Lab 05: Beer's Law and Molecular Spectroscopy

INTRODUCTION

Goals

- Describing how light absorption by an electron cloud changes when the system changes from a single atom to a molecule.
- Constructing a calibration curve for an instrument to perform an indirect measurement.
- Determining the concentration of a solution by the amount of light it absorbs.

Background

Light and its interactions with matter: When light interacts with matter, there are several possible outcomes: absorption, reflection, refraction, scattering, and transmission. While reflection, refraction, and scattering are all interesting phenomena in physics, we will focus solely on absorption and transmission here in this experiment.

One fact that is seemly counter-intuitive is that the color of a solution we observe is not the color of the light that is absorbed, rather the color of the light that is transmitted. For example, a colorless solution is one for which all light is transmitted, and none is absorbed. **Note**: a very common misconception is that the color we observe is that of the *reflected* light. While that is true for opaque solids, the color of solutions is due to transmitted light.

Consider the cartoon in Figure 1: a sample is exposed to broad spectrum visible light (red, orange, yellow, green, blue, violet) and some of the light is absorbed (red, orange, and yellow). The transmitted light wavelengths are green, blue, and violet. As a result, the solution will appear more blue like, because the light at the red end of the spectrum is absorbed.

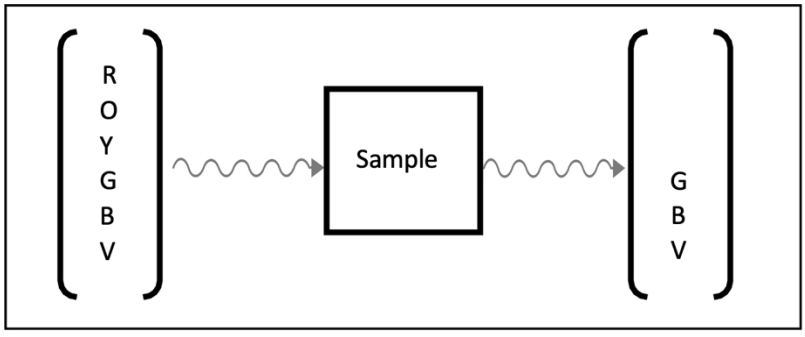

Figure 1. Illustration of absorption/transmission and the color of solutions.

Light is absorbed when it is in resonance with a transition in the atom or molecule, meaning that the frequency of the light is the same as the fundamental frequency of the molecular or atomic electronic oscillations. For example, in last week's experiment, we saw that when light is absorbed, a 1s electron wave can transform into a 2p wave in the hydrogen atom, and this transformation only occur when the frequency of the light is the same as the frequency of the atomic electronic

transitions. When we expand the system we study from a single hydrogen atom to a more complicated molecule, a similar process can also occur. The only difference is that now the nature of the electron wave and its transformation is more complex than the atomic electron waves we have studied thus far. However, the principle behind the atomic and the molecular absorption is the same: light is absorbed when, and only when, it is in resonance with a transition of the electron wave in the atom or molecule, and the frequency of the light determines what transformations occur to the matter (Figure 2).

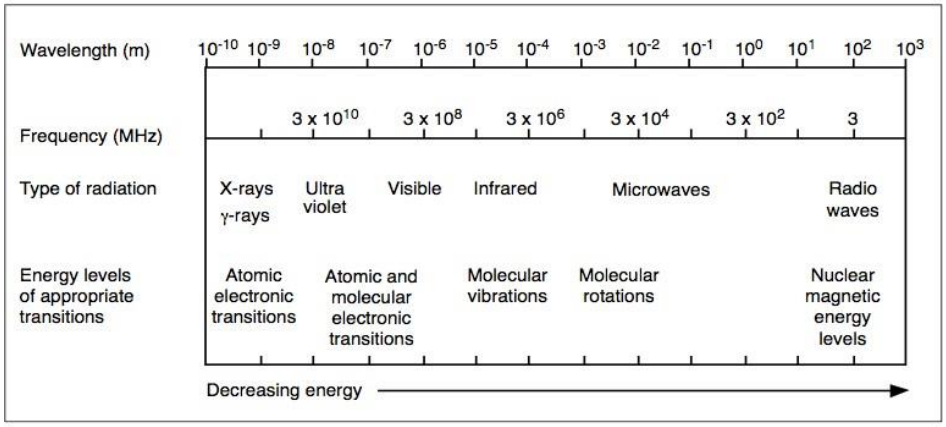

Figure 2. Electromagnetic spectrum in order of decreasing energy, and the types of transitions associated with absorption of light at these frequencies.

Light only transfers energy in quantized amounts known as photons. The photon energy, E_{photon}, is the amount of energy that the oscillating electric field from light can transfer to affect a change in the matter at its resonant frequency, ν.

$$E_{photon} = h\nu = \frac{hc}{\lambda} \tag{1}$$

As shown in Figure 2, the energies of the light in UV and visible light region of the spectrum are of the same magnitude as the energy differences between electronic energy levels in atoms and molecules. Consequently, when doing the molecular absorption spectroscopy, the absorption spectrum is usually recorded from 180 to 900 nm, hence the name “UV-vis spectroscopy”.

Anatomy of absorption spectrophotometers:

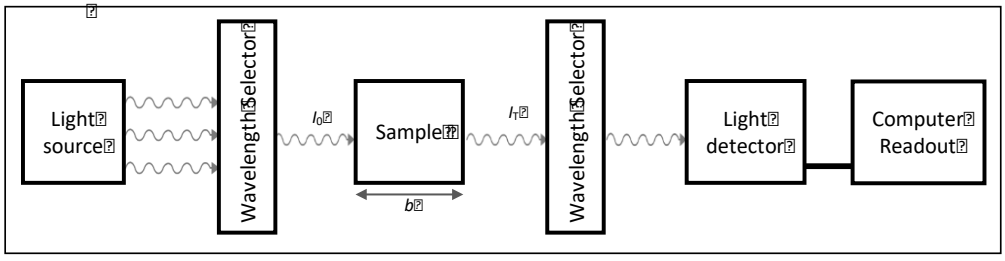

Figure 3. Box diagram for a spectrophotometer showing the key components: light source, two monochromators, sample, detector, and readout.

The actual internal components of a spectrophotometer are highly dependent on the type (UV-vis, AA, fluorescence, IR, etc.) and model, and they include things like beam splitters, mirrors, lenses, and much more. That said, most absorption spectrophotometers have five key components that

are common to all instruments: light source, wavelength selectors (monochromators), sample, detector, and readout.

The primary function of an absorption spectrophotometer is to measure the **transmittance**, T, of monochromatic light (light with a single wavelength) through a sample:

$$T = \frac{I_T}{I_0} \tag{2}$$

where I_0 is the intensity of the incident light that hits the sample and I_T is the intensity of the light that is transmitted through the sample – i.e., the light that is not absorbed, reflected, or refracted by the sample. Since most light sources give off a spectrum of light, a monochromator (wavelength selector in Figure 3) is used to isolate a single wavelength of light to be studied at any given time.

Note: These instruments are designed to minimize the reflection and refraction of light. Before measuring the sample, we measure a blank solution to further compensate for reflection and refraction. Therefore, it is safe to assume that any light not transmitted by the sample is in-fact absorbed. The theoretical maximum value for the transmittance of a sample is 1 (100%, all light transmitted) and the minimum value is 0 (0%, no light transmitted).

Beer-Lambert's Law: In general, spectrophotometers rarely report transmittance of a sample; rather, they typically report the **absorbance**, A, of a sample, which is a derived quantity related to the logarithm of the transmittance:

$$A = -logT = -log\frac{I_T}{I_0} \tag{3}$$

The importance of the absorbance is found in that it directly relates to the concentration of the absorbing species in the sample. This relationship is known as the **Beer-Lambert Law**, or Beer's Law, and is given by the equation:

$$A = \varepsilon bc \tag{4}$$

where A is the absorbance, c is the concentration of the absorbing species, b is the path length of the sample (usually in cm), and ε is the molar absorptivity. The molar absorptivity, sometimes referred to as the molar extinction coefficient, is a measure of how much light of a specific wavelength will be absorbed by a specific chemical species, and ε is an intrinsic property of that species at that wavelength. Because A is dimensionless (has no units), the units of ε must cancel out the units of both b and c. For this lab we will measure concentration as the moles of the solute dissolved per liter of solution ($\frac{\text{moles}}{\text{L}}$ or M, "molar"). The extinction coefficient ε you measure will therefore be in units of $\frac{\text{L}}{\text{cm moles}}$ or M^{-1} cm^{-1}.

For low-concentration solutions, where the extinction coefficient ε remains constant, Beer's Law holds true. As solution concentrations increase, however, ε can begin to change. Making a calibration curve and assessing its linearity is the only way to guarantee that you are working with solutions that are obeying Beer's Law. We will discuss the "calibration curve" shortly.

Vibrational and rotational states lead to curves in spectra of molecules: As discussed above, the absorption of light occurs because of resonance between the frequency of the oscillations of the electric field of light and a change in the electron clouds. Just like atoms, a molecule only has

a limited number of molecular orbitals with fixed orbital energies. As a result, we would expect a limited number of transitions to be possible, which means that only certain frequencies of light should be absorbed by the molecules. Moreover, given that the different transitions are at vastly different energies - separated by tens and hundreds of nanometers in wavelength - we would expect that the absorption peaks in UV-vis spectroscopy should be sharp peaks (just like the line spectra that are observed in atoms). However, in the UV-vis spectra of molecules, this is not the case – a sample absorption spectrum for a dye molecule is depicted in Figure 4.

Consequently, we are now tasked with trying to reconcile these two, seemingly disparate observations: (1) that absorption only occurs because of resonances between light and fixed states of the electron cloud and (2) that we see broad peaks in molecular spectra, which means that many wavelengths are absorbed. The answer is that there must be many more energy states for the molecules and that these states lead to a larger number of absorptions between many different possible states of the molecule's electron cloud.

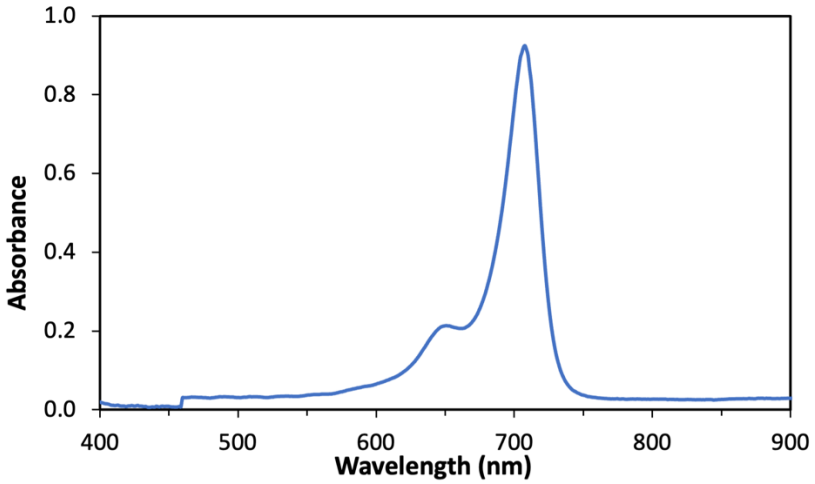

Figure 4. Absorbance spectrum of a dye molecule with a maximum absorbance wavelength, λ_{max} around 700 nm. Notice that the peak for this absorption is very broad, absorbing light from below 600 nm until around 750 nm.

The simple distinction between atoms and molecules is that molecules, having more than one nucleus, can also experience vibration and rotation, leading to many possible slightly different states of the electron cloud. As a result of these variations and rotations, many more different possible energy states exist near the resonance condition for the electronic states of the electron cloud, and the narrow absorption peak that we expect to observe for atomic spectra broadens out into the molecular absorption spectra that we observe in Figure 4.

The absorption spectrum of the dye molecule in Figure 4 shows that the **wavelength of maximum absorbance** (λ_{max}) is around 700 nm. Or in other words, the molar extinction coefficient ε is the largest at λ_{max}. Very little light passes through the dye sample at this wavelength, because the light at this wavelength is in resonance with an electronic transition of the dye's molecular electron wave. Many students mistakenly relate the energy of this transition to the number of photons absorbed (or the height of the peak). In reality, the energy of the transition is related only to the wavelength of the peak, and the height of the peak is affected only by the molar extinction coefficient ε.

Creating a calibration curve for Beer's law: Rather than measure both the path length for the cuvettes (b in Equation 4) and extinction coefficient (ε in Equation 4), it is often easier to construct what is known as a calibration curve from your spectrophotometric data. In general, calibration curves are often used to account both for variation between instruments/measurement set ups (such as the path length b, or the intensity of the light sources) and for the constants related to the sample or system being studied (such as ε at the specific wavelength being measured). In this experiment, you will construct a calibration curve for the expected absorbance of a solution as a function of the concentration of the solute.

As described above, Beer's law (Equation 4) is linear when the concentration of the light-absorbing molecule is low. Therefore, a plot of the measured absorbance of a solution against the concentration can be fit with a **linear regression**, and the slope of that fit line is equal to εb. This mathematical model for your system will hold true as long as you keep it within the bounds of the model. The light-absorbing molecule and solvent should be kept the same as your calibration samples, the concentrations you attempt to model should stay within the range that you used for the fitting, the path length should remain constant (do not use a different width of cuvette), and the wavelength at which you conduct the measurements should not change. Within those criteria, you will be able to predict either the absorbance of an arbitrary solution given its concentration, or the concentration of a given solution from a measurement of its absorbance.

Consider the example data for the absorbance of a hypothetical solution shown in Table 1. Note that since pure solvent is used as part of the calibration of the spectrophotometer, we can include it in the data for the calibration curve as having no absorbance at a concentration of zero grams solute per liter of solution, or zero moles solute per liter of solution.

Table 1. Example data of absorbance at λ_{max} as a function of concentration of a solute.

Concentration (g/L)	Concentration (moles/L)	Absorbance
0.0	0.000	0.001
25.0	0.100	0.100
74.9	0.300	0.300
124.8	0.500	0.500

Fitting a linear regression to the data (preserving the digits from the g/L measurements) yields a line with the equation:

$$A = \left(1.00 \frac{\mathrm{L}}{\mathrm{mole}}\right) c - 8.1 \times 10^{-5} \tag{5}$$

Note that the intercept is very small compared to the slope – almost to the point we could ignore it. It is left in for completeness' sake but may be safely ignored within the precision of the measurements. In fact, it is common practice to use a software option while doing the fitting to force the intercept to equal 0 to better match Beer's law (instruction on how to perform a linear regression is attached at the end of post-lab worksheet).

The slope is the product of both the extinction coefficient ε and the path length of the cuvette b, so the units of centimeters cancel out. Don't forget though that this constant εb is only valid for

this specific experiment in the specific spectrophotometer you use – you will have to re-do the calibration if you use a different instrument, solvent, cuvette, or absorbing molecule.

From this equation, we can predict concentrations from solutions with absorbances within the range of the calibration. For example, if a sample with unknown concentration is measured in the spectrophotometer at the same wavelength to have an absorbance of 0.200, then we can calculate the concentration to be:

$$A = \varepsilon b c$$

$$0.200 = \left(1.00 \frac{\text{L}}{\text{mole}}\right) c$$

$$c = \frac{0.200}{1.00 \frac{\text{L}}{\text{mole}}} = 0.2 \frac{\text{moles}}{\text{L}}$$

A graphical representation of the calibration curve and this prediction is shown in Figure 5.

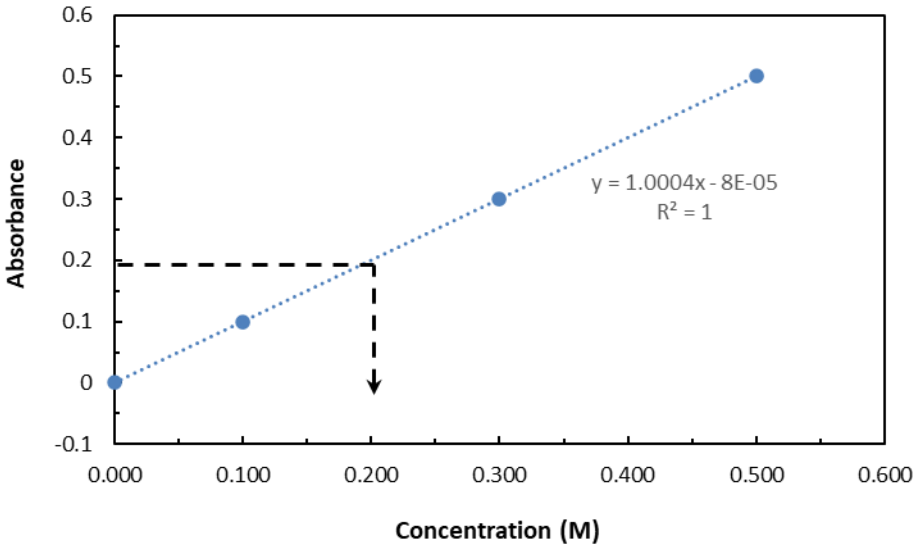

Figure 5. Absorbance of a hypothetical light-absorbing molecule as a function of its concentration in moles per liter of solution. The dotted line represents the calibration curve obtained from linear regression, and the dashed arrow represents the process of predicting the concentration of a sample with a measured absorbance of 0.200.

PREPARING FOR LAB 05: BEER'S LAW

- Read through the lab procedure, and in your lab notebook create an outline or flowchart of the procedure (don't copy it verbatim!) – you should be able to perform the experiment from this outline.
- Also note down each measurement or calculation you will perform, leaving space to write down those numbers, equations, or data tables with proper units. Leave room to write qualitative observations at every step. It's better to leave more space than you need than to run out or try to cram in observations/measurements!
- Bring your laptop with Excel/spreadsheet software with you for creating the calibration curve.

EXPERIMENTAL, LAB 05: BEER'S LAW

Safety

Copper (II) sulfate is toxic and harmful to the environment (it is used commercially as an herbicide). Dispose of any leftover solutions in the proper waste containers.

It is also a skin irritant and may be absorbed through the skin. In case of an exposure, rinse the affected area thoroughly with water.

Materials

- Spectronic 200 Spectrophotometer
- 1 cm Cuvettes
- Cuvette rack
- Beakers
- 100 mL volumetric flasks
- Pipettes
- Weigh paper/weighing boat
- Magnetic stir plate
- Stir bar
- Stirring rod
- DI water in wash bottle
- Unknown concentration $\mathrm{CuSO_4}$ solution
- Solid copper (II) sulfate pentahydrate $\mathrm{CuSO_4 \cdot 5H_2O}\ (s)$

Procedure

Use of the Spectrophotometer: (this instruction will be provided in lab)

Check that your spectrophotometer is plugged in and the switch on the back is turned on. Check the main compartment to make sure it is free of any cuvette from previous experiment. Press the "Enter" button (the button at the center of the 4 arrows) and wait until the instrument is done initializing, which should take 1 to 2 minutes. After it is initialized, it should display the "Home menu". From the home menu, use the arrow keys to scroll down until "Spec 20D + Emulation", then hit enter to select it.

Click the right arrow to set the measurement at the "Absorbance" mode (not transmittance). Select the wavelength of light λ being used for the absorption measurement – the knob marked λ will change the wavelength in increments of ±10 nm when turned normally and increments of ±1 nm when pressed and held down while turning. The λ of the maximum absorption for the copper (II) sulfate solution is 600 nm in this spectrophotometer.

Before making any measurements, you will need to "zero" the spectrophotometer, meaning setting the instrument to count a cuvette containing just the solvent (in this case, water) as zero absorbance to avoid any background readings that can interfere with the absorbance of the compound of interest.

To zero the spectrophotometer, fill a clean cuvette with DI water, and place it in the main compartment on the right side. One side of the cuvettes has a small, downward facing triangle near the top. Make sure that side of the cuvette is pointed toward the light source on the spectrometer – the left side of the sample compartment. Close the sample compartment and press the button marked "0.00" and wait until the instrument's calibration procedure is complete. You can then remove the cuvette with the DI water (called the "blank" for your measurement). The spectrophotometer should now allow you to read the absorbance A of whatever sample is placed in the compartment.

Important: Each time you change the wavelength of your measurement, you will have to re-zero the spectrophotometer with your blank.

Preparing your calibration solutions: You will prepare a set of solutions to provide data points for your calibration curve with standardized $CuSO_4 \cdot 5H_2O$ concentrations between $0.1\ M$ and $0.5\ M$. Since this procedure will create more than enough of each solution for your measurements, create just one of the calibration solutions and share it with the rest of the class. Make sure you report the exact concentration you create so the other groups can use it in their calculations.

For example, if your lab has 8 groups, each group could create one solution with concentrations of 0.1 M, 0.15 M, 0.2 M, 0.25 M, 0.3 M, 0.35 M, 0.4 M, and 0.5 M $CuSO_4 \cdot 5H_2O$ (s) respectively for 100 mL of solution. (The molar mass of copper sulfate pentahydrate is 249.685 g/mol.)

1. Pick one of the calibration solutions to prepare. Weigh the correct amount of $CuSO_4 \cdot 5H_2O$ (***s***) and record the **exact mass** you used in grams (it just needs be close to your target value).

   If you spill any of the $CuSO_4 \cdot 5H_2O$ (s) in the balance room, brush it into a weigh boat and dispose of it in a large beaker in your fume hood. Mark the beaker as waste and pour any waste solutions in there instead of going to the waste barrel for each solution.

2. Transfer the solid to a clean, dry beaker. Rinse any solid from the weigh paper/boat into the beaker with a small amount of DI water to make sure you do not leave any behind (ensuring the exact amount you measured is transferred into the beaker is known as a quantitative transfer).

3. Fill the beaker with about 50 mL of DI water and dissolve the copper (II) sulfate completely with a magnetic stirrer or stirring rod.

4. Pour the solution into a 100 mL volumetric flask. Using very small amounts of water to rinse off the beaker and transfer the rinse into the volumetric flask as well. (Make sure to NOT add so much water that exceeds 100 mL!)

5. Gradually fill the volumetric flask up to the mark on its neck with DI water.

6. To ensure proper mixing, cap the flask securely with the stopper. Mix the solution thoroughly by inverting it ten to twelve times. Make sure your finger is holding the cap in place during the process.

7. Label the flask with its concentration in moles per liter.

8. Record the concentrations of all the calibration solutions from the class in your lab notebook.

9. Fill a separate cuvette three-quarters full with each of your calibration solutions and another cuvette with water. Use a separate, clean pipette for each solution! Put a small piece of parafilm wax on top of each to help prevent spills.

Important: Each group should have their own set of cuvettes with each of the calibration solutions and one with water as a blank. It is recommended to place them in order of increasing concentration in a test tube holder and label them at the top to keep them organized. **Handle the cuvettes only by the top** to prevent getting fingerprints on where the light will pass through. *For group discussion:* How will fingerprints on the cuvette impact the measured absorbance?

Creating the calibration curve (absorbance for a given concentration):

1. Make sure the spectrophotometer is warmed up, set it to a wavelength of λ = 600 nm, and zero it with your blank. Measure and record the absorbance of each of the calibration solutions. **Design a data table here to record your measurements.**

Be as consistent as possible with each measurement – pay attention to how you set the cuvette in the sample holder, which side of the cuvette is toward the light source, etc. – consistency is important for getting a reliable calibration curve.

2. Check that the absorbances you measured follow the same ratio as the concentrations for the solutions. If they don't, try re-zeroing the spectrophotometer and measuring them again. If they still don't match the ratio, consult with your instructor. A reliable calibration curve is important for the next part of the experiment!

3. Calculate the calibration curve from your measurements. There are instructions on the last page of the worksheet for using either your spreadsheet software or calculator to do so.

When using your laptop for the linear regression, remember to work off to a side of the lab where nobody is working on "wet" chemistry!

4. Test that using Beer's law on the calibration solution concentrations gives you the absorbances you measured with the value of the calibration constant εb. If it doesn't, check your calculations!

5. Note that this calibration curve is only valid for the experimental conditions you had set when you measured it – the exact instrument, the wavelength of light, the size of the cuvettes, the compound being measured, etc. If you need to change anything about the experiment, such as switching to a different spectrophotometer, you'll need to create a new calibration curve.

Re-creating the unknown solution:

1. From your instructor, obtain a sample of the $CuSO_4 \cdot 5H_2O$ solution with an unknown concentration in a cuvette.

2. Zero the spectrophotometer, measure the absorbance of the unknown, record it in your notebook, and return it to your instructor.

3. From that absorbance, calculate its concentration in moles per liter using your calibration constant εb and Beer's law. Check that this concentration falls within the range of concentrations you used for the calibration curve. If it doesn't, check the calculations for the calibration and the unknown's concentration again. **Leave space for sample calculation here.**

Calculate the mass of $CuSO_4 \cdot 5H_2O$ you would need to make 100 mL of solution at this concentration.

4. Make a 100 mL solution of $CuSO_4 \cdot 5H_2O$ with the mass you calculated in the previous step (repeat steps 1 to 4 from the section “Preparing your calibration solutions”).
5. Measure and record the absorbance of your recreation of the unknown solution.
6. If the absorbance of your recreated solution is more than 0.05 away from the original unknown, discuss with your group how to make a second try at the unknown quantitatively. For example, if your absorbance was too low, don’t just add more $CuSO_4 \cdot 5H_2O$ (s) until the absorbance matches; revise your calculation of what the concentration should be based on what you observed with your recreated solution.

   Check in with your instructor on your plan to make an improved recreated solution, then go ahead and do so! Measure and record the absorbance of your second try.

   If the absorbance is within 0.05 away from the absorbance of the original unknown, ask your instructor to check your data and write their initials/sign in your notebook.

Checking the absorption spectrum:

1. Make sure the spectrophotometer is warmed up, set to a wavelength of λ = 600 nm, and zeroed with your blank. Place one of your calibration cuvettes in the spectrophotometer’s compartment, close the lid, and read the absorbance of the solution from the display. Record which concentration you’re using in your notebook, the absorbance of the solution and the wavelength at which you measured it.
2. Repeat step 1 with wavelengths of 500 − 700 nm in steps of 20 nm – remember to zero the spectrophotometer each time you change the wavelength. Record in your lab notebook the wavelengths and its corresponding absorbance (**design a table to record your data**).

   Make sure you’re placing the cuvettes in the spectrophotometer the same way each time you switch them.

Remove any cuvettes you may have left in the spectrophotometer and turn it off if you’re the last one using it. Dispose of all $CuSO_4 \cdot 5H_2O$ solutions in your waste beaker, then pour the combined waste into the lab’s waste barrel. Wash, dry, and put away all glassware you used. The cuvettes can be disposed of in the trash after they have been **rinsed**. Wipe down your lab bench and show both it and your fume hood to your instructor before you leave.