Dilution, Calibration Curves, Linear Range and Linear Regression

Instructor notes: DI water is more acid than tap water, use indicator that doesn’t change color in the pH range of 5-7 (don’t use MR, BB and BG are better)

The purpose of this laboratory experiment is to practice making dilutions, to learn to use the Spec-20, to use graphing programs to draw a calibration curve, to add error bars and to perform a linear regression analysis.

Procedure:
1.) Turn on the Spec-20 and allow it to warm up for 20 minutes
2.) Obtain approximately 50 mL of the stock dye solution. Note the dye name, molecular weight and color.
3.) Calculate the molarity of the dye in the stock solution based on the given amount on the reagent bottle.

Qualitative Data Obtaining the UV-VIS spectrum of the dye:
1.) Pipette 2.00 mL of stock dye solution into a 100.00 mL volumetric and dilute up to the mark. Calculate the concentration of the dye.
2.) Obtain a set of cuvettes, make sure they are clean and free of scratches.
3.) Using distilled water in a cuvette as a blank, wipe it clean with Kim-Wipes, place into the Genesis-20 spectrometer, and press the “0 Abs” button to set the blank sample to zero.
4.) Put the diluted dye into another cuvette, wipe with Kim-wipes and place into the Genesis-20.
5.) Measure the absorbance of the diluted dye sample at 340 nm, then remove the dye cuvette and set it aside. Put the blank back into the instrument.
6.) In 10 nm increments, repeat step 3-5 until you cover the entire range of 340-700 nm on the Genesis-20 or until the dye absorbance drastically increases then decreases again. 
   You must reset the blank sample to zero each time you change the wavelength.
7.) Find the wavelength where the dye exhibits maximum absorbance (minimum transmittance).
8.) Set the wavelength of the Genesis-20 to the maximum absorbance wavelength and reset the blank sample to zero (See section below on the detection limit…now!!! Before continuing!!!)

Quantitative data: Preparing a calibration curve
1.) Obtain ~3 mL of the unknown dye solution.
2.) Obtain a perfectly clean and dry cuvette.
3.) Measure the unknown’s absorbance at the chosen $\lambda_{\text{max}}$.
4.) You should use the same cuvette for all quantitative measurements and measure from most dilute sample to most concentrated sample, with triple rinsing in between each concentration. (Why???) You must also make sure that the cuvette is always facing the same direction when placed into the instrument. (Why?)
5.) YOU ARE FIGURING OUT YOUR OWN DILUTIONS. YOU ARE GUESSING!!! YOUR INSTRUCTOR IS NOT GOING TO TELL YOU HOW MUCH TO USE, TRY TO VISUALLY MATCH THE COLOR! To make the best calibration curve, at least 5 different dilutions of stock dye solution must be measured. There must be at least 2 concentrations (absorbencies) lower than the unknown and 2 higher than the unknown. The data points must also follow a linear relationship and cover as wide a range as possible. You should make a rough graph of the data in your notebook as you perform the experiment so that you don’t make unnecessary dilutions and waste time. For example, your dilutions might be 0.10 M, 0.20 M, 0.40 M, 0.80 M and 1.60M and the unknown may be determined to be 0.55 M from the graph.

6.) Samples having Absorbencies of less than 0.005 or greater than 1.6 should not be used if it can be avoided. The linearity of your curve will be affected. (Why?)

7.) Make your dilutions from the stock dye solution. You may use your 2.00 mL dilution from the qualitative section of this experiment if it’s absorbance falls into the correct range, just measure it again after setting the blank to zero at $\lambda_{\text{max}}$

8.) To minimize error, you should make all dilutions from same stock solution. If your calculations lead you to need an amount smaller than the smallest volumetric pipette (0.1 mL) we have, you are advised to dilute the original stock solution and then make all dilutions from the diluted stock solution. For example, dilute the stock solution by 50% and use twice as much in each dilution.

9.) You should measure 3 different aliquots from each dilution to allow you to calculate the average and standard deviation.

**Estimating the detection limit:** (should be done at various points along the experiment)

1.) You should take at least 5 measurements of the blank solution at the absorbance maxima over the course of the experiment. After setting the blank to zero in Qualitative step 8, do not re-set the blank for the remainder of the experiment. Just measure the blank to get an idea of how much your blank might drift or fluctuate over the course of the experiment.

**Calculations and Laboratory Write Up:**

**Qualitative Section:**

1.) Graph Absorbance versus wavelength using Excel. You must choose XY scatter for your Chart Type. Note the Absorbance maximum on the graph. Using Beer’s Law to calculate $\varepsilon$ (the molar absorptivity) for your dye at $\lambda_{\text{max}}$: $A = \varepsilon bc$ assuming a path length of 1 cm and the concentration that you calculated. Don’t forget the correct units on $\varepsilon$.

**Quantitative Section/Using Excel Spreadsheet**

2.) Subtract the average blank measurement from all other measured absorbances.

3.) Calculate the average absorbance for each concentration as well as the standard deviation (in the y direction) and the uncertainty in the volumes (in the x direction, figure out how to propagate the error for concentration).

4.) Follow the example in chapter 4 of your textbook for the rest of the steps. They have also been outlined below.
5.) Make 4 columns in your spreadsheet: Concentration, Absorbance and Standard Deviation (Y); Uncertainty (x). Discuss the magnitude of the standard deviations and site sources for these deviations.
6.) Plot the data in Excel. You must use XY scatter as the chart type. Do not allow the program to connect the dots. Just have it graph the points. (Absorbance vs. Concentration)
7.) Put the standard deviations on the graph by right clicking the mouse one time while pointing at the data. Go to FORMAT DATA SERIES. Click on the tab that says Y-error bars. Go to custom. Click on the small grey box by the + error bars and the program will take you back to your spreadsheet. Using the left mouse button, highlight the standard deviation column and hit enter. Do the same for the – error bars. Repeat for the X-error bars.
8.) *You may use excel to calculate the equation of the line via the linear regression, Right click the mouse on the graphed points, select add trend line, Linear Regression, Options Tab-- display equation on chart and R values. However, for full credit, you must show the linear regression calculations. You should get close to the same answer that Excel gets.
9.) Use the equation of the line to determine the concentration of the unknown.
10.) Find the uncertainty (s_x) in the calculated concentration (chapter 5 in your text). This equation propagates your calibration curve errors in the x direction. (Your text gives you the excel equation for this calculation) Calculate the 95% confidence level.
11.) What is the linear range of the data? Are any of your data points outside the linear range of the data? Explain
12.) Does your intercept go through zero? If not, discuss the nature and source of this error (note, there is no y-intercept in the Beer’s Law equation)

**Detection limit calculation:**
13.) Calculate the detection limit as discussed in class. z = 3, S_reag is the average blank signal and σ its standard deviation. Find (SA)_DL from the equation below, then put (SA)_DL into your equation of the line for y and calculate the detection limit (x).

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(S_A)_{DL} = S_{reag} + z\sigma
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* Note: Since we have measured the uncertainty (standard deviation) at each point along the curve, the linear regression line is best calculated as a “weighted linear regression”. This is much more complicated and the calculation of the uncertainty in the final concentration is not presented in your text due to the complexity.