INVESTIGATION

What Is the Relationship Between the Concentration of a Solution and the Amount of Transmitted Light Through the Solution?

CENTRAL CHALLENGE

You will be guided through an investigation to formulate your own answer to the question, "How can light be used to determine concentrations of chemical species in solutions?"

CONTEXT FOR THIS INVESTIGATION

Measuring how much of which wavelengths of light are absorbed by a substance, and getting useful information about that substance from the results, is the scientific discipline of spectroscopy. The visible spectrum is the only part of the electromagnetic spectrum that we can access with the equipment found in a typical school chemistry laboratory. The basic principles of spectral analysis that you learn in high school can also be applied to the more sophisticated instrumentation required to access the ultraviolet, infrared, and x-ray regions. What you learn by performing this lab will help you to understand more sophisticated instruments that you may encounter in a college or university.

In a visible spectrophotometer, we shine a beam of light into a solution containing the sample, and detect how much of it comes out of the other side of the solution. By comparing the amount of light transmitted by the pure solvent to the amount transmitted when the sample is dissolved in it, we can calculate a quantity called the *absorbance*. Spectrophotometers can report measurements as percent transmittance (%T) or directly as absorbance. In this investigation, you will be guided to discover the relationship between transmittance and concentration and, ultimately, the relationship between transmittance, absorbance, and concentration of solution.

PRELAB GUIDING QUESTIONS/SIMULATIONS

Your teacher will guide you through a series of questions to identify the relationship (if any) between the transmittance of light through a solution and the concentration of the solution.

Step 1: Your group will be given a known concentration or stock solution of the blue #1 dye (Brilliant Blue) molecule dissolved in water (see Figure 1). The blue #1 dye molecule is the only chemical species in solution. You will be measuring the percent transmittance of light through a solution containing only this chemical species.



Figure 1. FD&C blue #1 (Brilliant Blue) molecule

Step 2: Your teacher will tell you the appropriate wavelength at which to take transmittance measurements using the SPEC 20 (or other spectrophotometer/ colorimeter).

Step 3: Your teacher will assign your group to gather data as to the percent transmittance of the molecule in solution at various concentrations. Your group will need to make a specific dilution or set of dilutions according to this assignment. Circle and then make the dilution(s) to which you are assigned below:

Collect percent transmittance data for the following dilutions of stock/water:

10 mL stock/0 mL water

8 mL/2 mL

6 mL/4 mL

4 mL/6 mL

3 mL/7 mL

2 mL/8 mL

1 mL/9 mL

0 mL/10 mL

Step 4: Collect the percent transmittance values of your group's assigned diluted solutions using the SPEC 20 (or other spectrophotometer). Calculate the molar concentration of each of your assigned diluted solutions and record both pieces of

data in a data table in a central location in the lab. Use the original stock solution molarity and $M_1V_1 = M_2V_2$ to determine the concentrations of each of your dilutions.

Note: You will need to convert %T to a decimal and record that information in the data table as well.

Solution	Dilution Ratio mL stock/mL water	Molar Concentration (µM)	Measured percent transmittance	Measured %T in Decimal Form
1. (stock solution)	10 mL/0 mL			
2.	8 mL/2 mL			
3.	6 mL/4 mL			
4.	4 mL/6 mL			
5.	3 mL/7 mL			
6.	2 mL/8 mL			
7.	1 mL/9 mL			
8.	0 mL/10 mL			

Table 1

Step 5: Determine the relationship between transmittance and molarity of the solution by graphing the data in the data table. Plot transmittance (as a decimal) on the y-axis and concentration in μM on the x-axis. Discuss your proposed relationship between transmittance and molarity of the solution with other groups and with your teacher.

Step 6: Work with your group to make a series of plots to determine if there is a linear relationship (which has a positive slope and goes through zero) between transmittance and the molarity of the solution. Your teacher may assign you one type of plot to make from the following list:

- 1/T versus [dye]
- $1 \times 10^{\mathrm{T}}$ versus [dye]
- logT versus [dye]
- –logT versus [dye]

If there is a linear relationship between two values, that goes through zero and has a positive slope, then an equation can be constructed and the scientist can predict values of one variable given the other. Discuss with the other groups and with your teacher which treatment of the data (using the plots above) showed such a linear relationship between transmittance and concentration. Your teacher will further explain to you the relationship between transmittance, absorbance, and concentration.

Record that relationship in your lab notebook.

PREPARATION

Materials

Spectrophotometer SPEC 20, SPEC 200, or colorimeter	Cuvettes	Disposable pipettes
Stock solution of known concentration of blue dye #1 liquid food dye.	Blue-colored sports drink containing blue #1 dye	Distilled water
5–10 Test tubes (20 mL)	Graduated cylinders (10 mL)	Test tube rack

Safety and Disposal

The food dye and sports drink can be flushed down the sink with plenty of water.

INVESTIGATION

Many common sports drinks contain blue #1 dye. Use the relationship between transmittance, absorbance, and concentration (as well as your plot or calibration line and slope from your prelab) to determine the concentration of this dye in the sports drink.

Procedure

Obtain a sample of the blue-colored drink. Design a data-collection and dataanalysis procedure to determine the molarity or concentration of blue #1 dye in the sports drink.

Data Collection and Computation

- 1. Determine the molar concentration of blue #1 dye in the sports drink. Show all work.
- 2. Determine the mass of blue #1 dye found in 500 mL of the drink. Show all work.

Argumentation and Documentation

In the conclusion of your lab, justify the procedure you chose, the instrumentation you used, and the selection of the kind of data needed to determine the concentration of blue #1 dye in a blue-colored sports drink containing only this dye.

POSTLAB ASSESSMENT

 Suppose a solution was too concentrated for an accurate reading with the spectrophotometer. The concentrated solution was diluted by placing 1.00 mL of the concentrated solution in 4.00 mL of water. The solution was then placed in the spectrophotometer, an absorbance was obtained, and after a few calculations the molar



concentration was calculated to be 3.5×10^{-6} M. What was the concentration of the original stock solution before dilution?

- **2.** If a 0.10 *M* solution of a colored substance has a maximum absorbance at 500 nm and an absorbance of 0.26 at this wavelength, what will be the measured absorbance of a 0.20 *M* solution at 500 nm?
- **3.** The spectrophotometer really measures the percent of light that is transmitted through the solution. The instrument then converts %T (transmittance) into absorbance by using the equation you determined in the prelab section. If the absorbance of a sample is 0.85, what is the percent light transmitted through the colored sample at this collected wavelength?

SUPPLEMENTAL RESOURCE

Link

"USGS Spectroscopy Lab." United States Geological Survey. Accessed July 31, 2012. http://speclab.cr.usgs.gov/