Project 1: Investigation of Analytical Uncertainties

LABORATORY REPORT: Informal Report

PRE-LAB ASSIGNMENT: CLASS DAY 1

- Read the entire laboratory project and sections a1A – a1B (pp. 967–983) and 6D (pp. 157–159) in Skoog *et al*.[1]
- Prepare, on a typed sheet of paper, the Project Objectives of this lab (please consult the appendix for an example); on the same sheet, complete the assignment below:

1) Prior to the pre-laboratory discussion, find the chemical formula, molecular weight, and Safety Data Sheet (SDS) for the disodium salt of Erythrosin-B (#198269) on the Sigma-Aldrich web site. Read the SDS and record the HMIS Classification and health hazards.

PRE-LAB ASSIGNMENT: CLASS DAY 2

2) The following problem has been taken from Skoog *et al*.: Six bottles of wine of the same variety were analyzed for residual sugar content with the results below. a) Find the mean, variance, and standard deviation for each set of data. Show a complete set of sample calculations for Bottle 5. b) Pool the data to obtain a standard deviation for the method.

Bottle	Percent (w/v) Residual Sugar
1	0.99, 0.84, 1.02
2	1.02, 1.13, 1.17, 1.02
3	1.25, 1.32, 1.13, 1.20, 1.12
4	0.72, 0.77, 0.61, 0.58
5	0.90, 0.92, 0.73
6	0.70, 0.88, 0.72, 0.73

[1] Skoog, Holler, and Crouch, *Principles of Instrumental Analysis*, 6th ed., Thomson Brooks/Cole, Belmont, CA, 2007.

INTRODUCTION

In this experiment you will investigate the uncertainties associated with an instrumental analysis. Although every effort is made to minimize them, uncertainties creep into all analyses at the sampling, sample preparation, and measurement steps. In order to reduce the overall uncertainty, the step with the largest uncertainty must be identified and measures taken to reduce the uncertainty at that step.

In statistics the uncertainty is usually reported as the **standard deviation** σ or the **variance** σ^2. When small numbers of samples are measured, as is often the case in chemistry, $\boldsymbol{s}$ and $\boldsymbol{s^2}$ are used for standard deviation and variance rather than σ or σ^2. Assuming that only random errors are involved, the total variance for an analysis is the sum of the variances at each of the three steps. Thus,

$$s_{tot}^2 = s_{samp}^2 + s_{prep}^2 + s_{meas}^2 \quad (1)$$

Although the standard deviations are not additive, the value of *s* at each level is often calculated and reported because the standard deviation has the same units as the measurement itself.

For this experiment, the three steps of your analysis will consist of (i) sampling a solid, (ii) dissolving the sample in a solvent, and (iii) measuring the absorbance with a spectrophotometer. The magnitude of the variance for each step will be investigated using a three-level nested design experiment. In Level I, several independent samples are taken of a solid. Subsamples of each sample are independently prepared for analysis in Level II, and several independent measurements are made on each subsample in Level III. Data from such an experiment are shown in the accompanying spreadsheet in Figure 1.

The absorbance measurements in Level III are used to find the concentration of the compound Erythrosin-B (ErB) in solution. A calibration curve is necessary to convert the absorbance measurements into concentrations. According to Beer's Law, absorbance is directly proportional to concentration,

$$A_\lambda = \varepsilon b c = \varepsilon \ell c \quad (2)$$

where the proportionality constant is ε, also known as the **molar absorptivity**; the **pathlength** through the sample is *b* or ℓ; and the **concentration** of the absorbing species is *c*. Because the absorbance is a unitless quantity, ε must have units that cancel the units of *b* and *c*. In the common case where *b* (or ℓ) has units of centimeters (cm) and *c* has units of molarity (M), then ε will have units of liters per mole centimeter (L mol^{-1} cm^{-1} or M^{-1} cm^{-1}). Note that ε and therefore *A* are wavelength dependent, so the wavelength must be specified. Often the absorbance is measured at the wavelength of maximum absorption, λ_{max}. When concentration units other than molarity are used, the symbol *a* is used for the proportionality constant.

The calibration curve is constructed by measuring the absorbance (dependent variable) of solutions with different known concentrations of ErB (independent variable) and plotting the data on a scatter graph. A straight line is fitted to the data. This line should go through the origin if the solvent itself does not absorb at the wavelength of interest. The slope of the best-fit line is□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□□ chosen □□□□□□□□□□□□□□□□□□□□□. Statistical functions in spreadsheet software can calculate the slope, and the uncertainty (standard error) associated with the slope.

EXPERIMENTAL: ORGANIZATION

Students in each laboratory section will be designated as Investigator (R) 1 through Investigator *N* where *N* is the number of students in the section, or R1 through R*N*. Each investigator will contribute one point to the calibration curve and analyze one random sample of the solid unknown mixture. Record in your notebook the investigator assignments for your section.

EXPERIMENTAL: DAILY PLAN

Prior to beginning laboratory work, students should have in their notebooks a daily plan based on pre-lab discussions. Have the instructor check your plan every day before you begin work. For example, on the first lab day the plan should include the assignment of investigators and the distribution of responsibilities for generating a calibration curve. Investigators should also specify which pipet and volumetric flask combination they will use to make their assigned concentration from a 1×10^{-4} M (100 μM) stock solution of ErB.

EXPERIMENTAL: CALIBRATE A GLASS PIPET AND PIPETTOR (SEE EXPERIMENT 2, APPENDIX 2)

Each investigator will calibrate a 1-mL volumetric pipet and an adjustable micropipettor with a maximum volume of 1000 μL. You will test the accuracy and precision of the pipet and pipettor by dispensing water into a beaker or weigh boat on an enclosed analytical balance.

Practice transferring 1 mL of water with a Class A volumetric pipet. Then test your technique by taring a small beaker on an analytical balance and transferring 1 mL of water, including touching the pipet tip to the inside of the beaker to draw out the correct volume. Record the mass in your lab notebook; without taring, add at least three subsequent aliquots, recording the mass each time. The tolerance (error) of volumetric pipets can be found on page A4 of this manual.

Practice dispensing volumes with the 1000 μL adjustable micropipettor. Test the pipettor at both the minimum and maximum volumes. Select a pipettor volume, tare a weigh boat, dispense an aliquot of water into the weigh boat, and record the mass in your lab notebook. Without taring, add at least four subsequent aliquots, recording the mass each time. Pipettors should deliver aliquots that are accurate and precise to within 3% of the selected volume.

Record the temperature of the lab room.

EXPERIMENTAL: CALIBRATION CURVE

The unknown contains Erythrosin-B (ErB), a water soluble acid-base indicator that is red-orange in solutions with pH > 3. A stock solution of ErB with accurately known concentration will be provided. Each investigator will prepare, using volumetric glassware, one calibration standard from the stock solution and measure its absorbance at λ_{max} on a Cary UV-Visible spectrophotometer. The standards should span the range from about 2×10^{-6} M to 2×10^{-5} M (2 μM to 20 μM).

The instructor will demonstrate how to use the Cary spectrophotometer for both wavelength scans and quantitative absorbance measurements. All investigators within one lab section should use the same instrument. Each investigator must first scan the solution over the visible region (750 nm –350 nm) in double-beam mode using glass cuvets. Use the instrument software to find λ_{max}.

1. *Include in your informal report a copy of the visible scan.*

Investigators must compare scans so that all quantitative absorbance measurements are taken at the same wavelength, λ_{max}. When measuring the absorbance, be sure to zero the instrument and take multiple readings (5 to 10) of your standard. Post the absorbance data (reported as mean ± standard deviation) and exact concentration for the class. Use the values from all investigators to construct a calibration curve as described under data analysis.

EXPERIMENTAL: UNKNOWN PREPARATION AND ANALYSIS

The unknown is a mixture of two solids, NaCl and ErB. Each investigator will take a random sample of about 0.5 g from the unknown mixture. The samples will be labeled R1 through R*N* as previously assigned. After grinding with a mortar and pestle, investigators will prepare two solutions from their sample, R1A and R1B for example. To prepare the solutions, accurately weigh on an analytical balance two 0.2 g subsamples, quantitatively transfer them to different 50 mL volumetric flasks, and dilute to volume with water. Have the instructor check your filled volumetric flask before proceeding.

Measure the absorbance of four different aliquots of each subsample solution: Zero the instrument before measuring each subsample solution (but not each aliquot); then for each aliquot rinse and fill a cuvet with the subsample solution. Use the same cuvet for all measurements and take multiple readings (replicates) of each of the aliquots. In total, the class will collect four sets of replicate data for each of the 2*N* subsamples. Label the aliquots by investigator, R1A1, R1A2, etc., and record the absorbance data. The average absorbance for each aliquot will be used in further calculations.

DATA ANALYSIS: CALIBRATION OF A GLASS PIPET AND PIPETTOR

Find the density of water at the laboratory temperature. Convert each of your mass readings to the equivalent volume of water.

2. *In your informal report, note the lab temperature and the density of water at this temperature. Cite your reference for the latter value.*

3. *Build a data table that includes:*
    a. *the pipet or pipettor volume, V_{sel}*
    b. *average volume dispensed ± standard deviation = $V_{disp} \pm s$*
    c. *% error = $100 \times (V_{disp} - V_{sel})/V_{sel}$*
    d. *% relative error = $100 \times s/V_{disp}$*

4. *Comment on the accuracy and precision of the volumetric pipet compared with the pipettor at both volumes. Which pipet is more susceptible to human error (technique)?*

DATA ANALYSIS: DETERMINATION OF % ERB

Use the class data to generate a calibration curve in Excel by fitting the data to a *line with a zero intercept*. Use Regression Analysis to find the standard errors of the slope and regression. Plot the data with *y*-axis (absorbance) error bars and show the equation on the chart. Everyone must use the same value of the slope in subsequent calculations; confirm with the other investigators that your fits yield the same result (with correct significant figures given by the standard error).

5. *Include in your informal report a data table with the exact concentrations and reported absorbances (± standard deviation) for the ErB standards.*

6. *Include in your informal report the calibration curve with absorbance error bars and best-fit equation. Note in the caption if the error bars are too small to be seen.*

Calculate the mass percent of ErB in the unknown from the average absorbance of each aliquot, the molar absorptivity, and the mass of the subsample. Errors arising from use of calibration curves will be investigated in the next project; for this project use significant figures for reporting the concentrations and % ErB. Post your results for the class in the Google Doc spreadsheet as soon as you have finished the calculations.

7. *Include in your informal report a data table with the average absorbance, the calculated concentration, and the % ErB for each aliquot. Provide a reference to the exact page in your notebook where the sample calculation can be found.*

DATA ANALYSIS: NESTED EXPERIMENT CALCULATIONS

In order to assess the sources of error in this experiment, you must determine the standard deviations of measurement at each stage of the process: s_{samp}, s_{prep}, and s_{meas}. This determination will require a sequence of calculations that are best carried out with a spreadsheet. The data needed to construct the example spreadsheet in Figure 1 will be provided by the instructor; you should build the example spreadsheet using the procedure detailed below and then populate the spreadsheet with your data. In this procedure you will work systematically from Level III to Level I of the spreadsheet, and in the process find s_{samp}, s_{prep}, and s_{meas}.

The **variance** s_i^2 for one set of replicates (R1A1-R1A4) is given by the usual equation:

$$s_i^2 = \frac{\sum_{j=1}^{n} \left(d3_{i,j}\right)^2}{n-1} \qquad (3)$$

In equation (3), $d3_{i,j}$ are the deviations from the mean for each percent ErB value within set i of replicate aliquots, and $\boldsymbol{n = 4}$, the **number of replicates in each set**. Therefore, in your spreadsheet you can use the statistical functions in Excel to calculate the average and variance for each set of replicate aliquots (Level III columns in Figure 1).

The **variance in measurement** is the same as the variance at Level III of the experiment, and is equal to the average variance among the $2N$ sets of replicates, where $\boldsymbol{N}$ **is the number of investigators**:

$$s_{meas}^2 = s_{III}^2 = \frac{1}{2N}\sum_{i=1}^{2N} s_i^2 \qquad (4)$$

Find the *variance in measurement* by calculating the average of the variances of the sets of replicates, i.e. equation (4). Calculate the corresponding *standard deviation in measurement* by simply taking the square root of the variance.

At Level II the variance is equal to the average variance between the N pairs of subsamples:

$$s_{\mathrm{II}}^2 = \frac{1}{N}\sum_{k=1}^{N} s_k^2 = \frac{1}{N}\sum_{k=1}^{N}\left[\sum_{l=1}^{2}\left(d2_{k,l}\right)^2\right] \tag{5}$$

Here, s_k^2 is the variance for a related pair of replicate averages, like R1A and R1B. Note that $d2_{k,l}$ is the difference between one of the replicate averages (e.g., R1A) and the average of the two subsamples, (R1A and R1B). Since there are only two values in this variance calculation, the sum of the squares is divided by 2 – 1 = 1 (Level II columns in Figure 1).

The **variance at Level II** contains both the preparation and measurement variances:

$$s_{\mathrm{II}}^2 = s_{\mathrm{prep}}^2 + \frac{s_{\mathrm{meas}}^2}{n} \tag{6}$$

In equation (6), the measurement variance is divided by n to account for the *number of replicate values in a given set* used to find each average in Level III. The **variance in preparation** can then be calculated from equation (7):

$$s_{\mathrm{prep}}^2 = s_{\mathrm{II}}^2 - \frac{s_{\mathrm{meas}}^2}{n} \tag{7}$$

Calculate the corresponding *standard deviation in preparation* by simply taking the square root of the variance.

At the top level, Level I, the variance for the set of N averages by investigator contains terms for measurement, preparation, and sampling:

$$s_I^2 = s_{samp}^2 + \frac{s_{prep}^2}{2} + \frac{s_{meas}^2}{2n} = \frac{\sum_{k=1}^{N} (d1_k)^2}{N-1} \quad (8)$$

The quantity $d1_k$ is the difference between the average % ErB for a particular investigator (R1, etc.) and the average % ErB of the N investigators. The $2n$ and 2 in the middle equality are the number of values averaged in Level III and II, respectively, that are part of the Level I averages. You can use the statistical functions in Excel to calculate the average and variance at Level I and then find the **variance in sampling** as

$$s_{samp}^2 = s_I^2 - \frac{s_{prep}^2}{2} - \frac{s_{meas}^2}{2n} \quad (9)$$

Calculate the corresponding *standard deviation in sampling* by simply taking the square root of the variance (Level I columns in Figure 1).

When you have duplicated the example spreadsheet exactly, modify it for your class data. Adjust the number of investigators (N) if necessary and enter all of the % ErB from the class.

8. *Include a printout of your spreadsheet in your informal laboratory report. Be sure to format your spreadsheet so that all the data and calculated values are clearly labeled and easy to find.*

The average at Level I is the result one would obtain from a simple analysis of N samples of the mixture; thus further statistical analysis at Level I is useful. The 95% confidence interval for Level I and for the whole data set below should be calculated with the "Confidence.T" function in Excel.

9. *In addition to the average and variance at Level I, calculate the standard deviation and 95% confidence interval for the set of N values and include them on the spreadsheet.*

10. *Which step (measurement, preparation, or sampling) had the largest variance? Why might this be the case? What would you need to do experimentally to improve the precision of the analysis?*

11. *Using the standard deviations of measurement, preparation, and sampling, how many significant figures can be used to report the average at each step?*

DATA ANALYSIS: WHOLE DATA SET CALCULATIONS

Because of grouping, the average, standard deviation, and 95% confidence interval calculated above are likely different than that for the entire set of 8*N* values. Calculate a mean, standard deviation, and 95% confidence interval treating the 8*N* data points as one set of data.

12. *Include the results of the whole data set calculation on your spreadsheet.*

13. *Compare the 95% confidence interval of Level I with that of the whole data set. Which is smaller? If larger data sets have better precision, why do chemists usually work with a small number of replicates?*

DATA ANALYSIS: SUMMARY

14. *Provide a Summary paragraph as described in "Writing in the Chemistry Laboratory: The Informal Report."*

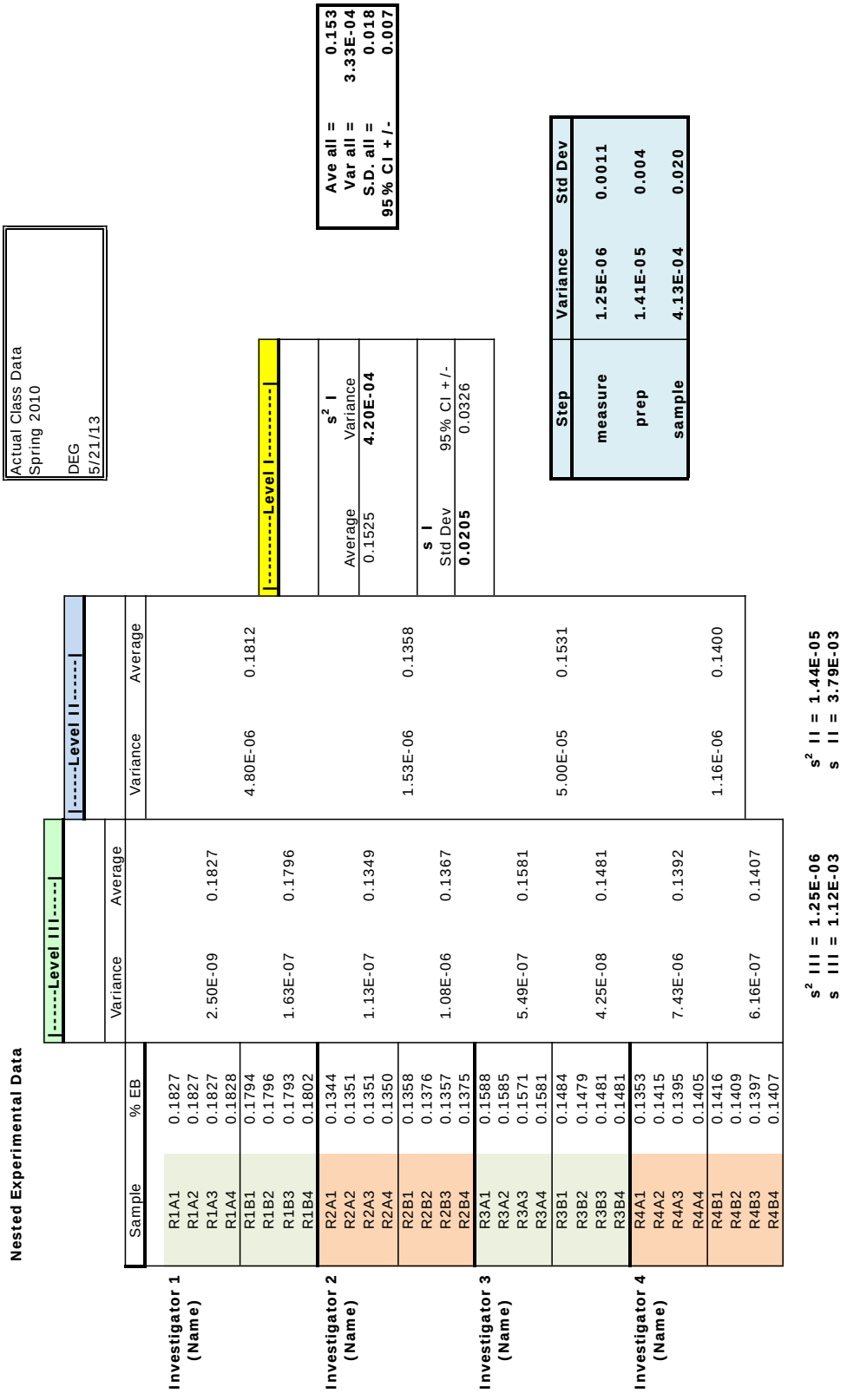

Figure 1. Spreadsheet illustrting nested experimental data and calculations.