

Measuring Nickel in an Unknown Solution (2 week lab)

Before lab (week 1):

1. Read lab, watch video, do reading
2. Write procedure and prepare data tables in your bound lab notebook.
3. Calculate exact concentration of $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ from Lab 1.
4. Fill in the tables (step 2 of each procedure) using $M_1V_1=M_2V_2$, so you know how much of what you need to add to make your solutions for analysis.

Remember: You will only be allowed your lab notebook, writing implement, and calculator during the lab period.

Before lab (week 2):

1. Read procedure for week 2.
2. Prepare your lab notebook with a procedure and data tables.

Introduction:

As we have discussed in class, measurements are meaningless unless they are calibrated against known standards. This means that in order to have confidence in your results, you must first have confidence that your measurements are accurate and precise. A good first step in this process is to calibrate your instrumentation with standards of known composition. The purpose of this lab exercise is designed to familiarize you two calibration methods (calibration curve and standard addition method) and make measurements using the UV-Vis spectrophotometer.

Calibration curves are the easiest way to correlate an instrument reading to a concentration value. To do this, you make standards of a variety of known concentrations and these measurements can be graphed as concentration versus the intensity of instrument response (in the case of UV-Vis, this is absorption). There should be a very linear relationship between detector response and concentration, generally quantified as r-squared, because of Beer's Law.

Beer's law states that absorption is equal to the concentration times path length times the molar absorptivity. Thus, when the line of best fit ($y=mx+b$) is determined for the calibration curve, y is absorption, x is concentration, b should be close to zero (when the concentration is zero, the absorption should be zero), and m is the slope is the molar absorptivity times the path length (usually the path length =1 cm). A linear calibration curve is the first step to

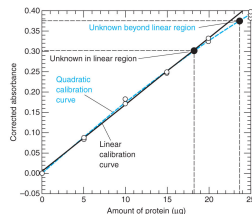


Figure 1: Calibration curve (Harris, 8th ed).

taking both accurate and precise instrument readings (Figure 1). We will be working on generating calibration curves during week 1.

During week 2, we will be using an alternate calibration method, called Standard Addition (Figure 2). This method is preferred when the analyte is contained in a complex matrix- meaning the analyte is mixed with something that may effect the apparent concentration. In this case, the standard addition method is used to make an accurate determination of the analyte concentration. In this method, an instrument response is measured as an increasing known amount of a standard is added to the sample solution. Only small volumes of a concentrated standard solution are added so the sample matrix is not significantly altered. This data can be fit with the line of best fit ($y=mx+b$) where y= instrument response, x= concentration of analyte from added standard, and m is still the molar absorptivity times the path length. The intercept, b is no longer zero because of the matrix effects and analyte in the initial sample solution. The concentration of the analyte in solution can then be calculated by calculating the x-intercept of the line. This type of quantitative analysis is a very important technique to acquire as samples in real life often have matrix effects associated with them and being able to compare the analytical signal between the sample and standards is vital to being able to quantify analytes of interest.

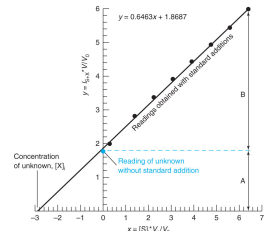


Figure 2: Standard Addition (Harris, 8th ed).

This lab will not only give you a chance to work on your laboratory techniques (i.e. preparing solutions and making necessary dilutions), but also provide you with the skills necessary to measure concentrations of an analyte in an unknown solution with or without matrix effects. In addition, you will gain hands on experience calibrating a widely used instrument (UV-VIS spectrophotometer). All these skills are necessary if you are considering work in any analytical, environmental, industrial (and so many more!) field.

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Procedure

Week 1: Quantifying Nickel using the Calibration Curve Method

5. Get two unknowns from your TA. Record the ID #s.
6. Using your 0.1 M $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ solution from week 1, determine how much $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ stock solution to achieve the final concentration specified with a total volume of 5 ml. You must add a volume of EDTA equal to the volume of $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ stock.

[Ni] (M) ideal	0.1 M $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ stock	0.1 M EDTA	RO H_2O (mL)	Total (ml)	[Ni]* (M) actual
0.05	2.5	2.5	0	5	
0.04				5	
0.03				5	
0.02				5	
0.01				5	
0.005				5	

* denotes calculated value

7. Measure solutions on the UV-Vis spectrophotometer at the absorbance max and a baseline absorbance (480 nm). Be sure to make triplicate measurements of each solution so that you are able to calculate a standard deviation for each point.

Make measurements for all samples in random order. Rinse the inside of the cuvette two or three times with the first solution. Then fill it 2/3 full, check for physical problems, and scan an absorbance spectrum. Record the absorbance values at 592 nm and at 483 nm and calculate the corrected absorbance. Pour the contents of the cuvette into a waste beaker, rinse the inside of the cuvette two or three times with the next sample, and repeat the above operations. Record your data in a table.

8. Absorption spectra, needed for the lab writeup, are available on the webpage for the blank, standards, and unknown solutions.

Week 2: Quantifying Nickel using the Standard Addition Method

1. Get one unknown from your TA. Record the ID #.
2. Using your 0.1 M $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ solution from week 1, perform serial dilutions according to the following table:

	Unknown sample (mL)	0.1 M $\text{NiSO}_4 \cdot 6\text{H}_2\text{O}$ stock (mL)	0.15 M EDTA (mL)	RO H_2O (mL)	Total (ml)	[Ni]* (M)
1	0.5	0	0.5	4	5	
2		0.1			5	
3		0.5			5	
4		1			5	
5		1.5			5	
7	0.5	2	2.5	0	5	

* denotes calculated value

3. Measure solutions on the UV-Vis spectrophotometer at the absorbance max and a baseline absorbance (480 nm). Be sure to make triplicate measurements of

each solution so that you are able to calculate a standard deviation for each point.

Calculations and Discussion Questions:

Calculations:

1. Generate a calibration curve in excel and perform a least squares fit. Determine the line of best fit with the equation $A=\epsilon[\text{Ni}] + b$. Determine the errors associated with A, ϵ , b. Determine R². Show your calculations on a separate sheet of paper.
2. Determine the concentration and uncertainty of Ni in your unknown. Show your calculations.

Discussion questions:

Compare and contrast the use of calibration curve and standard addition within the context of your results. Be specific and quantitative where possible.

1. Report the equations ($y=mx+b$) and R² values for each method in a table. In your best judgment, will these calibration curves yield accurate and precise results?
2. Report your unknown concentrations for each unknown and each method in a single table. What differences do you see? Why? If necessary, use statistical tests to determine if any observed differences are statistical. Be sure to show your work on attached calculations page.
3. What do you think main source of error in your experiment was? How would each of these things have affected your results? What would you do different next time?

Lab write-up: Due Friday

Annotated Figures: Bring annotated figures to class on the Friday before the report is due. These are print out copies of your figures and tables for a lab report. Either on these print outs or on an accompanying sheet, you will make a table (shown below) to record a one sentence summary of the figure, observations (to go into the results section of your report), interpretations (to go into the discussion), and a sentence conclusion. These will be checked off in class and provide a starting point for group discussion for writing the lab report.

What does the figure show?	
Observations	Interpretations
Conclusion	

Lab write-up: Due Monday

See example on blackboard.

1. **Purpose:** write a 1-sentence purpose for the entire lab.
2. **Results:** tabulate your measurements and results; show your graphs (1. Overlain spectra, 2. Calibration curve, 3. Standard addition), and briefly discuss your results.
3. **Discussion:** respond to the questions above in paragraph form (not Q1: answer, answer). Devote 1-2 paragraphs to discuss each question.
4. **Conclusion:** Write a short paragraph concluding what you learned.
5. **Group dynamics:** let me know how you feel your group worked together.
6. **Appendix 1- Calculations:** You can send me your spreadsheet.