fluorescent Compound

Grade Level: 6 (5–8)

Time Required: 30–45 minutes
**Group Size:** 4-5 students or demonstration performed by instructor for entire class

**Expendable Cost per Group:** < $1

**Summary:** In the second activity of this series, students continue to explore **serial dilution**, an important technique in physical science and engineering. In this case, they will use a fluorescent compound as the dye which they will track through a series of dilution steps. They will observe how the color intensity, or **saturation**, of each subsequent solution changes. They will also keep a running calculation of the **concentration** dye in each serial dilution. Finally, they will use a UV lamp to investigate whether the fluorescent dye can be detected after it disappears from view under normal lighting conditions. This activity is best performed after the investigation titled *Color Switching Molecules – the Magic of Fluorescence* (Matthew D. Cathell).

**Engineering Connection:** Engineers and scientists must often adjust the concentration of chemical solutions. For instance, an engineer might have a solution of 5 grams of polymer dissolved in 1 liter of solvent, but might need a solutions with concentrations of 1 g/L, 0.1 g/L, and 0.01 g/L. Using a technique called serial dilution, the engineer can easily and accurately produce these lower concentration solutions in a step-by-step fashion. Biomedical engineers use this technique when they have suspensions of cells (collections of cells floating in water or other liquids). By serially diluting these cell suspensions, engineers reduce the number of cells floating per unit volume of liquid.

**Keywords:** concentration, dilution, solutions, solute, solvent

**Educational Standards:**
Science:
Math:

**Learning Objectives:**
After this lesson, students should be able to:
- use serial dilution to make solutions of various concentrations from an initial solution
- answer the question: “If we can no longer see something, does it mean it’s not there anymore?”
- determine whether fluorescence is sensitive enough to detect the presence of a compound when it is at a concentration lower than the detectable limit in regular lighting
**Materials List:**
Each group needs:
- 2 larger plastic or glass container (~500 mL in volume)
- 4–8 smaller clear plastic or glass vials (15–50 mL in volume)
- pipette
- graduated cylinder
- water-soluble fluorescent dye, such as Atomic Glow dye from stevespanglerscience.com
- water

To share with the entire class:
- ultraviolet lamp

**Introduction / Motivation:**

Last time we investigated serial dilution, we used a food coloring dye. The dye was initially visible to our eyes, but became more and more faint as we diluted the solution, until it finally seemed to disappear.

But was it completely gone?

Today we investigate the serial dilution of a fluorescent dye. Fluorescent dyes are useful because they can be detected using UV light long after they become too dilute to observe in regular room lighting.

The critical question we wish to investigate today is this: “Is it possible that a dissolved material is still present in water, even after we can no longer detect it with our eyes under normal conditions?”

*See also Student Handout in Color Switching Molecules – the Magic of Fluorescence* (Matthew D. Cathell)

**Vocabulary / Definitions:**

<table>
<thead>
<tr>
<th>Word</th>
<th>Definition</th>
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<tbody>
<tr>
<td>concentration</td>
<td>The amount of a substance (solute) dissolved in a liquid (solvent) in a chemical solution</td>
</tr>
<tr>
<td>dilution</td>
<td>The process of reducing the concentration of unit solute per</td>
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unit solvent in a solution

dilution factor  The total number of units volume in which your sample will be dissolved. For instance, a dilution factor of 10 means we have combined 1 unit of our sample with 9 units of solvent (1+9=10).

fluorescent dye  A dye which emits visible light when excited with a higher energy light (often UV radiation)

Procedure:

Before the Activity:

Review the basics of solutions (solute dissolve in solvents to become solutions). Also, review PART I of this activity, in which students serially diluted food coloring dye until it seemed to disappear. In this PART II, students will investigate whether a fluorescent dye can be detected using a UV light source after it appears to disappear in normal room lighting.

It will likely be most practical to produce a bulk dye solution that will be used by the entire class. Plan to make about 10 mL for each group. If this step is done without the students, record the concentration of dye powder in the solvent and give this information to the class.

Students should be reminded that, of all the visible colors in the spectrum, violet light has the most energy and red light has the least. Fluorescence will always follow this pattern: higher energy light (absorbed light) → fluorescent molecule → lower energy light (emitted light). For instance, a particular molecule may absorb green light (higher energy) and fluoresce by emitting yellow light (lower energy).

Much of the procedure for this ACTIVITY II is shared with ACTIVITY I. The steps have been slightly changed and are repeated below.

The following steps are written with the assumption that a water-soluble dye is being used.
With the Students:

1. Divide the students into groups. Each group should get 2 larger containers to hold pure water (and rinse water) and 4–8 smaller containers for their solutions.

2. Each group should obtain a vial of dye solution. They should record the initial dye concentration in their notebooks, as well as any visible color they see.

3. Next, use a pipette to remove 1 mL of solution from the vial #1 and place it in vial #2. After rinsing the graduated cylinder, students should measure 9 mL of pure water and also place it in vial #2. They have just diluted the first solution by a dilution factor of ten (1 part dye solution to 9 parts pure water).

4. After gently swirling vial #2, students should observe the color of the liquid in vial #2 and compare it with the liquid in vial #1. They may notice a slight decrease in color intensity in the vial #2 (or they may not, depending upon how much dye the initially used).

5. At this point, the students should calculate the concentration of their new solution in vial #1, in units of g/mL.

6. Have the students continue with the serial dilution process. Each time they make a new solution, they will take 1 mL from the vial immediately proceeding the current vial (for instance, vial #3 will have 1 mL of solution from vial #2, not from vial #1). They will add 9 mL of pure water. Each serial dilution will reduce the concentration by a factor of 10. Have them continue to record a concentration and color comparison for each solution.

7. With each serial dilution, the concentration of dye goes down by a factor of 10.

8. When the students finally arrive at a solution that appears completely without color, they should note the concentration. At this low concentration level, the dye is no longer present in quantities large enough for their human eyes to see.

9. Now, have each group take their most dilute sample and place it under an ultraviolet light source. A vial of pure water (with no dye) should also be placed under the UV lamp to act as a control. The students should observe that the dilute dyed sample is still fluorescing, even though it has no perceivable color in normal room lighting.
10. Have the students continue diluting their solution until it no longer exhibits any noticeable fluorescence. Have them note the concentration, and compare it to the concentration when the dye was last visible in regular room light.

**Safety Issues:**

***SPECIAL NOTE IF ORGANIC-SOLUBLE FLUORESCENT DYES ARE USED*** Several commonly available fluorescent dyes, including fluorescein and Nile red, have poor solubility in water. These dyes work best in solvents like acetone. If organic-soluble dyes are used in this activity, it is more appropriate as a **demonstration** rather than an investigation performed by the students. Organic solvents can cause serious injury, particularly if splashed into the eyes. Even if students are not performing the experiment themselves, extreme care must be taken to ensure safety of both students and instructor. Strict adherence to safety eyewear is essential. Students should also be reminded to never taste any of the materials, and to promptly wash their hands after handling the chemicals.

Most battery-powered “black lights” do not emit dangerous amounts of ultraviolet light. However, more powerful high intensity UV lamps (for laboratory use) can cause eye injuries to students if they gaze directly into the light. Students should be instructed to **not look directly at the light source**, but rather, to simply shine the light on their liquid samples and observe the effects.

**Troubleshooting Tips:**

- In order to most accurately perform serial dilution, the pipette and graduated cylinder should be rinsed after each dilution cycle.

- It may be necessary to dim the room lights in order to best observe fluorescence.

**Assessment:**

- What is the basic idea behind fluorescence? Are all molecules fluorescent?

- When the solution is so dilute that it appears colorless, does this mean there is no longer any dye present in the water?

  **Answer:** No. Just because we can no longer see a substance with our eyes doesn’t mean it isn’t there, in some small quantity. We have confirmed this using a UV light to cause fluorescence of the dye compound after it seems to disappear from view in normal lighting.
Other:
A water soluble dye for this activity can be obtained at
http://www.stevespanglerscience.com/product/atomic-glow

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