Introduction to Spectrophotometry: Creating a Beer's Law Calibration Curve for FCF Brilliant Blue

Experimental Procedures

Part I. Building a spectrophotometer using your phone as a detector.

A spectrophotometer is a device that measures the amount of light for a particular range of wavelengths. Spectrophotometers are widely used to determine the concentration of unknown samples. This is done by shining light through samples containing colored compounds. Colored compounds have their color because they absorb some of the wavelengths (colors) of light better than others. For instance, a colored solution that is blue, appears this way because when all the colors of the visible spectrum are shined on it a lot of the light that gets through is blue and the other colors were mostly or completely absorbed by the solution. The visible spectrum is sometimes represented as three blocks Red (wavelength 600 nm-700 nm) Green (wavelength 500 nm-600 nm) and Blue (wavelength 400 nm-500 nm). This way of representing it is often associated with the abbreviation RGB. So, in RGB terms a blue solution absorbs all or most of the green and red light and transmits (allows to pass through) a substantial amount, but not all of, the blue light.

In this first part of the experiment you will construct a spectrophotometer using pieces of Lego. The source of light will be the LED flashlight in your kit. It puts out approximately equal amount of RGB light, that is why the light from it appears uncolored to our eye. The light from the flashlight that is able to reach the sample will be determined by the light filter you place in the light path. For this experiment you will use a light filter that allows light in the range from 410-440 nm. This is all in the blue region of the visible spectrum. The amount of selected light that gets through the sample will be measured using the camera of your phone. You will need to **download the free app Color Assist from the App store** if you have an iPhone or if you have an android phone you will need to **download Color Grab (color detection) from the Google play store**. In Both apps go into setting choose to display RGB values.

Part I. Constructing the Spectrophotometer

Watch the instructional videos on constructing the Lego spectrophotometer and using it to make measurements. Note: the cuvettes you will use are the small rectangular containers not the round cuvettes shown in the video. Also, instead of placing double stick tape on both edges of the Lego that hold the cuvette only place the double stick tape on one side.

https://www.youtube.com/watch?v=uJvw9VWPyOg&t=7s

https://www.youtube.com/watch?v=YtYRBvQso9I&t=397s

iPhone users only

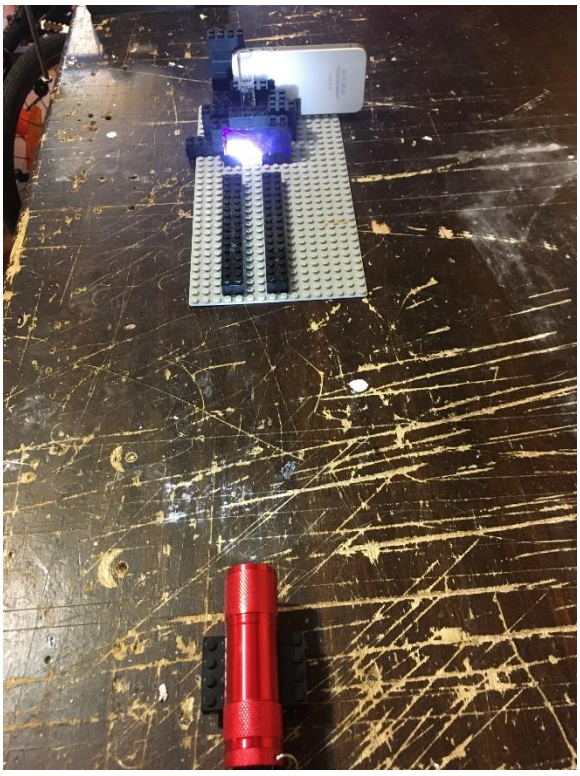
https://www.youtube.com/watch?v=yLchyiYgbLw&t=108s

Android users only

https://www.youtube.com/watch?v=BI93aZHSZ7s

https://www.youtube.com/watch?v=b7gCI5WHW-0&t=110s

Figure 1. Lego spectrophotometer.



Part II. Preparing dilutions of McCormick Blue Food Coloring and determining their absorbance

1. Using a dropper add 20 drops of McCormick Food coloring to the 100 mL volumetric flask.

2. Fill the test up with water close to the fill line on the neck of the flask. Use a dropper to carefully add water so that the meniscus is just at the level of the line.

3. Place the lid on the flask and gently invert it several times to mix the components.

4. Pour this solution into the mixing container or a Dixie cup so you will be able to use a dropper to access it.

5. This solution you have prepared appears blue because of the presence of a compound known as Brilliant Blue FCF. The concentration of the solution you have prepared is 10 mM (1.0×10^{-2} M). Label this solution as 10 mM *Brilliant Blue FCF Stock Solution*.

6. Label ten 15 mL screw lid test tubes #1-10 using your permanent magic marker.

7. Label 11 cuvettes #1-10 using your permanent magic marker. Label the 11^{th} cuvette *Blank*. Write in the area close to the top of the cuvette. Note the cuvettes are the smaller tubes with no markings on them.

8. Prepare 10 solutions with varying concentration of Brilliant Blue FCF by mixing each of the volumes of the Brilliant Blue FCF Stock Solution and water shown below into a 15 mL screw lid test tube. The total volume of solution in all trials (V_{dil}) is 5.0 mL, so when adding the water it is not necessary to measure it before adding, just carefully add water until the total volume is 5.0 mL using the marks on the tube. Do this using a dropper. Once prepared, transfer each solution into the cuvette labeled with its corresponding Trial #.

Trial #1 – 0.50 mL (13 drops) Brilliant Blue FCF Stock Solution + 4.5 mL Water

Trial #2 – 1.0 mL (26 drops) Brilliant Blue FCF Stock Solution + 4.0 mL Water

Trial #3 – 1.5 mL (39 drops) Brilliant Blue FCF Stock Solution + 3.5 mL Water

Trial #4 – 2.0 mL Brilliant Blue FCF Stock Solution + 3.0 mL Water

Trial #5 – 2.5 mL mL Brilliant Blue FCF Stock Solution + 2.5 mL Water

Trial #6 – 3.0 mL Brilliant Blue FCF Stock Solution + 2.0 mL Water 4.5 mL Water

Trial #7 – 3.5 mL Brilliant Blue FCF Stock Solution + 1.5 mL Water

Trial #8 – 4.0 mL Brilliant Blue FCF Stock Solution + 1.0 mL Water

Trial #9 – 4.5 mL Brilliant Blue FCF Stock Solution + 0.5 mL Water

Trial #10 – 5.0 mL Brilliant Blue FCF Stock Solution

Blank – 5.0 mL of water

9. Calculate the millimolarity (mM) of each of the solutions prepared. V_{dil} = 5.0 mL for all trials. V_{conc} is the volume of the Brilliant Blue FCF added to each test tube. The dilution factor $\frac{V_{conc}}{V_{dil}}$ has no units so V_{conc} and V_{dil} can be plugged in using whatever units are most convenient, milliliters in this case.

$$mM_{dil} = \frac{V_{conc}}{V_{dil}} \times mM_{conc}$$

   Enter this data into both Table 1 and Table 2 and show a sample calculation in the designated area.

10. Prepare an unknown solution by taking your mixing container (screw lid container with blue cap). Filling about half full with tap water (don't read the markings on the container at all) and adding 5 drops of McCormick Blue Food Coloring. Tighten the lid on the container gently invert several times to mix.

11. Fill up a cuvette with this unknown solution and label it Unknown using your permanent magic marker. Write in the area close to the top of the cuvette.

12. Place the cuvette containing 4 mL of water that you used to align the spectrophotometer into the cuvette holder.

13. Record the B value reported by color assist in the space below the data table (or a spreadsheet) as *Relative blue light intensity for the Blank trial (I_o)*. This is theoretically

the maximum amount of light we expect could get through any sample we use with a similar cuvette using water as the solvent.

14. Place cuvette for trial #1 into the cuvette holder and record the B value reported as *Relative Blue Light Intensity (I) for trial #1*. Repeat this step for the cuvettes containing trials #2-10 and the unknown you prepared in step 11.

15. Calculate the transmittance (T) for trials #1-#10 and the unknown sample using the relationship

$$T = \frac{I}{I_o}$$

16. Calculate the absorbance (A) for trials #1-#10 and the unknown sample using the relationship

$$A = -\log (T)$$

17. On one graph plot both Transmittance and Absorbance for trials #1-#10 (not the unknown) on the y axis and concentration on the x axis. Label the y axis *Transmittance and Absorbance*. Note: both absorbance and transmittance are dimensionless quantities---they have no units.

18. Interpret the trend in both sets of data by constructing a straight line or curve that you think the data would follow if there was no random error in the experiment. This line should follow the overall profile of the data and have about half of the data points above it and about half below it. To construct a straight line use your ruler as a straight edge laying it flat on the page. To construct a curved line, place the edge of the ruler on the surface of the page and bend it to follow the trend in the data.

19. Find the location on the y axis that corresponds to the absorbance you calculated for the unknown. Using a ruler and pencil draw a horizontal line from this point on the y axis to the point where it intersects the line you constructed for the trend in the absorbance data. Draw a vertical line from this point of intersection down to the x axis. Read the point this line intersects the x axis, treating the axis like a measurement device to determine how many significant figures to record.

20. Record the value where the horizontal line crossed the x axis as the *Concentration* for the unknown sample in the data table.

Table 1. Data for Brilliant Blue FCF solutions prepared in trials #1 - #10 and unknown. Concentration for trials#1 - #10 calculated from the dilution formula. Concentration for the unknown determined graphically (described in the procedure).

Trial #	Volume Brilliant Blue FCF Stock (mL)	Total volume (mL)	Concentration (mM)
1			
2			
3			
4			
5			
6			
7			
8			
9			
10			

Table 2. Concentration and Spectrophotometric data for Brilliant Blue FCF solutions prepared in trials #1 - #10 and the unknown.

Trial #	Concentration (mM)	Relative blue light intensity (I)	Transmittance ($T = I/I_o$)	Absorbance ($A = -\log(I/I_o)$)
1				
2				
3				
4				
5				
6				
7				
8				
9				
10				
Unknown				

Relative blue light intensity for the *Blank* trial (I_o) _____

Sample Calculations

1) Concentration of Brilliant Blue FCF in Trial #1

2) Transmittance for Trial #1

3) Absorbance for Trial #1

Transmittance and Absorbance Curves for FCF Brilliant Blue Dissolved in Water