Introduction to our spectrophotometer

(Self-Guided)

Color is created when a white light strikes a molecule that then reflects light of a certain wavelength and absorbs all the others. A spectrophotometer is an instrument that measures the amount of light absorbed by a sample. It does this by shining light of a particular wavelength into a sample and measuring how much light comes all the way through. Samples are held in cuvettes between the light source and the detector.

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Measuring absorbance

Here are two important things to remember about spectrophotometers. First, different compounds absorb different wavelengths of light. Red pigments absorb blue light (light of ~300 nm wavelengths) and blue pigments absorb red light (light of ~600 nm wavelengths). Therefore all spectrophotometers have ways of adjusting the wavelength of light shining into the sample. The second important point is that the amount of light absorbed by a sample is directly proportional the concentration of that sample. This is a very useful relationship, making the spectrophotometer a valuable research tool.

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Spectrophotometer

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Cuvette Holder

Part 1: Using the Spectrophotometer

In this assay you will calibrate your pipets by measuring the absorbance of the XC dilutions you made. Beer's Law, which relates absorbance to concentration, will be derived as part of experimental module 2. Here, you'll see that the graph of absorbance versus volume of 0.01% XC is a straight line….or at least it should be!

1. Using your P1000, measure 1 ml of water into a plastic cuvette. This cuvette will serve as your blank for the spectrophotometer.
2. Confirm that the machine is set to read absorbances at 600 nm.

3. Put your blank into the spectrophotometer at position 1, which is furthest back in the instrument. Be sure the window of the cuvette and not the frosty sides are in the light beam that travels from left to right.
4. Close the door of the spectrophotometer. Click “blank” (lower left of the screen). A “reading blank” message should appear. When the message is gone, then the blank is set.
5. Replace the blank with your first sample. Close the door of the spectrophotometer. Click “read samples” (upper left of the screen). Write down this value.
6. Repeat with all your samples.
7. Remove your last sample. Close the door of the spectrophotometer.
8. The XC dilutions can be washed down the sink and the cuvettes can be discarded in the sharps bin.

Part 2: Considering your data

1. Use Excel to prepare a graph of absorbance versus volume of 0.01% XC. Some sample graphs are reproduced below and you should generate similar ones with your data. Be sure to include a trendline, displaying its equation as well as the r-squared value on the graph. The r-squared value reflects how well the data points fit the equation. A perfect fit will give an r-squared value of 1. If you are uncertain how to make such a graph using Excel, be sure to ask for help. We will use Excel a lot this semester, in particular during experimental module 2.
2. If the pipets were well calibrated and the measurements were done carefully, then the points should fall close to a straight line, and the r-squared will be close to 1. If one point seems way off, you can repeat the three measurements for that pipetman. If the second set of data does not look linear, we can clean the inner workings of your pipetman before you try the assay again.
3. There should also be good agreement between the 20 ul measurements made with the P20 and the P200 as well as the 100 ul measurements made with the P200 and P1000. Is there?

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P20 calibration

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P200 calibration

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P1000 calibration

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