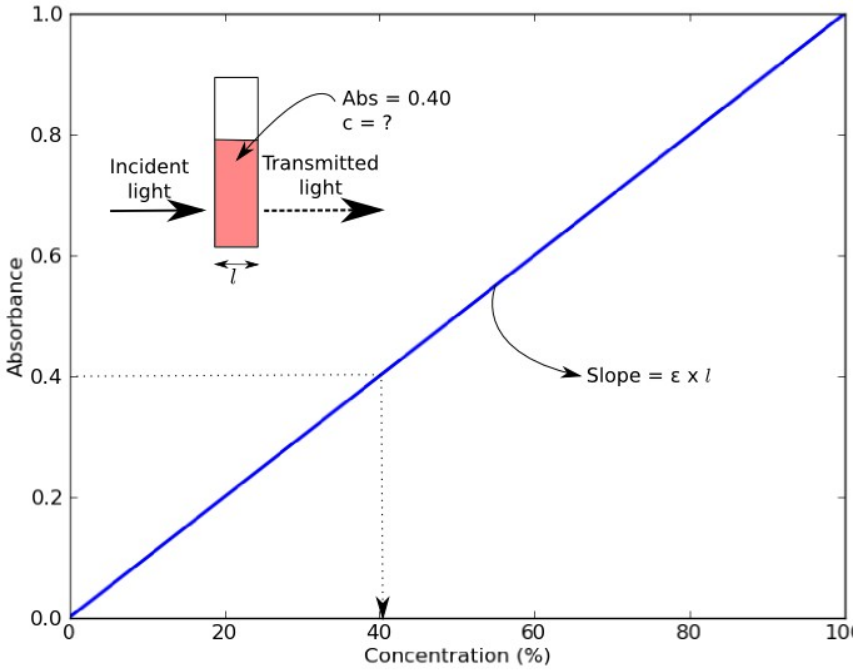
Beer's Law and Molar Extinction Coefficient

Background and Objectives

Colorimeters (and spectrophotometers) measure absorbance of light of a specific wavelength by a solution. Absorbance values can be used to determine the concentration of a chemical or biological molecule in a solution using the **Beer-Lambert Law** (also known as Beer's Law). Beer's Law states that absorbance of a sample depends on the molar concentration (c), light path length in centimeters (l), and molar extinction coefficient (ε) for the dissolved substance at the specified wavelength (λ)[1].

Beer-Lambert Law: $$Abs = \varepsilon\, c\, l$$

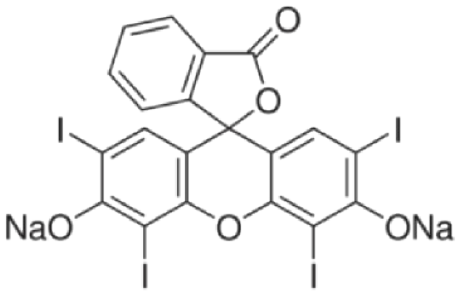
An example of a Beer's Law plot (concentration *versus* absorbance) is shown below. The slope of the graph (absorbance over concentration, Abs/c) equals the molar absorptivity coefficient, ε x l. The **objective** of this lab is to calculate the molar extinction coefficients of three different dyes from their Beer's Law plot.



1 **Path length** (distance that light travels through the solution) is determined by the cuvette that the sample is placed in. Most colorimeters and spectrophotometers, including the one in this kit, use cuvettes with a path length of 1 cm. **Molar extinction coefficient** is a measure of how strongly a substance absorbs light at a particular wavelength, and is usually represented by the unit $M^{-1}\ cm^{-1}$ or $L\ mol^{-1}\ cm^{-1}$.

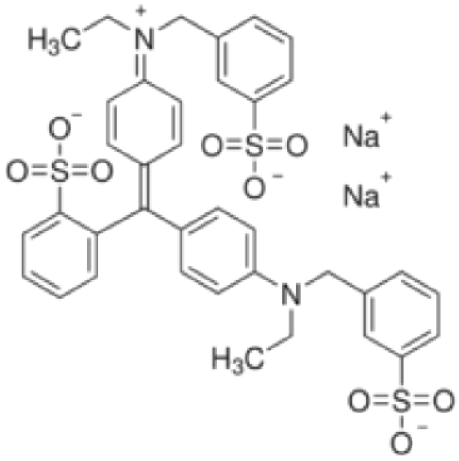
Food dyes are used to color a variety of food products such as sweets, cereal and sports drinks and are often used in high school and undergraduate labs[2]. The 3 dyes used in this lab were chosen as they absorb in the range of the colorimeter LED wavelengths.

Erythrosin B



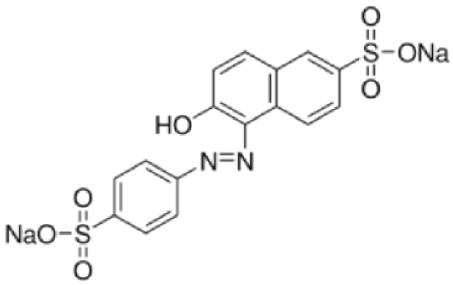
FD&C Dye: Red #5
Sigma cat # 198269
Wavelength: 522 nm
Mol. Wt: 879.86

Erioglaucine



FD&C Dye: Blue #1
Sigma cat # 861146
Wavelength: 628 nm
Mol. Wt: 792.85

Sunset Yellow



FD&C Dye: Yellow #6
Sigma cat # 465224
Wavelength: 482 nm
Mol. Wt: 452.37

2 For example: Sigman and Wheeler 2004, J. Chemical Education **81** (10): 1475-1478; Henary and Russell, 2007, J. Chemical Education **84** (3) 480-482.

Materials

The following list of materials is required for this lab. More details can be found in the Appendix (online).

- Assembled Educational Colorimeter kit from Lab 1
- Powdered food dyes erythrosin B, erioglaucine and sunset yellow
- Analytical scale
- 3 x 250 mL volumetric flasks
- 15 x test tubes (>5 mL)
- 1 mL fixed volume pipette
- 16 x cuvettes
- Water

Methods

This lab uses the Educational Colorimeter Plotting program. Before starting the lab, download the software and review the operation of this program (details online and in your User's Manual).

Step 1: Prepare 1 mM stock of dyes

- Erythrosin B (FW: 879.86): e.g. 0.218 g in 250 mL distilled water
- Erioglaucine (FW: 792.85): e.g. 0.198 g in 250 mL distilled water
- Sunset Yellow (FW:452.37): e.g. 0.113 g in 250 mL distilled water

Step 2: Preparation of standard curve

1. Dilute the 1 mM stock solutions as shown in Table 1 using a 250 mL volumetric flask. Label these flasks **working stock**;
2. For each of the 3 dyes, prepare a series of standard curve dilutions as shown in Table 2 using the test tubes. Label tubes #1-5 for each dye;

Step 3: Measure absorbance with the colorimeter and plot data

1. Launch the colorimeter plotting program. Calibrate the device with a cuvette containing water as described in Lab 1.
2. Starting with erythrosin B, measure the absorbance for each standard curve solution with the appropriate color channel[3], and enter the corresponding concentration in the program;
3. Once all the samples are measured, click on the "Plot" button. Repeat measurements for erioglaucine and sunset yellow. Record values for the slope in Table 3.

3 To determine which color channel to use, measure absorbance at all 3 wavelengths as described in Lab 1.

Table 1: Preparation of working solutions

Dye	Volume of 1 mM stock	Concentration of working stock	Color channel/ wavelength
Erythrosin B	1 mL in 250 mL	4.00 µM	**Green/528 nm**
Erioglaucine	2.5 mL in 250 mL	10.00 µM	**Red/625 nm**
Sunset Yellow	10 mL in 250 mL	40.00 µM	**Blue/470 nm**

Table 2: Preparation of standard curves

Tube #	Volume of working stock	Erythrosin B	Erioglaucine	Sunset Yellow
1	1 mL + 4 mL H_2O	0.8 µM	2 µM	8 µM
2	2 mL + 3 mL H_2O	1.6 µM	4 µM	16 µM
3	3 mL + 2 mL H_2O	2.4 µM	6 µM	24 µM
4	4 mL + 1 mL H_2O	3.2 µM	8 µM	32 µM
5	5 mL + 0 mL H_2O	4 µM	10 µM	40 µM

Table 3: Molar extinction coefficient

	Plotted Slope (µM vs. Abs)	**Molar extinction coefficient** (M^{-1} cm^{-1})	**Reported value** (Sigma spec sheets)
Erythrosin B	0.056	56,000 at 528 nm	$\geq$ 82,500 (524-528 nm)
Erioglaucine	0.098	98,000 at 625 nm	$\geq$ 80,000 (627-637 nm)
Sunset Yellow	0.020	20,000 at 470 nm	$\geq$ 20,000 (479-485 nm)

Sample Data

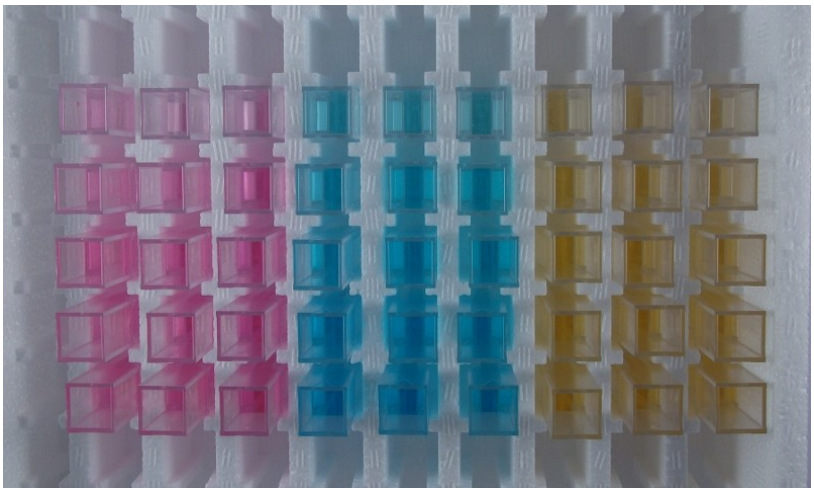


Fig 1: Image of cuvettes with 3 different food dye standard curves

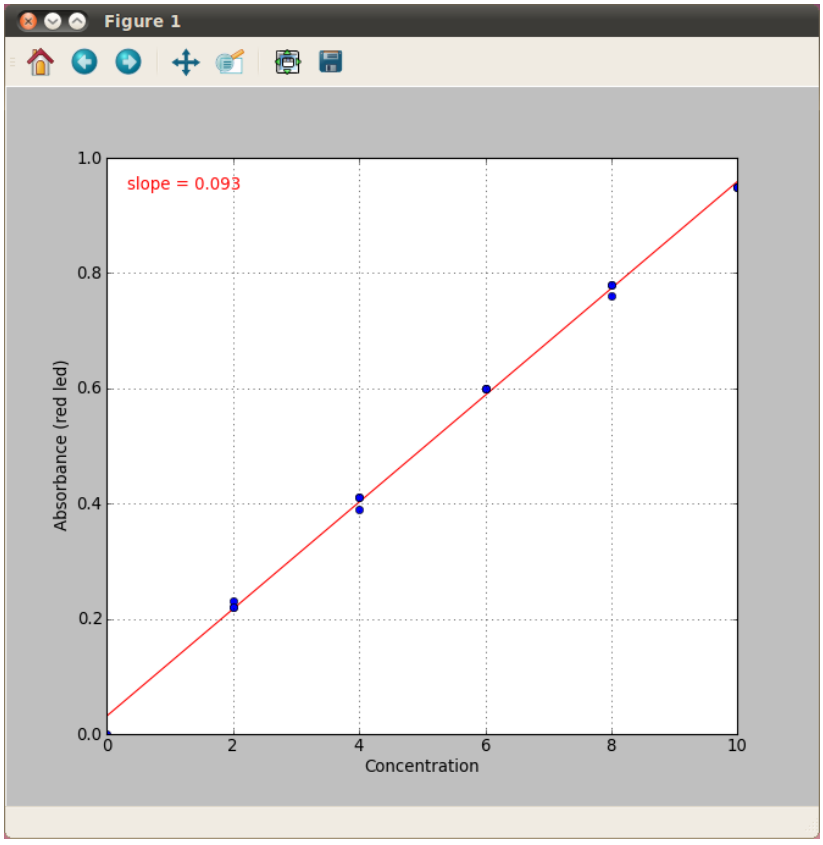


Fig 2: Sample data - Erioglaucine standard curve