

Guided Inquiry External Calibration Activity (Used in Experiment 8):

Objectives:

- Learn how to make and calculate the concentrations of standard dilutions from a stock solution.
- Learn about external calibrations and how to quantitate a sample with them.
- Learn how to perform a linear regression in EXCEL.
- Learn how to confirm that your data truly fits a linear model.
- Learn how to find unknown concentrations from regression analysis of an external calibration.
- Learn how to find the confidence limits for unknown concentrations using regression data.

Background Resources

Chapters 4 and 5 in Quantitative Analysis by Daniel Harris

Instructions

Download and read the Experiment #8 Experimental Handout on eLC. Then read each section of this guided inquiry and answer any questions along with your work. You will record your answer in an EXCEL file that is provided. The worksheets of this file will be uploaded and graded on GradeScope. Look in the Experiment 8 folder on eLC to find the file.

Regression Analysis

Regression methods allow researchers to model trends in data between two or more variables. The model can be based on many different mathematical functions: linear, polynomial, power, logarithmic, etc. In this lab, we will look at how the absorbance of a solution varies with the concentration of an iron o-phenathroline complex. The condition under which the complex is formed are covered in the lab's experimental handout, but it should be emphasized that proper sample preparation is crucial before an analysis. Sample preparation encompasses equilibria such as complex formation, redox chemistry and buffers.

The absorbance of a solution is governed by the Beer-Lambert Law and is specific to the wavelength of light used to measure the absorbance. The molar absorptivity (ϵ , $M^{-1} \text{ cm}^{-1}$), pathlength (b , cm) and concentration (c , M) can be multiplied to give the unitless absorbance:

$$A = \epsilon bc$$

The absorbance is measured by an instrument called a spectrometer. This instrument uses an appropriate light source and selects the wavelength to use for the measurement. A sample cell or cuvette with a known pathlength, b , is then used to measure the intensity of that wavelength of light making it through a blank sample, P_o . The intensity of the light through a sample containing the absorbing analyte is then measured as P . Using these values, we can get transmittance and percent transmittance of the solution

$$T = \frac{P}{P_o} \quad \text{and} \quad \%T = T \times 100\%$$

The transmittance is related to the absorbance by the following relationship

$$A = -\log\left(\frac{P}{P_o}\right) = \log\left(\frac{P_o}{P}\right)$$

Problem:

A blank gave an intensity of 628 mV at a wavelength of 450 nm in a 2.00 cm cell. A sample gave an intensity of 247 mV. Download and load into EXCEL the file 'Guided Inquiry External Calibration Activity EXCEL Student' from eLC. **** Do your calculation in this EXCEL answer sheet on the worksheet named 'Problems 1-3.'**

1. What is the transmittance of the solution at this wavelength? (Show work and units)
2. What is the absorbance of the solution at this wavelength? (Show work and units)
3. The molar absorptivity of the analyte is $3.45 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$. Find the concentration of the analyte.

The absorbance of various standards of the iron o-phenanthroline complex can be measured to quantitate the iron in an unknown. These relationships are integral in understanding this lab. In the following, we will not use absorbance but look at emission of light from a sample. This should not be a roadblock as you can apply the same concepts to the absorbance and concentration data in the lab.

Definition: A stock solution is a concentrated standard solution used to prepare, by dilution, lower concentration standard solutions for analysis.

Definition: A standard solution contains a well known concentration of an analyte (ion or compound) and is used as a standard for an external calibration. A special standard called a 'blank' does not contain any analyte.

Definition: An external calibration assumes that the measured analytical signal is proportional to concentration. A series of standards are made containing the analyte and their analytical signal measured. Any matrix effects are usually ignored, e.g. the unknown is pond water and distilled water is used as the solvent for the standards. Signal versus concentration data are plotted and a regression performed to find the best-fit mathematical model (usually linear).

Performing an External Calibration

Let's suppose ACME Chemical is performing an analysis for sodium in water samples. The sample is aspirated into a flame photometer, which excites the sodium ion in the sample and causes them to emit light at 589.5 nm. A simple flame photometer measures this emission and records a signal level, in arbitrary units, which is proportional to the concentration of sodium ions. For the results to be valid, a series of standard solutions must be made by diluting from a stock solution. The signal for each is measured and an external calibration performed. After that, unknowns can be analyzed and their concentration determined from the calibration model.

Definition: Dilution factor, DF, is an expression, which describes the ratio of the final volume to the aliquot volume.

$$DF = \frac{\text{Aliquot Volume}}{\text{Final Volume}}$$

The dilution factor is derived from the well-known dilution equation: $M_1V_1 = M_2V_2$. Here V_2 is the total volume of the dilution (Final Volume) and V_1 is the volume of the aliquot of the stock solution that is used. Of course, M_1 and M_2 are the concentrations of the concentrated solution and diluted solution, respectively.

Preparing Dilutions

0.6355 grams of primary standard sodium chloride (MM = 58.4428 g/mol, 39.3372% Na^+ , 60.6628% Cl^-) was dissolved in a 500.00 mL volumetric flask.

4. Determine the molar concentration of the SODIUM ION in this stock solution. Show all work and units. ** Do your calculation in the EXCEL answer sheet on the worksheet named 'Standards.'

This Na^+ stock solution is used to make the following standard solutions for an external calibration. Each dilution is made by adding the appropriate stock volume to a 100 mL volumetric flask and diluting to the mark with distilled water. The solution is thoroughly mixed by inverting it 10x.

5. Find the molarity of the sodium ion for each standard using the dilution equation: $M_1V_1 = M_2V_2$. Do these calculations in the worksheet named 'Standards.'

Standard	Stock Volume (mL)	Na Standard Concentration, M	Emission Signal, millivolts (mV)
A	0		0.230
B	2.00		166.7
C	5.00		369.6
D	10.00		910.4
E	15.00		1131.9
F	20.00		1675.8

6. For dilute solutions the density is ~ 1.000 g/mL, so $1 \text{ ppm} \equiv 1 \text{ mg/L}$. What is the ppm concentration for standard B? $\text{AW Na} = 22.9900 \text{ g/mol}$. Do this calculation in the worksheet named 'Standards' in the appropriate area.

Look at the worksheet named Sodium Iron Analysis. The yellow highlighted cells are where you will input data or formula. Do not mess with the other cells. In Cells A13 to A18 copy the concentrations you calculated on the Standards worksheet as well as the signals on the same work sheet.

An *external calibration* curve is a plot of an instrumental signal versus the concentration of the analyte. You should be able to make such a plot on your own. A model is usually used to fit the data and the fit process is called a *regression* as discussed previously.

7. Look at the graph on the right of the worksheet. Will a linear model fit this data, i.e. $y = mx + b$? Explain. Answer in cell E1 on the worksheet.
8. In cell E2 symbolically rewrite the equation for the best-fit line that models the data using 'S' for the signal and 'c' for the concentration use m and b as they are defined.

The regression data parameters can be calculated by using the LINEST function in excel. Highlight cells B22 through C24. Enter " $=\text{LINEST}(\text{Emission}, \text{Concentration}, \text{TRUE}, \text{TRUE})$ ". The press and hold CONTROL-SHIFT-ENTER. The regression parameters should appear.

Definition: The correlation coefficient, r , is a measure of the goodness of fit between two variables. It can be any real number in the range $\{-1 \dots +1\}$. $r = +1$ is perfect positive correlation between x and y (directly proportional and slope is positive). $r = 0$ means there no correlation between x and y . $r = -1$ indicates a perfect inverse relationship between x and y and a negative slope. Generally if $r \geq 0.85$ you can say there is a correlation.

Definition: The coefficient of determination, r^2 , represents the proportion of the variance in the dependent variable, y , that is predictable from the independent variable, x . A value equal to 0 means you cannot predict the x variable from the y . A value of 1 means that you can find the x value from y with no error. A value between 0 and 1 tells you the probability (percentage) that the variance in y is predictable from x . For instance, a value of 70% indicates that 70% of the variance in Y is predictable from x .

9. Examine the 'External Calibration Graph' and determine *visually* if the value correlation coefficient, r , is positive or negative. Explain.
10. What is the coefficient of determination? What does it tell you?
11. Calculate r from the coefficient of determination (you may have to add a sign!). What do these two values tell you about the goodness of your fit?

Definition: Residual: The difference between the y value, y_i , and its estimation from a regression fit, \hat{y} , is called the residual or residual $= y_i - \hat{y}$.

Definition: A residual plot is constructed by plotting the residuals for each y value against their corresponding x values. If the plot is 'random', this indicates a good linear fit. A parabolic relationship (or inverted parabolic relationship) indicates a poor fit. The fit assumes the data's variance around the regression is the same for all x-values and is randomly distributed. The latter is a call homoscedasticity.

Show the calculation (with units) of the residual value for the first y data point in the calibration plot. Do it on paper here and look at the formula used to find it. There is nothing to enter on the EXCEL file here.

To confirm the goodness of the fit, look at the residual plot on the EXCEL sheet.

12. Does this plot, your r value and the r^2 value confirm a linear relationship based on the definitions above? Explain either way.

Unknown Analysis

An unknown analysis is now possible. Three separate unknowns, A, B and C were measured in triplicate thus $k = 3$. The average values for each one were 205.6, 750.7 and 1200.1, respectively.

Confirm on paper the calculation of the unknown concentration B using the value of the slope and intercept (show units). The slope, m, and intercept, b, can be found in the Regression Data parameters given on the Sodium Analysis worksheet.

Enter each of these values into the 'Mean measured y' cell (B32) and enter 3 for 'k' in cell B33. Fill in the table below from the data calculated for each unknown. This table can be found on the 'Unknown Analysis' worksheet.

Unknown	Mean Signal, $k = 3$	Derived x, M	Uncertainty in derived x, u_x	95% Confidence Limit
1	205.6			
2	750.7			
3	1400.1			

Format each unknown concentration to the correct number of significant figures based on the 95% confidence limit.

13. Comment on the variation in the uncertainty and confidence limit as the signal of the unknown increases. Note any trends. Answer this in cell A11.
14. Based on what you observed, what region of the calibration plot will give the minimum area? Is this where you would like your unknown signals to be? Explain. Answer this in cell A15.

15. Use concentration of the unknown #1 and its uncertainty, find the relative uncertainty of the concentration for unknown #1. Answer this in cell A19.
16. Let's say that the original unknown was diluted by a factor of 10, and this was the solution that was analyzed above. Use the answer to question 15 to find the absolute uncertainty in the concentration of the undiluted unknown. Find the 95% confidence limit of this concentration as well. Answer this in cell A23.

Explain how increasing each of the following will affect the confidence limit of the measurement.

17. Number of Data Points used in the regression analysis. Answer this in cell A27.
18. Replicate measurements of the unknown. Answer this in cell A32.
19. The slope of the calibration plot. Answer this in cell A39.
20. The standard deviation of the data point y-values. Answer this in cell A46.