

CHEM 142 Experiment: Quantitative Analysis of Blue Dye in Commercial Drinks Using Visible Spectroscopy

Introduction:

Spectroscopy is a technique that uses the interaction of energy with a sample to perform an analysis. The data that is obtained from spectroscopy is called a *spectrum*, which is basically a plot of the intensity of energy detected versus the wavelength (or frequency or wavenumber) of the energy. One might ask, “What information can be obtained from a spectrum?”

A spectrum can be used to obtain information about atomic and molecular energy levels, molecular geometries, chemical bonds, interactions of molecules, and related processes. Often, spectra are used to identify the components of a sample (qualitative analysis). Spectra may also be used to measure the amount of material in a sample (*quantitative analysis*).

Several instruments can be used to perform a spectroscopic analysis. In simplest terms, spectroscopy requires an energy source (such as lamp that gives off certain wavelengths of light, or a laser) and a device for measuring the change in the energy source after it has interacted with the sample (often a spectrophotometer or interferometer).¹ In this experiment, we will employ a specific type of spectrophotometer called single-beam UV/vis spectrophotometer, which can provide both ultraviolet (UV) and visible (vis) light. The idea is to shine visible light (wavelength range between 400 to 800 nm) from a *source*, split it into component wavelengths using a *monochromator* (mono means one; chromo means color), select a narrow band (or color) of wavelength through a slit, then pass the monochromatic light to a solution of a colored compound, such as FD&C Blue 1 dye (Figure 1). The intensity of light from the monochromator (I_0) is reduced as it passes through the sample solution (I) due to the presence of light-absorbing species, in this case the dye. The ratio between light intensities, I/I_0 , is related to a property of light called **absorbance, A**.

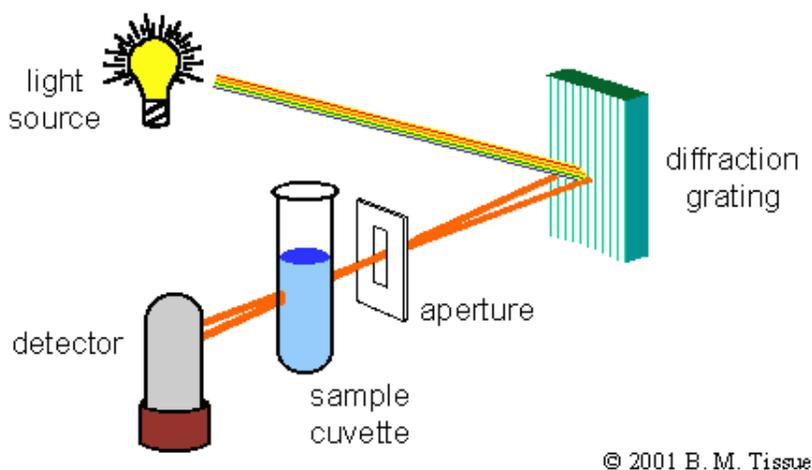


Figure 1. Schematic of a single-beam UV-Vis spectrophotometer. Image available at <http://www.files.chem.vt.edu/chem-ed/spec/uv-vis/singlebeam.html>

The amount of light absorbed by the dye, A , can be used to determine the amount of dye in the sample by comparing the absorbance data of the sample with those of carefully prepared solutions of blue dye of known concentrations called *standard solutions* using a mathematical relationship called **Beer-Lambert's Law**,

$$\text{Beer-Lambert's law: } \mathbf{A = \epsilon bc} \quad (1)$$

where A is the *absorbance*, c is the *concentration* of standard or sample solutions, b is the length at which light passes through (equal to the width of the cuvette or sample holder) and ϵ is a constant called *molar absorptivity* (or molar extinction coefficient). The value of ϵ depends on the wavelength used for analysis and the nature of species in solution.

Equation 1 tells us that a plot of absorbance, A , on the y -axis against concentration of standard solutions, c , on the x -axis will yield a straight line with a slope equal to (ϵb) . Such plot is called a **standard curve**, which is obtained by measuring the absorbance signal from a series of standard solutions of known concentration. The standard curve is then used to determine the concentration of dye (c_{unk}) in the sample solutions by locating the absorbance measurement of the unknown on the y -axis (A_{unk}) and following a line until it intersects the standard curve. The corresponding value on the x -axis is the concentration of substance in the unknown sample (Figure 2). This process is called linear interpolation.

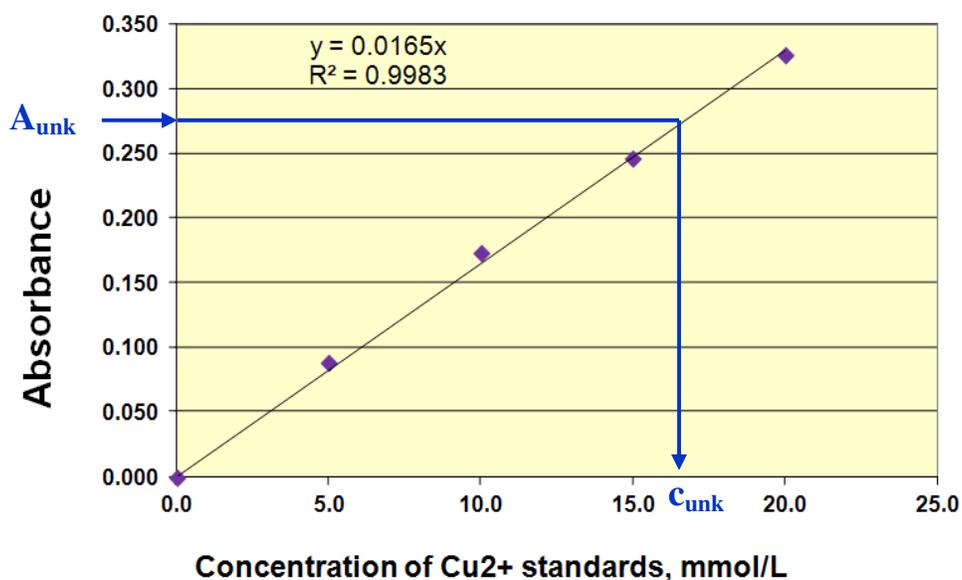


Figure 2. Example of a standard curve

The best-fitted (diagonal) line in the standard curve above is determined by a process called linear regression. During pre-lab discussion, your instructor will show you how to (1) generate a standard curve and (2) perform linear regression in Excel.

Objectives: (1) To prepare standard solutions of food dyes FD&C Blue 1 by dilution of stock solutions; (2) to generate a standard curve from absorbance of visible light data for standard solutions using a desktop spectrometer; and (3) to apply Beer's law on the absorbance data in order to determine the amount, or concentration, of blue dye in commercial drinks.

Materials:

- 5.00 x 10⁻³ M stock solution of FD&C Blue 1 (Use 0.101 g dye, MM 792.84, per 25 mL solution in water; this will be sufficient for 3 lab sections)
- 25-mL volumetric flask, 4 per group
- 100-mL volumetric flask, 1 per group
- 5.00-mL pipet, 1 per group
- P1000 autopipet, one per class
- Plastic centrifuge tubes
- Plastic cuvettes (for visible absorbance spectroscopy measurements)
- Lambda XLS+ spectrometer (one per group)
- Commercial drinks containing food dye FD&C Blue 1

Experimental:

A. Preparation of standard solutions of the blue dye FD&C Blue 1 by dilution

1. Using a P1000 autopipet, measure 1.000 mL of the stock solution of FD&C Blue 1.
2. Transfer into a 100-mL volumetric flask. Add water up to the 100-mL mark. Cap and shake well. This will be your new stock blue dye solution.
3. Calculate the molarity (concentration) of your new blue dye stock solution following equation 2 below and enter your result next to Table 1, *Data and Results* section.

$$\text{Molarity of new stock} = \frac{\text{Molarity of original stock solution} \times \text{Volume of original stock taken}}{\text{Total volume of solution}}$$

$$M_{\text{blue dye stock}} = \frac{(5.00 \times 10^{-3} \text{ M})(1.000 \text{ mL})}{100.0 \text{ mL}} \quad (2)$$

4. You will be preparing four dilutions of the new blue dye stock solution into separate 25-mL volumetric flasks by following steps (a) through (c) below.
 - (a) Using a 5.00-mL pipet, measure 1.00 mL of the new blue dye stock and transfer into a 25-mL volumetric flask. Dilute with water to the 25-mL mark using a transfer pipet. Cap and shake well. Transfer contents into a plastic tube and label as Standard 1. Calculate the molarity of Standard 1 following equation 3 and using the newly-calculated molarity of your new stock from Table 1. Enter your result in Table 1. Since you took 1.00 mL of the stock and diluted it to a final volume of 25.00-mL, this is called a *1:25 dilution*.

$$M_{\text{std 1}} = \frac{M_{\text{blue dye stock}} \times 1.000 \text{ mL}}{25.00 \text{ mL}} \quad (3)$$

- (b) Repeat 4(a) using the same pipet and stock solution, but this time measuring 2.00 mL of the stock into another 25-mL volumetric flask. Dilute to the line as before with water. This is called a *2:25 dilution*. Cap and shake well. Label as Standard 2. Calculate the molarity of Standard 2 following equation 3, but using the 2.00 mL volume of stock solution. Enter result in Table 1.
- (c) Repeat the procedure above to prepare Standards 3 and 4 using 3.00 mL and 5.00 mL volumes (*3:25* and *5:25* or *1:5 dilutions*, respectively), respectively, of stock solution, and two clean 25-mL volumetric flasks. Calculate the molarity of Standards 3 and 4 using equation 3 and the appropriate volume of stock solution used for each standard. Enter results in Table 1.

B. Preparing a standard curve from absorbance measurements of standard solutions

1. Use transfer pipets to fill plastic cuvettes up to the line with each of the standard solutions. Use the marks on the cuvette tray to remember which solution is in what cuvette. Fill another cuvette with deionized water. This will serve as your reference or “blank” solution, meaning one without the blue dye.
2. Following the instructions on the use of Lambda XLS+ spectrometer, set the wavelength to 630 nm, then adjust the absorbance of the blank solution to zero. This process is called “zeroing the blank”.
3. Replace the blank with Standard 1 and measure its absorbance at 630 nm. Record your absorbance data in Table 1.
4. Repeat step 3 until all the standards’ absorbance has been measured and recorded in Table 1. You will use this data to construct a standard curve after the experiment.

C. Quantitative analysis of blue dye in commercial drinks

1. Measure the absorbance of your commercial drink samples at 630 nm following the same procedure for standard solutions.
2. Complete Table 2 in the *Data and Results* section.
3. Using your standard curve (don’t forget to include the line equation in your graph) calculate the molar concentration of blue dye in your commercial samples.

Reference:

1. Helmenstine, Anne Marie, About.com, “Spectroscopy Introduction: Introduction to Spectroscopy and Types of Spectroscopy” available at <http://chemistry.about.com/od/analyticalchemistry/a/spectroscopy.htm>

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Partner _____

Data and Results

Table 1. Molar concentration and absorbance of various dilutions of blue dye

Molarity of new blue dye stock solution: _____ mol/L

Standard number	Molar concentration ($M_{\text{std } x}$), mol/L	Absorbance at 630 nm
1		
2		
3		
4		

Table 2. Absorbance data for commercial drinks

Sample (Brand Name)	Absorbance at 630 nm	Molar concentration of blue dye, mol/L

Construct a standard curve in Excel using data in Table 1, making sure you include the following.

- Label the x -axis “Concentration of Blue Dye Standards, mol/L”
- Label the y -axis “Absorbance at 630 nm”
- Use the graph title “Standard Curve for Blue Dye”
- Perform a linear regression (set the intercept to zero) and include the line equation in your graph.
- Using the line equation and the measured Absorbance (y), calculate the molar concentration (x) of blue dye in each of your commercial samples. Complete Table 2 above.

Include one sample calculation of molar concentration below:

Sample calculation:

Rank the drinks from lowest to highest blue dye concentration:

Ranking:

Attach your Standard Curve to this Data sheet.

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Postlab Assignments

1. Using your own standard curve, the line equation you generated and Beer's law equation (Equation 1) calculate the molar absorptivity (ϵ) of blue dye. For all your measurements, the size of the cuvette used is 1.00 cm. Use this as the value of b in Equation 1.

2. The absorbance (A) of light is related to the amount of light transmitted through the equation:

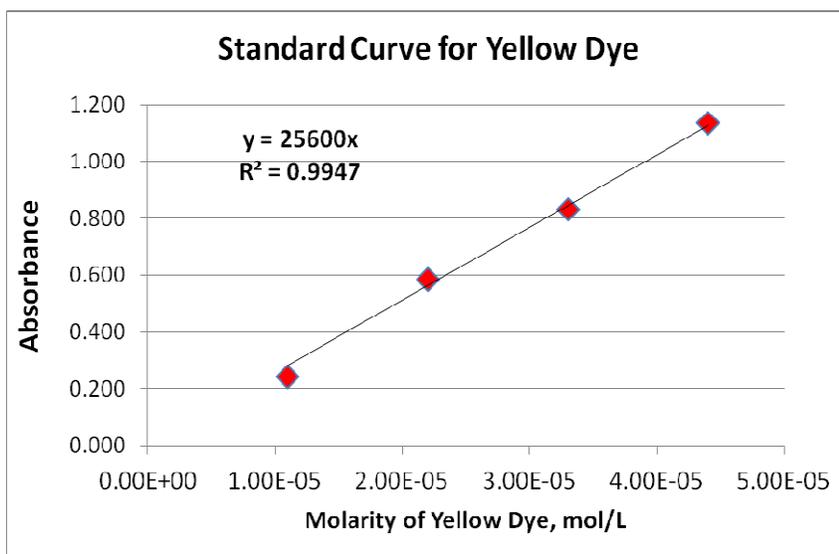
$$A = -\log T \quad \text{or} \quad T = 10^{-A} \quad (4)$$

The standard curve below is generated after analyzing a series of standard solutions of FD&C Yellow dye at a certain wavelength.

(a) If a commercial "vitamin" water sample containing yellow dye has a percent transmittance, % T , of 43, what is the molar concentration of the yellow dye in the vitamin water? [Hint: You have to use equation 4 first, then Beer's law (equation 1)]

(b) If another commercial drink has a transmittance of 73% at the same wavelength, how much yellow dye does it contain?

Show one sample calculation below:



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Prelab assignment

1. Using the line equation $y = 0.0165x$ in Figure 2, where y = absorbance and x = concentration in mmol/L, calculate the concentration of Cu^{2+} in a sample whose absorbance is 0.275. Show your work below and include the correct unit for concentration.

Use en.wikipedia.org to answer questions 2-3.

2. What does the acronym FD&C in FD&C Blue #1 represent?
3. Identify five commercial uses of FD&C Blue 1 other than its use in drinks.

Go to <http://www.fda.gov/food/foodingredientspackaging/ucm094211.htm> and answer Questions 4-6 below.

4. What is the FDA definition of “color additive”?
5. Give an example of colors that are “exempt” from FDA certification.
6. What is meant by FDA “certified colors”? What are the nine certified color additives approved for use in the United States?