Experiment 21: Calibration Curve Review

Purpose
The purpose of this experiment is to reintroduce students with working in a general chemistry lab and to review the basics of making and using a calibration curve.

Background
This absorbance of light will follow the Beer-Lambert Law (Beer's Law), which states that the absorbance of light by a solute in solution is directly proportional to its concentration. The mathematical equation is A = abc, where A is absorbance, a is the molar absorptivity coefficient, b is the path length, and c is concentration.

Calibration curves are graphs that show the relationship between concentration and absorbance of light by the solute. Absorbance is dependent on the concentration of solute in the solution. Therefore, concentration is on the x-axis and absorbance is on the y-axis. The data points obtained in lab are plotted using Excel or Google Sheets. The computer program will put a best-fit line through the data points. The equation of the linear line is obtained, and this equation shows the mathematical relationship in the

$y = mx + b$ format which translates to $A = mc + b$ *(this b is not path length)*

The spectrophotometers that are in the general chemistry laboratory cover the wavelength range of visible light. Therefore, in order to measure the absorbance of light by the solute, the solute must produce a colored solution.

The **first step** to prepare a calibration curve is to make the standard solutions. These are solutions of known concentration. The **second step** is to determine the best wavelength at which to measure the absorbance. The best wavelength is typically the wavelength at which the solute absorbs the maximum amount of light, which is referred to as λ_{max}. The **third step** measures the absorbance of light by the standard solutions and sample solutions, using the spectrophotometer set to λ_{max}.

Chemicals
Blue Dye # 1 2.0%wt
Deionized water

Equipment
50 mL volumetric flasks
10 mL graduated pipet
Parafilm
Plastic dropper
Small test tubes
Spectrophotometer

Procedure

Preparing Standard Solutions *(Solutions of known concentrations.)*
Use the provided stock solution to prepare the standard solutions. Calculate the needed volume of the stock solution for each standard solution, using the dilution equation: $M_c \cdot V_c = M_d \cdot V_d$ *(you are solving for Vc)*

The concentrations of the standard solutions should be:

0.60%wt 0.40%wt 0.20%wt 0.10%wt

1) Use the graduated pipet to deliver the correct amount of stock solution to each 50 mL volumetric flask.
2) Dilute to the calibration mark with deionized water. Make sure the bottom of the meniscus sits exactly at the calibration mark on the neck of each flask.
3) Cover the flask with Parafilm, and invert to mix, 30 – 50 times.

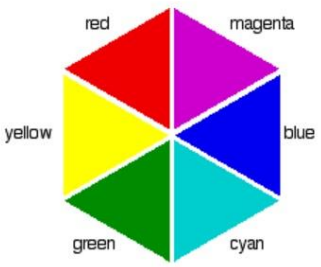
Determining the λ_{max} *(finding the best wavelength for absorbance of light by the dye)*

1) Use the 0.60% standard solution for this part of the experiment. Place this solution into a small test tube, about ¾ full. Put DI water into another test tube, also about ¾ full. The DI water is your blank that you will use to zero the spectrophotometer.
2) The dye looks blue because it absorbs light in the 560 nm – 650 nm region of visible light. Refer to Figure 1. You need to determine the best wavelength in this region at which the dye absorbs light. Turn on the spectrophotometer and set the wavelength to 560 nm.

color region	wavelength (nm)
violet	380 - 435
blue	435 - 500
cyan	500 - 520
green	520 - 565
yellow	565 - 590
orange	590 - 625
red	625 - 740

https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Map%3A_Organic_Chemistry_(Bruice)/13%3A_Mass_Spectrometry_Infrared_Spectroscopy_and_Ultraviolet_Visible_Spectroscopy/13.20%3A_The_Visible_Spectrum_and_Color

Figure 1



3) Put the test tube with the DI water into the sample compartment of the spectrophotometer, and close the lid. Press the zero Abs button. This sets the zero point on the spectrophotometer and subtracts out any absorbance of light by the glass and DI water.
4) Put the test tube of standard solution into the sample compartment and close the lid. Record the absorbance readout in your notebook.
5) Repeat steps 3 and 4 after increasing the wavelength setting by 10 nm. Continue to do this until you have absorbance readings up to 650 nm. You must set the blank each time the wavelength setting is changed.

6) Plot the absorbance vs. wavelength on graph paper (absorbance on the y-axis, wavelength on the x-axis). Report the best wavelength to use when measuring the absorbance of light by the blue dye solution.

Measure the absorbance of light for each solution (standards and sample)

1) Make sure the spectrophotometer is set to λ_{max} for the wavelength.
2) Use the DI water as the blank to zero the spectrophotometer.
3) Transfer each standard solution and unknown into its own test tube, about ¾ full.
4) Measure the absorbance of light for each solution, and record these values into your notebook.

Prepare a calibration curve

Follow the instructions in the Calibration Curve video and use Excel or Google Sheets to prepare a calibration curve. The concentration is the independent variable, so that is on the x-axis. Absorbance is the dependent value, so that is on the y-axis. Plot a scatter plot, linear, and put the best-fit line on the graph. Display the equation of the line on the graph, along with the R^2 value. The R^2 value is not part of the equation of the line, but instead is an indication of how well the data points fit on the line. In other words, how well did you prepare your standard solutions?

Determine the concentration of your unknown.

Use the equation of the line to solve for the unknown concentration. The equation of the line is in the y=mx+b format. Y is the absorbance and X is the concentration. m is the slope and b is the Y-intercept. You know the absorbance of the unknown solution, so solve for X. Show this calculation in your notebook, and report the concentration of the unknown with correct significant figures and units.

Graph Paper to find λ_{max} *(cut this out and put into your notebook)*