Quantification of Food Dyes in Sports Drinks

1

Absorbance Spectroscopy and Sports Drinks

How much food dye is present in sports drinks? This is the question you will answer in this laboratory experiment. You will use visible spectroscopy to determine the concentration of a food dye in a beverage of your choice. This requires that you understand how light and matter interact – specifically how light is transmitted and absorbed species in a solution. You will design a method to create standard dye solutions and use these standards to generate a calibration curve. You will then use Beer's law to quantify the amount of food dye in your beverage. You will use your results to comment on the safety of food dyes in beverages.

INTRODUCTION

Learning Goals

As a scientist, you are often tasked with identifying and quantifying unknown compounds or solutions. What and how much of an unknown compound is present in a sample? For example, a stream or river may need to be analyzed for the presence of certain harmful chemicals such pesticides. First, you would want to identify the pesticide species present in the waterway. You would then want to quantify the amount of each pesticide present. In this experiment, you focus on food dyes in beverages. You will quantify the amounts of food dye in a drink of your choice using UV-Vis spectroscopy and Beer's law.

Food Dyes

Food dyes are used in many common beverages and foods.[1] While food dyes serve no nutritional purpose, they provide an attractive color to many different sports or soft drinks, desserts, and even meat. Odds are you have consumer many beverages or food that contain food dye – a colored sports drink after a hard workout, a cupcake decorated with red frosting, or a piece of colored candy. Dying food and drinks is a not a new art. Indeed, food dyes have been reported since ancient times. While some historic food dyes were harmless (chalk was added to bread to make it 'whiter') there was a trend in the 17th and 18th century to use brightly colored but toxic inorganic salts (such as lead chromate, mercury sulfide) to improve the color of food.[2] By the mid-19th century nearly every food or drink was adulterated and the safety of the compounds added to food was receiving widespread attention.

The food dye landscape changed dramatically once the first synthetic dyes were produced. The first synthetic organic dye, "aniline purple" or "mauve", was created by William Perkin in 1856.[3] These synthetic dyes quickly replaced the old unsafe inorganic dyes in food products. However, the safety of these new dyes had not been proven. Over the next century, what was to become known as the FDA tested and approved or rejected numerous synthetic dyes for food consumption. However, the safety of food dyes is still under debate. For example, the FDA considers powdered Red 3 safe, but a different form of this same dye is banned because it is carcinogenic to rats.[4] In the 1970s, several studies found that FD&C Yellow 5 and Yellow 6 cause allergic reactions and hyperactivity in children.[5,6] Currently the United States permits seven synthetic foods dyes – FD&C Red 3, Red 40, Yellow 5, Yellow 6, Blue 1, Blue 2, and Green 3. You will investigate FD&C Red 40, Yellow 5, and Yellow 6 in this experiment.

[1] Sharma, V., McKone, H. T., & Markow, P. G. J. Chem. Ed. 2011, 88 (1), 24–28.
[2] Hutt, P. B.; Hutt, P. B., II. Food, Drug, Cosmet, Law J. 1984, 39, 19.
[3] Garfield, S. Mauve: How One Man Invented a Color That Changed the World; Norton: New York, 2000.
[4] Dept. of Health and Human Services, FDA. Federal Register 1990, 55 (22), 3515-3543.
[5] Feingold, B. F. Ann. Allergy 1968, 26, 309–313.
[6] Feingold, B. F. Am. J. Nurs. 1975, 75, 797–803.

Absorbance Spectroscopy

When an atom or molecule absorbs a single photon, an electron makes a transition to a higher energy orbital (see Figure 1). As the number of atoms or molecules increases more and more photons can be absorbed. Thus, the number of photons a sample absorbs is directly related to the number of atoms or molecules in that sample.

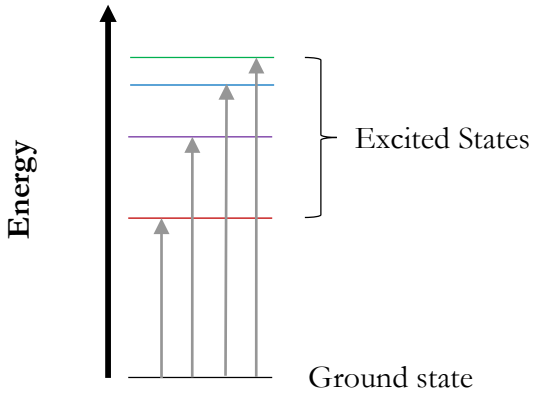


Figure 1: Transition of electrons from ground to excited states upon the absorption of a photon.

Absorbance is related to the number of photons a sample absorbs. The change in the number of photons of a light beam before (P_0) and after (P) it passes through a sample is related to the number of molecules the beam encounters in that sample. Absorbance (A) is defined as ratio of number of photons before and after the light beam passes through a sample:

$$A = \log\left(\frac{P_0}{P}\right)$$

Absorbance Measurements

How is absorbance used to determine the concentration of a colored solution? Typically, a cuvette containing the colored solution is placed in the spectrometer. Spectrometers consist of a light source, which produces a beam of light (P_0) that passes through a sample in the cuvette. The light that passes through the sample (P) is then split into its component wavelengths by a diffraction grating. A charge coupled device (CCD) detector then measures the number of photons that reaches it at each wavelength. A computer then converts this into an absorbance reading using $A = \log(P_0/P)$ as shown in Figure 2.

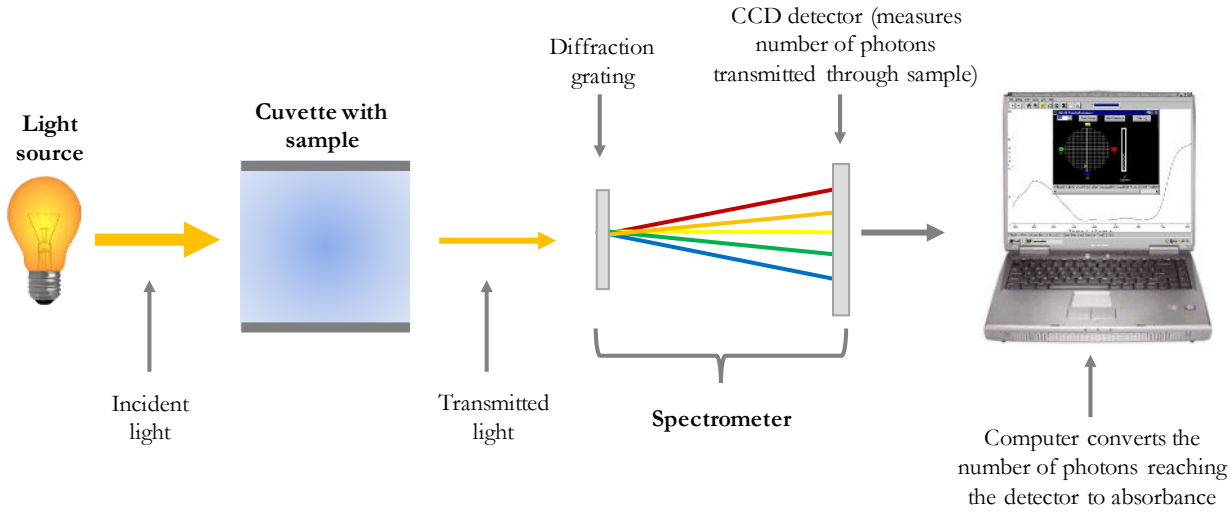


Figure 2: Schematic of an absorbance measurement using a spectrometer

Absorbance is an incredibly useful measurement because it is directly proportional to the concentration, c, of the light absorbing species in a sample. This relationship is known as Beer's law:

$$A = \varepsilon l c \qquad \text{(Beer's Law)}$$

Absorbance is dimensionless but is sometimes given 'dummy' units of absorbance units or AU. The concentration, c, of the sample is usually given in moles per liter (M). The pathlength, l, is the distance the beam of light travels through the sample is commonly given in centimeters. The pathlength is usually 1.000 cm – in this experiment you will place your samples in cuvettes that have a pathlength of 1.000 cm. Epsilon, ε, is the molar absorptivity (or extinction coefficient) and has units of $M^{-1}cm^{-1}$. The molar absorptivity depends on the wavelength of the light and is determined by measuring the absorbance of a series of standard solutions of known concentration. Once ε and b are known, you can determine the concentration of an unknown sample from its absorbance.

Beer's Law Calibration Curves

To determine the concentration of colored species in a solution you must calibrate your spectrometer using a set of known solutions. By measuring the absorbance of a set of standard solutions of known concentrations, you can create a calibration curve that shows how the response of the instrument (absorbance) changes with concentration. You can compare the response of the instrument for those known solutions with the response of the spectrometer for an unknown solution. This calibration curve correlates to how the spectrometer responds to various concentrations of standard solutions (see Figure 3). You can then use Beer's law to determine the concentration of your unknown solution.

However, Beer's law may fail if a solution is too concentrated or too dilute. Beer's law assumes a linear relationship between absorbance and concentration but that is only true for subset of absorbance measurements. Modern spectrophotometers are more precise (reproducible) at intermediate levels of absorbance (~0.3 to 2). If too little light passes through the sample to the detector (high absorbance), photon intensity is hard to detect. If too much light passes through the sample to the detector (low absorbance), it is hard to distinguish the transmittance of the sample from that of the blank (which shouldn't absorb many photons). You want your samples to have absorbance values that are neither too high nor too low – outside of this range absorbance and concentration are no longer linearly correlated. This means that you can only accurately determine the concentration of an unknown if its absorbance falls within the ***linear range***. While there are rules of thumb about linear absorbance ranges (e.g. ~0.3 to 1.5) the linear response range for a given species must be experimentally determined at the wavelength of interest.

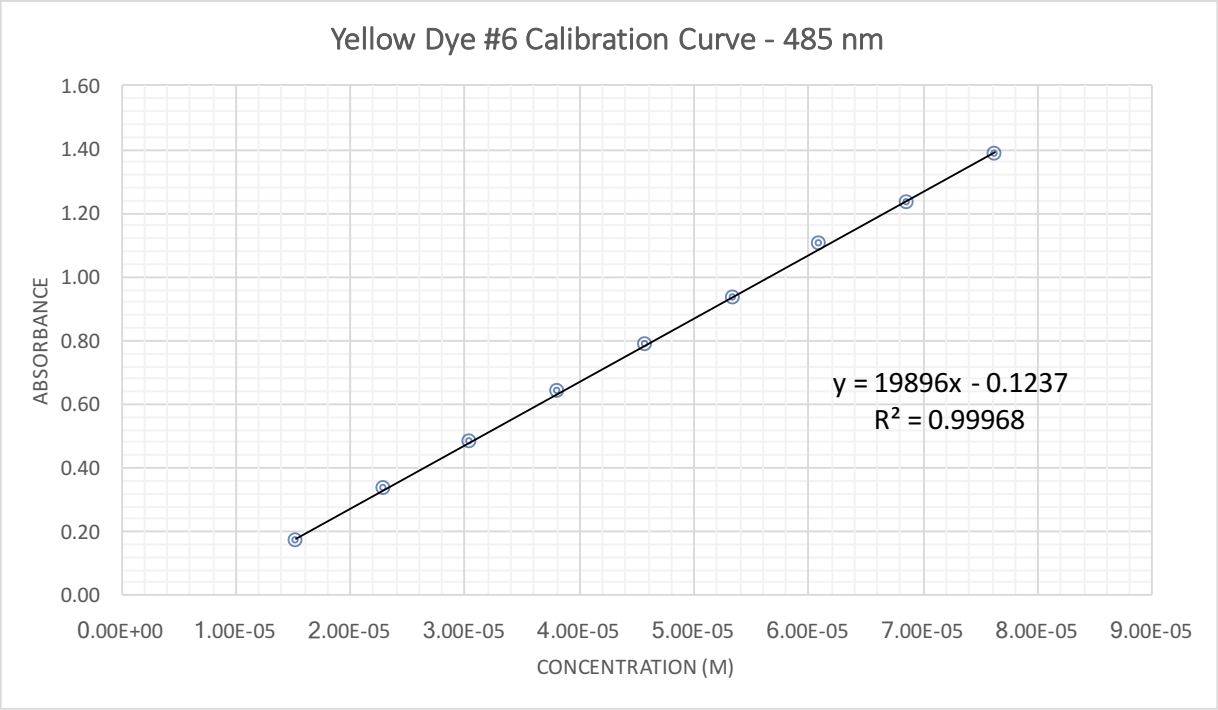


Figure 3: Calibration curve for FD&C Yellow #6

Additional Reading on Food Dyes

Sharma, V., McKone, H. T., & Markow, P. G. (2011). A Global Perspective on the History, Use, and Identification of Synthetic Food Dyes. *Journal of Chemical Education*, *88*(1), 24–28. https://doi.org/10.1021/ed100545v

EXPERIMENT PREPARATION

Before you come to lab

In preparation for this experiment, you should choose a colored drink or drink powder that you would like to explore in lab. You can choose this based on personal or professional interest (perhaps it's a drink that you particularly enjoy or one that you recently heard about in a news article or advertisement). Your instructor can also provide you with examples of appropriate drinks or drink powders to choose. Since you will be exploring yellow and red food dyes in this experiment your drink or drink powder should contain **one** of these dyes (FD&C Red 40, FD&C Yellow 5, or FD&C Yellow 6). You can see which food dyes a drink contains by looking at the ingredient list on the back of the container or bottle. Bring your chosen drink or drink powder to lab.

Please make sure that your chosen drink contains only **ONE** dye. It is not uncommon for drinks to contain multiple dyes. While it is possible to determine the amount the individual dyes in a mixture this requires a more complicated analysis than what you will do in this experiment.

Collaboration

This experiment requires you to collaborate with fellow students. Your instructor will assign you and your partner a food dye. You will create a calibration curve for your assigned dye and share this information with two other group (who were assigned different dyes). Between the three groups you will have complete calibration curves for all three dyes (FD&C Red 40, FD&C Yellow 5, or FD&C Yellow 6). You will then use this information to determine the amount food dye in the drinks you brought from home and will share your findings with your laboratory.

Safety

In preparation for this experiment, you should look up hazard information on the chemicals and equipment used in this laboratory. This might include looking at the Material Safety Data Sheets (MSDS) or Safety Data Sheets (SDS) for the chemicals used in this experiment. Additionally, the Pharos Project's Chemical and Materials database (https://www.pharosproject.net) provides a wealth of hazard and safety information on thousands of chemicals. You should identify any hazards associated with this chemical, necessary personal protective equipment (e.g. lab coats, goggles), and toxicology and ecological information. After completing this search, you should understand both how to handle chemicals safely in lab and how these chemicals effect the environment outside of your laboratory. Remember, the chemicals you use and the waste you generate don't stay in lab – they have to be disposed of after you complete your experiment.

PRELAB QUESTIONS

1. What drink or drink powder did you choose? What food dye does it contain (remember, it should contain FD&C Red 40, FD&C Yellow 5, OR FD&C Yellow 6)? Why did you choose this drink?
2. Read through the first part of the procedure (*Creating Standard Solutions*). How will you prepare you standard solutions? Describe the glassware you plan to use and the volumes of solution you will need for each standard. Calculate the final concentration of each standard assuming you add the exact planned volume for each solution.
3. Familiarize yourself with the spectrometer system you will use for this experiment. Using Figure 4 as a guide, explain how the spectrometer, light source, and computer (with spectroscopic software) will help you to measure the absorbance of food dye in a drink.
4. What is the purpose of using blank? What is the purpose of zeroing the spectrometer (or taking a dark reference spectrum)?
5. Describe the hazards associated with the chemicals for this lab. What precautions should you take when working with these chemicals? What would you tell someone who wanted to pour these chemicals down the drain (remember, many sinks eventually connect into your local rivers, lakes, and coastal waters)?

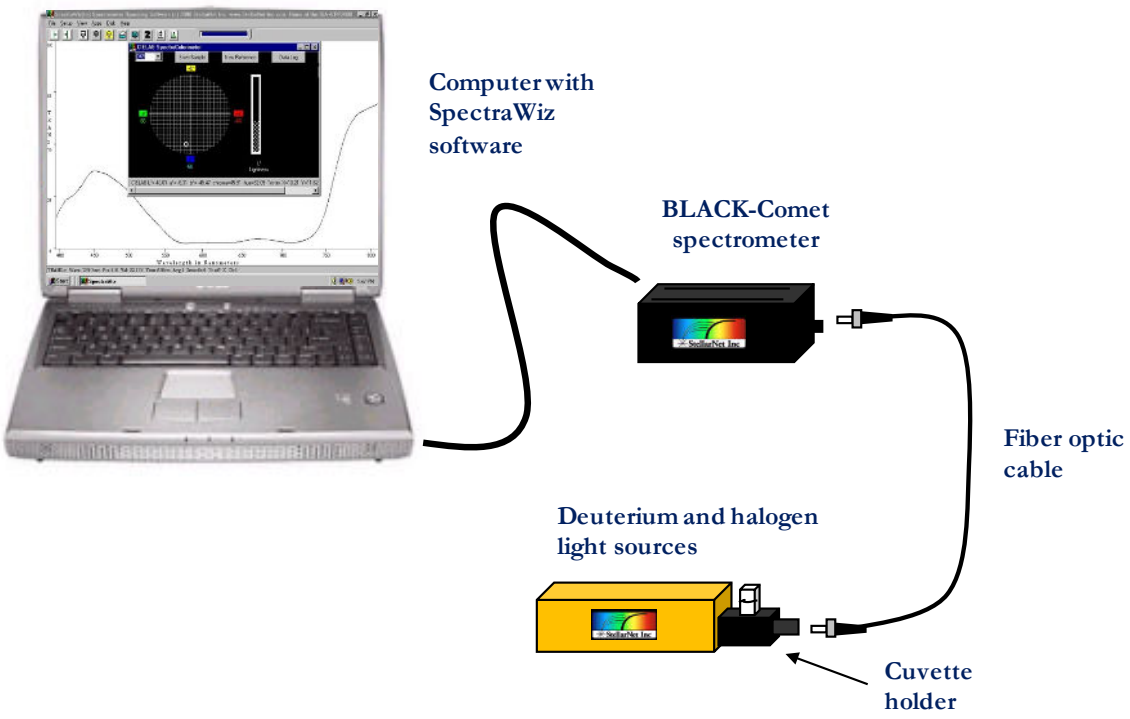


Figure 4: StellarNet UV-visible spectroscopy system

PROCEDURE

Material and Equipment

Chemicals

- ✓ 7.50×10^{-5} M FD&C Red 40 stock solution
- ✓ 7.50×10^{-5} M FD&C Yellow 5 stock solution
- ✓ 7.50×10^{-5} M FD&C Yellow 6 stock solution
- ✓ Water
- ✓ Drink or drink powder

Equipment

- ✓ Glassware for standard solution preparation
- ✓ Cuvettes with caps
- ✓ Lint free wipes
- ✓ Spectrometer (Black Comet)
- ✓ Light source (deuterium and halogen) and cuvette holder
- ✓ Computer or tablet
- ✓ Fiber optic cable
- ✓ USB cable

Creating Standard Solutions

1. Obtain approximately 30 mL of your assigned dye solution. Each dye solution should be approximately 7.50×10^{-5} M. Make sure to record the actual concentration of this **stock solution**. You will use this stock solution to prepare you standard solutions.

2. Prepare your **standard solutions** using the method described in your prelab using Table 1 below as a guide. Make sure you instructor has approved your solution preparation method.

   Standard solutions are solutions that contain known concentrations of analyte (the compound you are trying to measure). Since you don't know the concentration of dye in your drink, you want your standard solutions to span a range of concentrations to accurately measure the linear range of the calibration curve.

3. Transfer each standard solution to a cuvette. Fill each cuvette approximately ¾ full and cap. Do not shake or agitate the cuvettes as that can introduce air bubbles which will alter your absorbance readings.

   Make sure to check each cuvette is free of scratches or discoloration on the transparent sides. Gently clean the transparent sides using a lint-free wipe to remove any residual particulates. Make sure to not touch the transparent sides with your (ungloved) fingers. Your fingers can transfer small amounts of oil onto the cuvettes which will obstruct light passing through the cuvette and alter your absorbance readings.

4. Fill a cuvette approximately ¾ full with water and cap the cuvette. This sample will serve as your **blank solution**.

> **Blank solutions** are solutions that contain all the reagents and solvents used in analysis but not the analyte. They measure the response of the instrument to impurities or interfering species in the reagents.

Table 1: Standard solution preparation. The volume of dye for each standard is only a suggestion. You do not need to measure that exact volume. However, you do need to measure and record the actual volume of dye and water you use to create each standard solution.

Standard	Stock solution concentration (M)	Volume of dye (mL)	Volume of water (mL)	Total standard volume (mL)	Final concentration of dye (M)
#1		1.00	4.00	5.00	
#2		1.50	3.50	5.00	
#3		2.00	3.00	5.00	
#4		2.50	2.50	5.00	
#5		3.00	2.00	5.00	
#6		3.50	1.50	5.00	
#7		4.00	1.00	5.00	
#8		5.00	0.0	5.00	

Spectrometer Setup

1. Transfer your standard and blank cuvettes to the spectrometer. Make sure that your cuvettes are capped so you don't accidently spill solution on or near the spectrometer.
2. Review the spectrometer system setup. Make sure that the spectrometer system resembles Figure 5. Make sure that you can identify all the components of the spectrometer system.
3. Remove the cuvette holder cap. Place your **blank** cuvette into the cuvette holder and replace the cuvette holder cap.

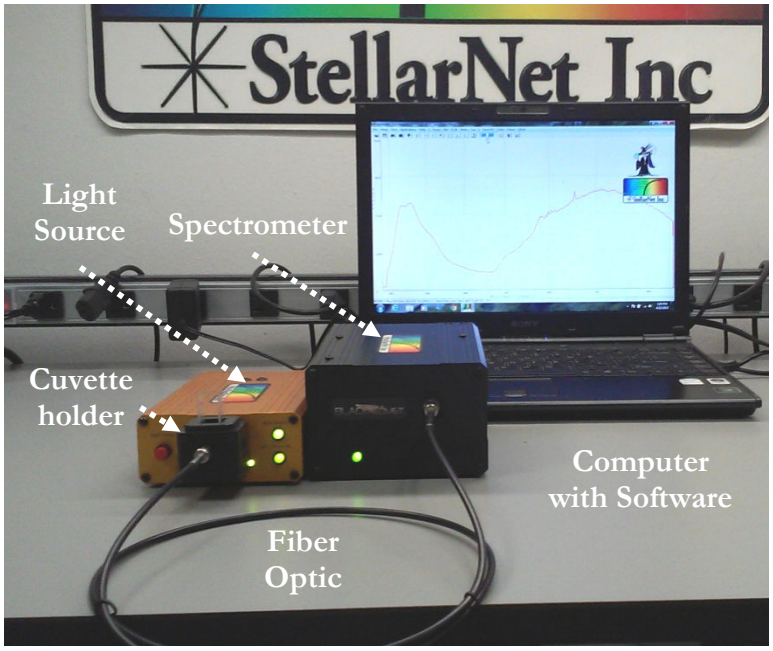
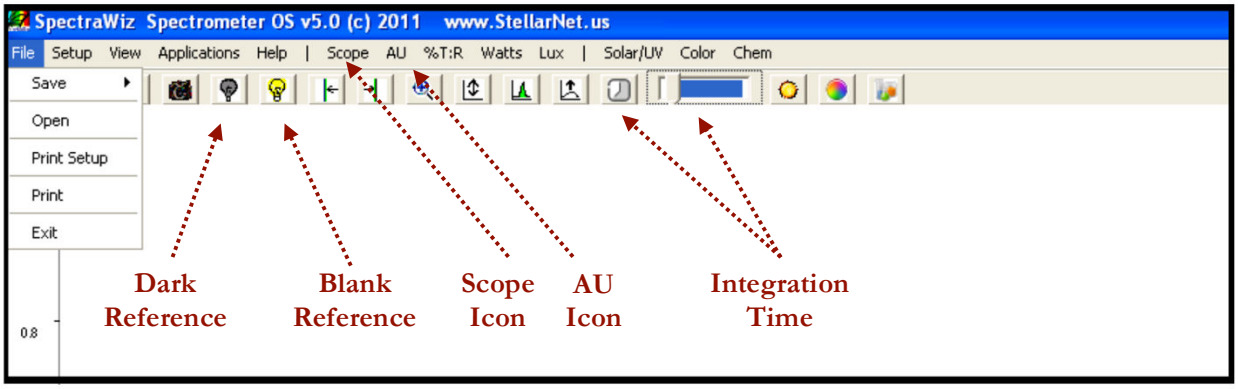


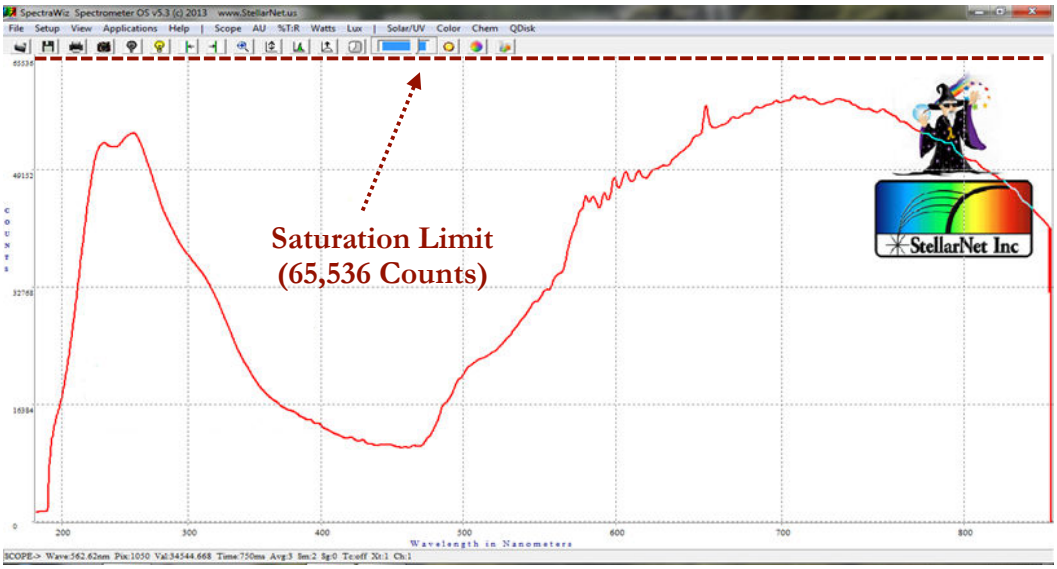
Figure 5: StellarNet spectrometer system (Black Comet spectrometer and SL5-CUV deuterium and halogen light source)

4. Turn on the computer. Open the StellarNet software by double clicking on the desktop icon ***SpectraWiz*** to open the application.

5. When you first open the software, you should see a screen that looks like:



6. Click on the **Scope** icon (highlighted above). This will show you the number of counts (photons) the detector receives. You should see a spectrum of counts versus wavelength.



7. You now need to make sure that there are not too many photons making it to the detector. Click on the **integration time** icon  (and type in the integration time you want – a value between 1 and 1000 ms) or use **sliding scale**  (both highlighted above). Set the integration time such that your spectrum is not saturated but as close to 65,000 counts as possible.

   If too many photons reach the detector it will be become saturated and you will not be able to get accurate absorbance readings for your samples. *Why is this?*

   This means you will want to be able to see a complete spectrum of your blank on the computer screen. In the picture above, the spectrum is correctly optimized to be right below the saturation limit (65,536 counts). If a portion of the spectrum is off the screen you need to reduce the integration time.

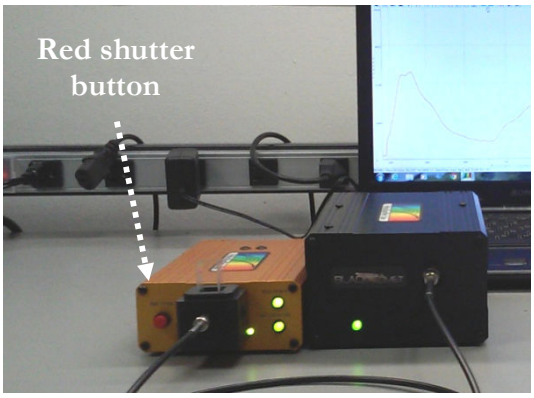
Blanking and Zeroing the Spectrometer

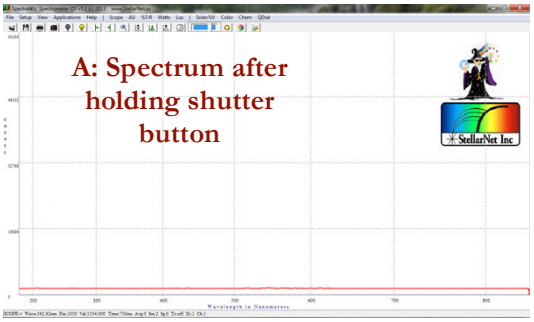
1. Make sure that your **blank cuvette** is in the cuvette holder and the cap is on the cuvette holder. **Blank** the spectrometer by clicking on the **yellow light bulb** icon in the toolbar at the top of the screen.

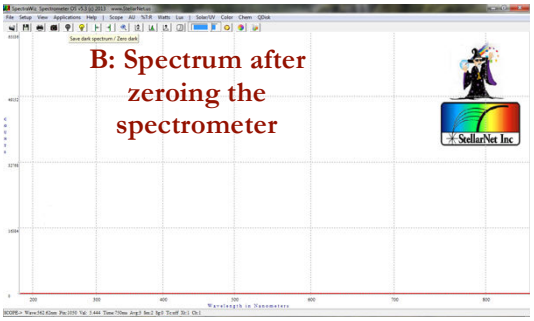
2. **Zero** the spectrometer. First, hold the red shutter button for at least three seconds. You should see the spectrum fall flat everywhere on your graph (A).

   Second, while still holding the shutter button, click on the **dark light bulb** icon in the toolbar at the top of the screen. The baseline will drop to zero (B).

   Finally, you can release the shutter button. You should see the spectrum return to its original profile.







3. Now that you have blanked and zeroed your spectrometer, click on the **AU (absorbance)** icon in the toolbar at the top of the screen to display a spectrum in absorbance units.

4. You should see a nearly flat absorbance spectrum once you switch to absorbance if you have blanked and zeroed the spectrometer correctly.

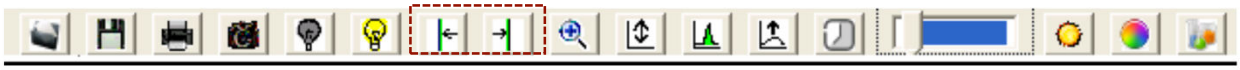
   Depending on the cuvettes you use, you may see a lot of fluctuations below 300 nm. This is simply due to the cuvette absorbing UV radiation at these wavelengths. *Why is this not an issue for your analysis of food dye?*

Taking Absorbance Measurements

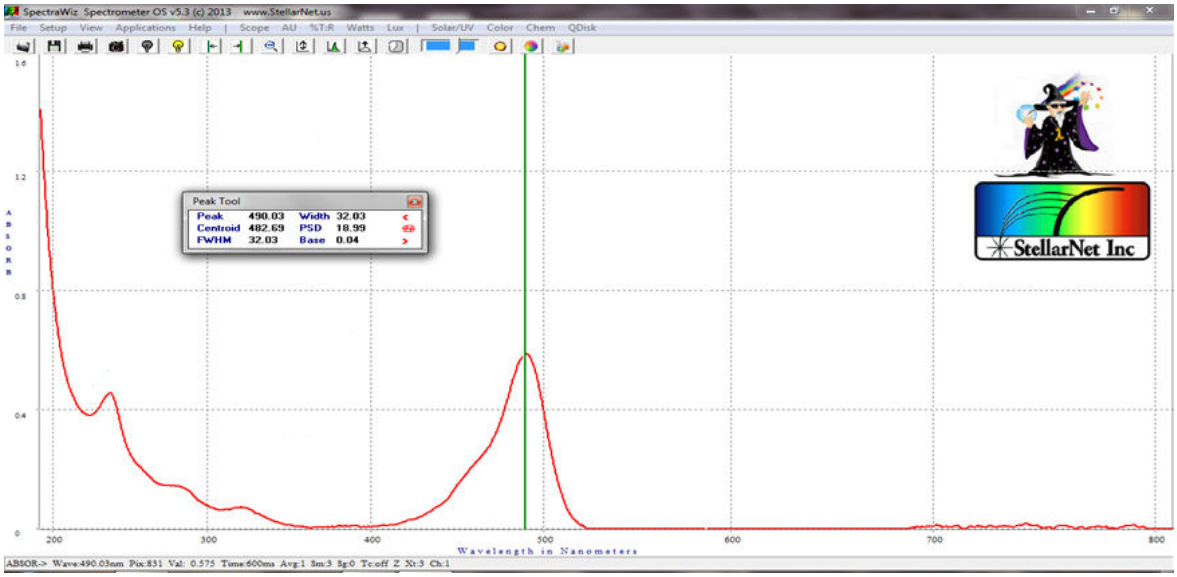
1. Remove your blank sample from the cuvette holder. Place the most concentrated standard cuvette in the holder and replace the cuvette cap. The instrument will now display absorbance in real time.

2. Look at the spectrum and select the wavelength of maximum absorbance. Sketch this spectrum in your lab notebook and label the wavelength of maximum absorbance. Make sure to label your axes. Use this wavelength for subsequent absorbance measurements.

3. To find a peak, use the mouse to **right click** on either of the following icons:



The left arrow will find a peak to the left of the vertical line in the spectra. The right arrow will find a peak to the right of the vertical line in the spectra.



4. You can also **right click** directly onto the spectrum to find the absorbance of a specific point. To find the specific wavelength your cursor is at, look to the bottom of the spectrum. **Wave** gives the specific wavelength and **Val** gives the absorbance for that particular point in the spectrum.

5. Record the absorbance for each one of your standard solutions.

Re-blank and zero between separate trials or if you have let the instrument sit for more than five minutes between sample. *Why is this necessary?*

Note the absorbance of your most concentrated solution. If the absorbance value if very high (above ~1.5 absorbance units) you may need to dilute you stock solution and remake your standard solutions. Discuss with you instructor the best plan of action.

6. You should have a completed table similar to the one below in your notebook.

Table 2: Sample data table for recording absorbance measurements of standard solutions

Standard	Wavelength (nm)	Absorbance (AU)	Concentration of dye (M)
#1			
#2			
#3			
#4			
#5			
#6			
#7			
#8			

7. Quickly sketch your calibration curve data using graphing paper, a graphing calculator, or a computer/tablet. You should produce a plot of absorbance versus concentration. Make sure that your standard solutions adequately cover the linear range (usually between ~0.5 and ~1.5 absorbance units). If you have few data points within the linear range (too high or too low of concentrations) discuss with your instructor which solutions you should remake to obtain a better calibration curve.

Sharing Data

1. Share your calibration curve data (the information contained in Table 2) with two other pairs of students that had different dyes than one you were assigned. Between the three pairs you should have a complete set of calibration curve data for FD&C Red 40, Yellow 5, and Yellow 6.

2. Make sure to note which spectrometers each group used to create their calibration curve. You will want to use the same spectrometer to analyze a drink containing that dye. For example, if the pair assigned to FD&C Red 40 used spectrometer #1 you will want to use spectrometer #1 for the subsequent analysis of any drink containing FD&C Red 40. *Why is this?*

Analysis of Your Drink or Drink Powder

1. Fill a cuvette ¾ full with your drink of interest and cap the cuvette. If you have brought a drink powder you will need to dissolve a portion of it in water before proceeding. Make sure to record the mass and volume you used to make this solution.

2. Record a spectrum of your drink (see the previous section for spectrometer operating instructions). Note the absorbance at the chosen wavelength (determine in the previous section) for the dye your drink contains. Dilute you drink quantitatively if the absorbance is above the linear range for that dye (usually above ~1 to 1.5 absorbance units).

3. Record the absorbance value of your (diluted) drink at the chosen wavelength. Use this data to calculate the concentration of food dye in your drink of interest.

DATA ANALYSIS

1. Calculate the actual concentrations of all the food dye standards you made AND all the food dye standards you used to analyze your chosen drink (these may not necessarily be the same).
2. Construct calibration plots of absorbance versus concentration for the dye standards you made AND for the dye standards you used to analyze your chosen drink.
    a. Use concentration and the absorbance data to construct two Beer's Law plots – one for the standards you made and one for the standards you used to analyze your chosen drink.
    b. Use linear regression to determine the slopes ($\varepsilon \cdot b$) of these curves.
    c. Calculate the extinction coefficient (or molar absorptivity) for the food dyes you analyzed.
    d. What is the R^2 value for your linear regression? What does this value tell you?
3. Are any of your standards too concentrated or too dilute?
    a. Make an argument for why you should exclude certain data points from your calibration curve.
    b. If you excluded certain data points, redo the analysis from *2a – 2c*. Does your extinction coefficient or R^2 value change? Why or why not?
4. Determine the concentration of food dye in your chosen drink.

DISCUSSION QUESTIONS

1. The blank used for all of your absorbance measurements was water. However, the drink you choose most likely did not only contain water and food dye.
    a. What other components were present in your chosen drink (you can find this information in the ingredients list usually on the back of the bottle or container)?
    b. Why were you able to use water as a blank for your analyzing your drink? What assumptions did you make? Were they good assumptions?
2. Comment on the accuracy and precision of your data.
    a. If did not use volumetric glassware to prepare you standards, what assumptions were you making about the total volume of your standard solutions?
    b. How many significant figures does concentration data have? What was the limiting piece of equipment for your volume measurements?
    c. What sources of error are present in your data? Is there anything you could do to prevent this in the future?

3. Per the FDA, the acceptable daily intake (ADI) for FD&C food dyes are shown in Table 3. What is the daily allowance for the food dye in your drink? How much would an average person need to drink to reach this daily limit? How much would child need to drink to reach this daily limit?

Table 3: Acceptable daily allowances for FD&C food dyes[7]

Color Additive	Acceptable Daily Intake (mg per person per day)	
	U.S. Population (60 kg person)	Children (30 kg child)
FD&C Blue No. 1	720	360
FD&C Blue No. 2	150	75
FD&C Green No. 3	150	75
FD&C Red No. 3	150	75
FD&C Red No. 40	420	210
FD&C Yellow No. 5	300	150
FD&C Yellow No. 6	225	113

4. Based on your calculations are you concerned about the presence of food dyes in commercial drinks?
5. If you could interview the scientists who determined the acceptable daily intake of food dyes, what questions would you ask them about their ADI conclusions?
6. What additional data or information would you like to know about food dyes to determine their safety as food additives?

[7] Food and Drug Administration. (2011). Background document for the Food Advisory Committee: Certified color additives in food and possible association with attention deficit hyperactivity disorder in children March 30-31, 2011. Retrieved from http://www.fda.gov/downloads/AdvisoryCommittees/CommitteesMeetingMaterials/FoodAdvisoryCommittee/UCM248549.pdf. Accessed March 1, 2017.