EXPERIMENT 7

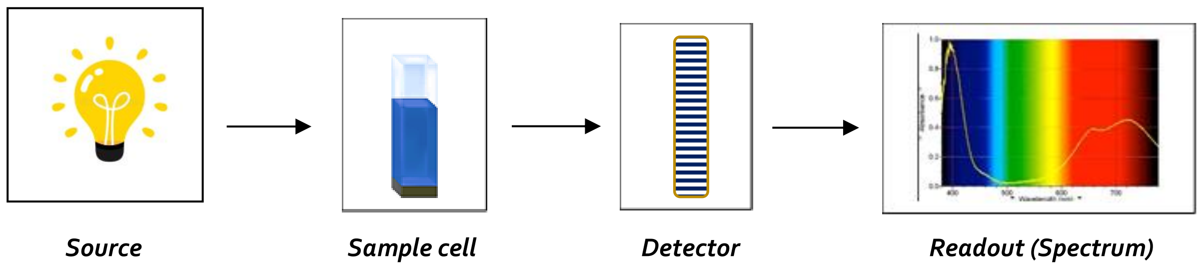
SPECTROPHOTOMETRY

Objectives:

This experiment will investigate the analytical technique spectrophotometry using commercial food dyes. The relationship between absorbance of a solution and the concentration of the analyte in the Beer-Lambert Law will be qualitatively and quantitatively investigated.

Background:

A spectrophotometer is an instrument used to measure the amount of light transmitted through a solution containing an **analyte**. The analyte is simply the chemical species being analyzed in the solution. In this case, the analyte is food dyes which are easily dissolved in the solvent (water) and readily interacts with light. During the analysis, light of a known wavelength is sent through a sample and the light coming through is measured by a detector.



- *Source* - light bulb that emits light over a range of wavelengths.
- *Sample cell* – Sample holder (cuvet) through which light passes
- *Detector* – Phototube sensitive to light
- *Readout* – Indicator of the quantity of light reaching the detector

The quantity of light reaching the detector is compared to the quantity of light originating from the source. The specific quantities of use are transmittance, T, percent transmittance, %T, and absorbance.

- **Transmittance**, *T*, is the ratio of the intensity of light reaching the detector, I, to the intensity of light from the source, I_o. This is a detected quantity. **Percent transmittance**, *%T*, is the percentage of source light intensity reaching the detector.

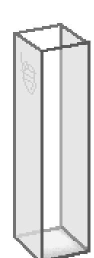
$$T = \frac{I}{I_o} \qquad and \qquad \%T = \frac{I}{I_o} \times 100\%$$

- **Absorbance**, A, is a measure of the light absorbed by the analyte and is calculated from the transmittance or percent transmittance.

$$A = -log_{10}(T) = 2 - log_{10}(\%T)$$

The Vernier SpectroVis spectrophotometer used in this procedure has only one detector therefore it is necessary to measure the intensity of light originating from the source prior to placing the sample in the sample cell. This process is known as **blanking the instrument**. The intensity of the light source and the sensitivity of the detector vary with wavelength and therefore must be measured. The blanking process will also take into account any scattering or absorption of light by the solvent and the sample holder. The SpectroVis measures the original signal at the detector at all wavelengths, records this information in memory and refers to it whenever making absorbance and %T readings of the analyte. It is common to blank the instrument using all components of the solution excluding the analyte. Typically, the blank contains only the solvent. This way, the transmittance of the solution reflects that of the analyte only.

Solutions investigated using the SpectroVis are placed in a sample holder called a **cuvet**. The cuvet used for this procedure is made of a disposable plastic and have an analysis width of 1.00 cm. It is important that no air bubbles are in the solution, no fingerprints or scratches are on the surface of the cuvet and that the outside of the cuvet be dry. All of these could lead to interference of the transmission of light through the sample.



Analyzing a sample by spectrophotometry results in an **absorption spectrum**. A spectrum is a plot relating the quantity of light absorbed by a sample to the wavelength of the light. It is created by scanning a sample through a range of wavelengths and measuring the intensity of light reaching the detector at each wavelength. An example spectrum is shown in Figure 1. The characteristic shape of a spectrum can be used to identify a compound. The peak in the spectrum represents the wavelengths of light that are being **absorbed** by the analyte. In this spectrum, wavelengths of light from 320 nm to 480 nm are being absorbed. The wavelength at the top of the peak is known as the **wavelength of maximum absorbance**, λ_{max}. In this spectrum, lambda max is 400 nm.

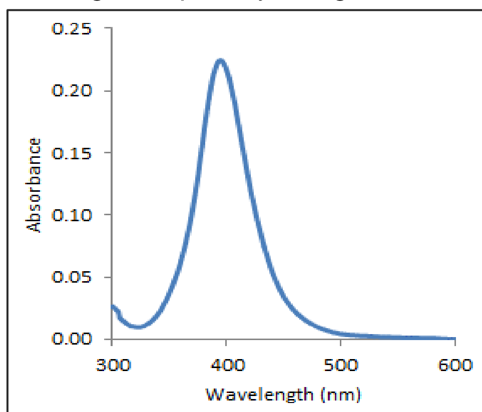


Figure 1: Sample absorption spectrum showing an absorption peak from 320-480 nm, lambda max at 400 nm.

The wavelengths not contained in the peak of the absorption spectrum are the wavelengths of light that are being **transmitted** through the sample solution. In the absorbance spectrum shown in Figure 1, wavelengths from 300 nm to 350 nm and 480 nm to 600 nm are being transmitted. **The colors of the transmitted wavelengths are responsible for the solution's color.** During this experiment, common food dyes will be used to investigate absorption spectra by identifying regions of absorption and transmittance and how the spectrum relates to the color of the dye solution.

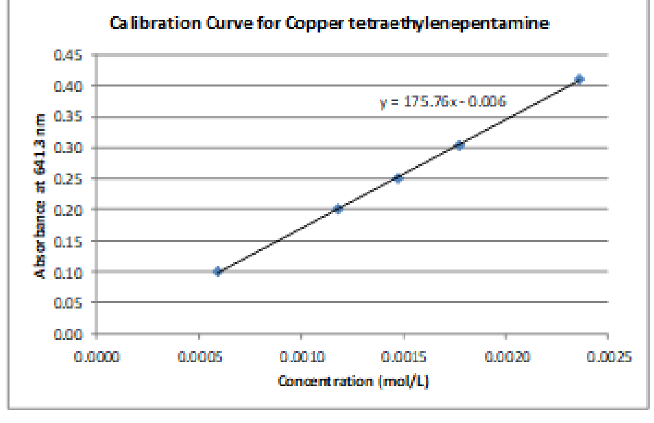
A spectrophotometer is routinely used to identify the concentration of a solution because the absorbance, A, of a solution at a given wavelength of light is directly proportional to the concentration of the analyte in solution according to the **Beer-Lambert Law**.

$$A = \varepsilon bc$$

A = measured absorbance
ε = molar absorptivity, L/(mol·cm) (unique characteristic of the analyte)
b = path length, cm (distance through the substance: 1.00 cm)
c = concentration, mol/L

The direct relationship between the absorbance and concentration is determined by measuring the absorbance of a solution of known concentration using a consistent experimental set-up. When a set of known concentration solutions, **standards**, are available a **calibration curve** can be constructed.

The calibration curve shown in Figure 2 was constructed by accurately preparing three standard solutions containing copper (II) tetraethylenepentaamine so the concentration can be accurately calculated then the absorbance of each standard solution is measured at the wavelength of maximum absorbance. The calibration curve was then prepared by plotting absorbance versus concentration (mol/L). A linear fit equation is used to determine the concentration of other solutions containing the same copper analyte by plugging the absorbance value for the unknown solution into y and solving for x.



A calibration curve can be prepared for any analyte. In this experiment a calibration curve for Yellow Dye #5 will be prepared by careful dilutions of a concentrated solution. The concentration of each standard will be calculated using the absorbance of the standard solutions and the molar absorptivity value for Yellow Dye #5. The equation for the linear fit of the calibration will be used to determine the concentration of Yellow Dye #5 in sports drink samples.

Tasks to be completed:

1. Prepare solutions of food dyes and collect an absorption spectrum of each using the SpectroVis spectrophotometer.
2. Prepare three dilutions of the yellow food dye solution and collect absorption spectra for each.
3. Calculate the concentration of each yellow dye dilution to construct a calibration curve in Excel.
4. Measure the absorption spectrum of an assigned sports drink sample and calculate the concentration of yellow dye in the sports drink.

Experimental Procedure: To be completed in groups of two students.

Set-up and Blank the Vernier Spectrophotometer

1. Start Logger Pro software on the computer.
2. Use the USB cable to connect a Vernier Spectrophotometer to the computer.
3. Blank the spectrophotometer. (Note: The LoggerPro software refers to the blanking process as *calibration*.)
   a. Prepare a **blank** by filling an empty cuvet ¾ full with deionized water, dry the outside with a Kimwipe. Verify that the cuvet is free of fingerprints, scratches, and air bubbles.
   b. Open the *Experiment* menu and select *Calibrate* → (*Spectrometer*). The following message appears in the *Calibrate dialog box: "Waiting … seconds for the device to warm up*." After 90 seconds, the message changes to: "*Warm up complete*."
   c. Place the blank in the cuvet holder of the spectrophotometer. If using a cuvet with frosted sides, align the cuvet so that the clear sides are in line with the light source of the spectrophotometer. Click "*Finish Calibration*", and then click OK.
   d. Place the cuvet of deionized water in the cuvet holder. It will be necessary to re-blank the instrument later in the procedure.

I. Obtain and Analyze the Absorption Spectra of Food Dyes:

1. Prepare samples of RED, BLUE AND YELLOW food dyes to analyze dye by dissolving 1 drop of a food dye in approximately 100 mL of deionized water. Use a separate beaker or Erlenmeyer flask for each solution and mix each solution well using a glass stir rod.

2. Obtain and analyze an absorption spectrum of each food dye sample by filling a cuvet ¾ full with a food dye solution and place it in the spectrophotometer. **Only ONE cuvet will be used for all food dye analysis.**

    a. Click ▶ Collect. A full spectrum graph of the food dye sample will be displayed. Click ■ Stop to complete the analysis. Hover the curser over the highest peak and record the wavelength on maximum absorbance on the data shet.

    b. Select, *Experiment* from the top menu then "Store Latest Run" to save the spectrum.

    c. Empty the cuvet and rinse with deionized water followed by two rinses with small amounts of the next food dye mixture to be analyzed. Repeat steps a through c to analyze all assigned food dyes.

    d. Right click on the graph and select *Graph Options* from the menu. Enter a meaningful title and select *Done*.

    e. In the Postlab, examine the spectrum. Note the regions of wavelengths in which the solution absorbs and wavelengths in which it transmits. Identify the regions by wavelength and corresponding color. How do these regions correspond to the visible color of the solution?

3. When finished analyzing all three dyes, prepare the graph and copy into a Word file to submit on-line for grading.

    a. Open Microsoft Word and orient the page in Landscape. Screenshot the LoggerPro plot and paste it into a Word Document.

    b. Add text boxes to label the red, yellow and blue spectra on the plot.

4. Open a new file in LoggerPro and prepare a sample of the GREEN food dye in the same manner by adding one drop of dye to 100 mL of deionized water.

    a. Follow the Set-up instructions to re-blank the spectrophotometer with deionized water.

    b. Acquire the absorption spectrum of green food dye in the same manner as above. Answer the appropriate questions on the Data/Report Sheet.

5. Screenshot and paste the absorption spectrum of green dye into the same Word document containing the absorption spectra of the red, yellow and blue dyes. Add a page break so each spectrum fills an entire page. Email a copy of the Word document to your lab partner. **Each partner in a group MUST submit their own graph.**

II. Prepare a Calibration Curve for Yellow Food Dye #5.

1. Prepare a buret with the yellow food dye solution used in Part I.

    a. Close the stopcock on a buret and add about 5 mL of the solution to the buret. Hold the buret horizontally and turn to completely coat the inside of the buret.

    b. Secure the buret in a buret clamp attached to a ring stand and open the stopcock to allow the rinse water to drain into a waste beaker.

    c. Close the stopcock and fill the buret with the yellow dye solution above the 10.00 mL marking using a supplied plastic funnel. Fully open the stopcock to allow about 5 mL of

the solution to rapidly run through the stopcock region to eliminate air trapped in the stopcock and stem. Re-fill the buret to the 10.00 mL marking.

2. Obtain a second buret. Rinse and fill with deionized water as above. Fill buret to the 10.00mL marking.

3. Prepare three dilutions by delivering the appropriate amount of dye solution or deionized water from the burets into clean, dry, 6” test tubes. Mix by covering the test tubes with Parafilm and inverting 5 times.

Trial #	Volume of Dye Solution	Volume of Deionized Water
Trial 1	10.00 mL	2.00 mL
Trial 2	6.00 mL	6.00 mL
Trial 3	2.00 mL	10.00 mL

4. Record Absorbance for each Trial at a wavelength near lambda max (427 nm).

   a. Open a new file in LoggerPro. Follow the Set-up instructions to re-blank the spectrophotometer with deionized water.

   b. Using the **SAME CUVET** as used in Part I, rinse the cuvet with deionized water from a wash bottle then a small portion of solution from Trial 1. Fill the cuvet ¾ full with Trial 1 solution and dry the outside of the cuvet. Place it in the spectrophotometer. Select ▶ Collect to obtain an absorption spectrum of the solution. After the spectrum appears select ■ Stop .

   c. Select the *Configure Spectrometer Data Collection* icon, , on the toolbar

   d. Select the *Abs vs Concentration* collection mode button.

   e. Verify that the wavelength selected is near 427 nm. You can click on other wavelength choices to select the wavelength closest to the analysis wavelength if necessary. Click OK to proceed. Select “No” in the dialog box that appears concerning the storing of the latest run.

   f. The absorbance of the Trial 1 should now appear in a box in the bottom left corner of the screen along with the analysis wavelength. Record both the wavelength (nm) and absorbance of Trial 1 in the appropriate space on the data sheet.

   g. Empty the cuvet into a waste beaker. Rinse the cuvet with deionized water and a small portion of solution from Trial 2. Place the cuvet into the spectrophotometer and record the new absorbance reading from the data box at the bottom of the page. THERE IS NO NEED TO PRESS THE COLLECT BUTTON- THE SPECTROPHOTOMER IS ACTIVE AND WILL DISPLAY THE NEW ABSORBANCE READING FOR THE TRIAL 2 SOLUTION IN THE DATA BOX.

   h. Repeat step g. for Trial 3.

   i. Using Excel, create a calibration curve. Use the linear trendline equation to determine the concentration of yellow dye in a sports drink sample.

III. Obtain absorbance spectrum of a Sports Drink Sample

1. Open a new file in LoggerPro. Re-blank the spectrophotometer with deionized water.
2. Bring a waste beaker and the cuvet used in Parts I and II to the reagent table. Rinse the cuvet with a portion of an ASSIGNED sports drink. Dry the outside of the cuvet then collect an absorption spectrum. Click stop when complete. Autoscale, if necessary, using the icon or choose Autoscale in the View menu.
3. Scroll through the data in the chart to the left and find the wavelength and closest to the Yellow Dye analysis wavelength used to collect data in Part II. Record the wavelength and absorbance value on the appropriate data sheet.
4. Scroll the data and find the wavelength closest to 631 nm. This is Lambda max for Blue food dye. Record the wavelength and absorbance on the data sheet.

6. When finished analyzing the spectrum, right click on the graph and select *Graph Options* from the menu. Enter a meaningful title and select *Done*. Screenshot and submit on-line for grading. Email a copy to your lab partner. **Each partner in a group MUST submit their own graph.**

Waste Handling and Clean Up:

- All liquids can be safely disposed down the drain.
- Rinse burets well with deionized water. Dry outside and leave at work space for instructor to inspect. Place buret clamp in the blue bin under the sink.
- Wash the plastic funnel and cap with soap and water then rinse with deionized water.
- Dispose of emptied cuvets in the trash receptacle.
- Unplug cord from the Vernier spectrophotometer and leave at the bench. DO NOT wrap cord around the spectrophotometer!
- Wipe down benchtop area, including sink area, with a damp sponge.

Data Analysis:

Determine molarity of the Yellow Dye #5 solutions.

Calculate the molarity Trial solution 1-3 using the Beer-Lambert Law. Molar absorptivity, ε, of Yellow Dye #5 is 2.73 x 10^4 L/mol·cm at 427 nm. Pathlength, dimension of the cuvet, is 1.00 cm.

$$A = \varepsilon bc \quad or \quad c = \frac{A}{\varepsilon b}$$

$$Molarity, mol/L = \frac{absorbance}{(2.73\ x\ 10^4 \frac{L}{mol \cdot cm})(1.00\ cm)}$$

Create a calibration curve in Excel for Yellow Dye #5.

1. Open a new file in Microsoft Excel. In Column A enter the concentration (mol/L) of each trial solution. DO NOT include units in the data entry. In Column B enter their absorbance values.
2. Create a scatter plot by highlight data in both Columns A and B. From the top menu, choose *Insert* menu, *Scatter Plot with Markers Only*.
3. To add Titles and Axis Labels: From the top menu, under *Chart Tools* choose the *Layout or Design* tab. Alternatively, click on *Add Chart Element*. Select *Chart Title* and chose a location for the title. Enter an appropriate title. (Remember- DO NOT restate the axis label as an appropriate title!) Select *Axis Titles* to enter axis labels. DO NOT forget to include units when needed.
4. Add a Line of Best Fit: Right click on one data point on the graph. Choose *Add Trendline* from the drop-down menu and choose Linear regression. Alternatively, choose *Add Chart Element, Trendline, Linear*.
5. Check the boxes to Display Equation and R-squared value on chart. Record the equation for line of best fit on the Data Sheet.
6. If a Legend is displayed delete it.
7. Screenshot the Calibration curve and submit online for grading. Email a copy to your lab partner. **Each partner in a group MUST submit their own graph.**

Determine the molarity of Yellow and Blue dyes in Sports Drinks sample

1. Calculate the concentration of yellow in the sports drink sample using YOUR calibration curve's equation for the linear fit trendline. Insert absorbance value near 427 nm into the y-value and solve for x (concentration, mol/L). Report the concentration to the correct number of significant figures.
2. Determine the concentration of blue food dyes in the assigned sports drink using the following linear fit trendline for Blue food dye. Again, substitute the absorbance readings recorded near 631 nm into y-value of the linear fit trendline. Report the concentration to the correct number of significant figures.

$$y = 129483x + 0.000131$$

Data Sheet Name:_______________________________________

Date ___________ CHM123L section _____ Instructor_______________

Partner Name _______________________________________

I. Absorption Spectra of Food Dyes

Red Food Dye Wavelength of maximum absorbance, λmax (nm) __________

Blue Food Dye Wavelength of maximum absorbance, λmax (nm) __________

Yellow Food Dye Wavelength of maximum absorbance, λmax (nm) __________

Green Food Dye Wavelength of maximum absorbance, λmax (nm) __________ and _________

II. Calibration Curve for Yellow Food Dye #5

Analysis Wavelength, nm ____________

Trial #	Volume Yellow Dye #5 solution, mL	Volume water, mL	Absorbance
1	10.00	2.00	
2	6.00	6.00	
3	2.00	10.00	

III. Determination of Dye Concentration in Sports Drinks

Assigned Sports Drink Name ____

Color of Sports Drink ______________________________

Wavelength of maximum absorbance, λmax, near Yellow dye (nm) __________

Absorbance near λmax, near Yellow dye __________

Wavelength of maximum absorbance, λmax, Blue dye (nm) __________

Absorbance, λmax, near Blue dye _________

Report Sheet

Name:_______________________________________

Date ___________

CHM123L section _____ Instructor_______________

I. Absorption Spectra of Food Dyes

a. Complete the table below for each food dye.

Analysis	Blue dye	Yellow Dye	Red dye
Wavelengths Transmitted *Example: 400-510 nm, 620-720 nm*			
Colors Transmitted *Example: violet, blue, orange, red*			
Wavelengths Absorbed *Example: 510-620 nm*			
Colors Absorbed *Example: Green and yellow*			

b. Does the transmitted wavelengths represent the visual color of the dye solutions?

Report Sheet (cont.)

2. Analysis of Green food dye:

   a. Describe the spectrum of the green food dye by filling in the following information:

Wavelengths Absorbed (nm)	Colors Absorbed	Wavelengths Transmitted (nm)	Colors transmitted

   b. The color green is a secondary color, comprised of two primary colors. Is the shape of the spectrum consistent with this observation? Why or why not? Explain in terms of absorbed wavelengths.

II. Construction of a Calibration Curve for Yellow Dye #5

1. a. Complete the table below for Trial 1-3 by copying the absorbance values of each Trial then calculating the molarity of yellow dye in each Trial. Show work in the table.

Trial #	Absorbance	Concentration of Yellow Dye #5, mol/L
1		
2		
3		

b. Create a calibration curve in Excel using Absorbance for y-values and concentration for x-values.

Equation for Linear fit from Yellow Dye #5 calibration curve ______________________________

Report Sheet Name:___________________________________

Date __________ CHM123L section _____ Instructor_______________

III. Determine the concentration of Yellow and Blue dye in your assigned sports drink sample.

2. Determine the concentration of dyes in the Sports Drink sample

   a. Using the trendline equation from your calibration curve, calculate the molarity of Yellow dye in the sports drink. Show work.

   b. Calculate the molarity of blue dye in your sports drink using information in the Data Analysis section. Show work. Use $y = 129483x + 0.000131$ as the calibration curve trendline for blue dye.