

Spectrophotometry

In this lab, you will use the SmartSpec Plus in groups of 2 to measure the absorbance of a dye solution. As always, this exercise should be documented in your notebook. We don't have enough specs for everyone to work at the same time, so be patient. While you wait for a spec, I suggest you:

- Prepare your samples (see below)
- Work on the lab math practice problems

Sample preparation for the SmartSpec:

1. Obtain 5 microcentrifuge tubes. Label them "undil", "2x dil", "4x dil", "8x dil", and "16x dil".

Tip: To help keep track of your tubes, and what you have done to each one, keep your tubes in a logical order, and place them in a rack in rows for "done" and "not done". For instance, empty tubes could be in the top row of the rack. When you pick up a tube and put something in it, put it into another row. If you pick it up again to add something else, put it down into yet another row. Developing this habit can save many mistakes, especially once protocols become more complicated!

2. Take 750 μL of blue dye solution from the class stock, and put it in tube "undil".
3. Take 750 μL of blue dye solution from the class stock, and put it in the "2x dil" tube. Get a new pipette tip each time you go into a new solution. Then, add 750 μL of water to the "2x dil" tube. Mix your solutions each time by pipetting up and down. (Because you mixed 1 part original plus one part water, you did a 2-fold dilution.)
4. Transfer 750 μL of "2x dil" to the "4x dil". Add 750 μL water to the "4x dil". (You just did a 2-fold dilution of something that was already 2-fold diluted. $2 \times 2 = 4$.)
5. Transfer 750 μL of "4x dil" to the "8x dil". Add 750 μL water to the "8x dil". (You just did a 2-fold dilution of something that was already 4-fold diluted. $2 \times 4 = 8$.)
6. Transfer 750 μL of "8x dil" to the "16x dil". Add 750 μL water to the "16x dil". (You just did a 2-fold dilution of something that was already 8-fold diluted. $2 \times 8 = 16$.)
7. To check your work, make sure that tubes "undil", "2x dil", "4x dil", and "8x dil" all have the same volume (750 μL). Tube "16x dil" should have twice that volume, or 1.5 mL.
8. Congratulations! You have just done a **serial dilution**. What you did is a 2x serial dilution (because you diluted 2x over and over). Often, people will do 10x (or 100x) serial dilutions to achieve a drastic change in concentration relatively quickly.

SmartSpec Readings

1. Turn on the SmartSpec, and allow it to warm up and go through its diagnostics.
2. Press the lambda (λ) button. (FYI: The Greek letter λ means wavelength.)
3. The screen should now say "Enter number (1-3) of wavelengths to read." Use the keypad to type 1, then press enter.
4. The screen should now say "Enter wavelength:". Type 620, then press enter.
5. The screen should now say "Do you want to subtract background reading? NO". Press enter to accept that choice.
6. The screen should now say "Ready to read absorbance".
7. First, you need to zero the spectrophotometer by giving it a cuvette filled with the solvent in which you diluted your sample. In this case, the sample is diluted in water. So, put 750 μ L of water into a plastic cuvette. Be sure you touch only the frosted parts of the cuvette, and not the clear portion through which the light beam passes. If you have any doubts that you may have left fingerprints on an important part of the cuvette, wipe it with a kimwipe.
8. Insert the cuvette into the spec so that the "V" shape on the cuvette is aligned with the arrow next to the cuvette chamber. (This ensures that the light is going through the proper path.) Close the lid.
9. Press "read blank", and wait while it reads.
10. Record the value for the blank in your data section. ($A_{620} = ???$)
11. Press the right arrow button to continue.
12. The screen should now say "Ready to read absorbance".
13. Obtain a new cuvette, and put in one of your samples. (I suggest starting with the most dilute sample, and working your way up. Again, it helps to go in some logical order.)
14. Place the cuvette into the spec, making sure orientation is correct.
15. Press "read sample". Record the value in your data section.
16. Repeat steps 13-15 until you have values for each of your samples.

Data Analysis (this is not part of the prelab and does not need to be copied into the lab book)

In the data/calculations section of your notebook, address the following:

- Report your absorbance values in a table.
- Recall Beer's law, $A = C \cdot l \cdot \epsilon$
 - A = absorbance (no units)
 - C = concentration
 - l = path length (10 mm in this case)
 - ϵ = extinction coefficient specific for a particular molecule at a particular wavelength
- Suppose that the extinction coefficient for this dye is 12 mL/(mg x cm)
- Calculate the concentration of dye in each of the tubes you prepared. (This may seem confusing, but take your time, and make sure your units cancel out. You should come out with an answer in units of mg/mL.)
- Now, use the concentrations in each of your tubes to back-calculate the concentration of dye in the original undiluted stock. For instance, if your 8x dilution tube had a concentration of 10 mg/mL, then the stock would have been $8 \times 10 \text{ mg/mL} = 80 \text{ mg/mL}$. Obtain an estimate of the original stock concentration from each of your absorbance measurements.
- Present your calculated tube and stock concentrations in a table.

Conclusion:

- Remembering that absorbance values are most trustworthy in the range of 0.1 – 1.0, are there any of your data points that can be deemed less accurate?
- Note which data points you consider trustworthy, and compute an average value of those data points. This is your best estimate of the original concentration! Did you have a lot of variation?