**Experiment 2**

SAMPLING IN ANALYTICAL CHEMISTRY

2 lab periods

*Reading: Chapter 4, pg 69-87, Chapter 27, pg 699-705, Quantitative Chemical Analysis, 8th Edition, Daniel C. Harris (7th Edition: Chapter 4, pg 53-68, Chapter 28, pg 644-650).*

**Objective**

In this experiment you will explore the role of sampling in analytical chemistry. In the first part of the experiment you will use a particle counting method to determine the fractional composition of a “toxic chemical” in soil. Harmless beads are used as models for both the “toxic chemical” and the soil. In this case, the sampling uncertainty is expected to dominate the measurement. In the second part of this experiment, you will sample cherry Kool-Aid for Allura Red, a food dye, and explore how sample treatment affects analytical results. You may do this experiment with a lab partner, but you will each need to write up and turn in individual reports.

**Suggested Schedule**

Lab 1 Half the class does Part I, experimental procedure and data workup, while the other half works on Part II.

Lab 2 Finish all experiments. **This is a good time to get help from the TA, if you don’t understand the calculations.**

**Introduction**

Chemistry laboratory classes typically focus on teaching students how to perform analyses on well-defined samples with known (to the instructor) concentrations of the target analyte. This is because real-life samples, although interesting, can present a variety of often unexpected analytical challenges. In real-life analytical practice, the sampling, sample preparation, and analytical measurement steps are all equally important. The sampling and sample preparation steps often introduce more uncertainty than the analytical measurement step.

Determining how best to acquire a representative sample from a heterogeneous material is sometimes quite difficult. Examples of heterogeneous mixtures include soils, food and consumer products, and biological tissue. How sampling is done depends upon the use to which the analytical data will be put, upon the accuracy and precision desired, and upon the costs incurred in obtaining suitable samples.

The sources of *variance* in any analysis are additive. Variance is simply the square of the standard deviation, s, of a measurement. The total variance for an analysis (s2*total*) is composed of the variance due to sampling (s2*sampling*), the variance due to sample preparation (s2*prep*), and the variance due to the analytical measurement (s2*meas*). The relevant equation is:

 s2*total* = s2*sampling* + s2*meas* + s2*prep* [1]

From equation 1 we see that all three contributions to the total variance are equally important. Analyses are usually designed to minimize each contribution to the total variance.

When the variance in sampling is large, a highly precise analysis is wasted, since *s*2*sampling* >> *s*2*meas* and*s*2*sampling* >> *s*2*prep*.Thus, *s*2*total* ≈ *s*2*sampling*. Generally, the variances due to the analysis and the sampling steps should be similar in order to minimize analysis costs; i.e. it is not cost effective to perform a highly precise analysis when the sampling variance is high.

In Part I of this experiment you will use a particle counting method to determine the fractional composition of a “toxic chemical” in soil. Harmless red beads are used as models for the “toxic chemical” and white (or colors other than red) ones for the soil. The fractional composition (fr) of red beads in a sample of red and white beads is:

fr = nr/(nr + nw) [2]

nr is the number of red beads and nw is the number of white beads in the sample. For a two-component system, such as our mixture of red and white beads, the theoretical relative standard deviation of sampling (theoretical RSD) is given by:

 theoretical RSD = [(1-p)/np]1/2 [3]

where p is the fractional composition (fr for red beads) and n is the number of beads sampled. Quantitatively, the number of particles times the percent RSD squared equals a constant value, called the *Ingamells’ sampling constant*:

n × (%RSD)2 = Ingamells’ sampling constant [4]

%RSD = RSD x 100, as you know.

In Part II of this experiment, you will analyze cherry-flavored Kool Aid for its dye content. Kool Aid is a heterogeneous mixture of sugar, fructose, some vitamins, calcium phosphate, and Allura Red (FD&C Red 40, a food dye). The sample preparation step simply involves weighing a portion of Kool Aid and dissolving it in water. Then, you will measure the absorbance of the Kool Aid solution at 504 nm (this may vary slightly), which is the wavelength of maximum absorbance for Allura Red.



Allura Red, aka Red 40, FD&C Red 40

The absorbance, A, of an analyte is related to its concentration, C, through the Beer’s Law equation:

A = εbC [5]

The molar absorptivity, ε, is a measure of how strongly a chemical species absorbs light at a given wavelength. The molar absorptivity of Allura Red is 25,900 M-1cm-1 at 504 nm, its wavelength of maximum absorbance. In this experiment, b, the pathlength of the cuvette, is 1 cm. The molecular formula for Allura Red is C18H14N2Na2O8S2. Its molecular weight is 496.43 g/mol. You will learn more about Beer’s Law later in the semester.

In Part II of this experiment the variance due to sample preparation, s2*prep*,should be small because sample preparation simply involves weighing the Kool Aid and dissolving it in water. Because of the way we are doing the experiment, the variance due to sample preparation is included as part of the variance due to sampling. Therefore, the total variance becomes:

s2*total* = s2*sampling* + s2*meas* [6]

Part III, Analysis of Unknown Concentration of Allura Red. A performance evaluation sample will be provided to you.

***Prelaboratory Assignment***

1. Describe the steps involved in sample preparation.

2. What is the purpose of a sampling step in an analysis? What factors would you consider in choosing a representative bulk sample of a mixture of particles? How might you reduce the sampling uncertainty in such a mixture?

3. In the analysis of some powdered milk for melamine, the standard deviation of sampling is ±4%, and the standard deviation of the measurement is ±3%. What is the overall standard deviation? To what value must the sampling standard deviation be reduced if we need an overall standard deviation of ±4% or less? Assume the standard deviation of the measurement cannot be reduced.

#### Apparatus

* 50 mL, 25 mL and 10 ml plastic beakers
* A flat tray
* 5 mm size red and white (or colors other than red) beads, ~150-250 mL
* 50-mL volumetric flasks
* Large watch glass
* Mortar and pestle
* Ocean Optics spectrophotometer and Vernier LabQuest
* **Note – Use the same spectrometer through this lab.**
* One or two plastic cuvettes

#### Chemicals

* Cherry flavored Kool Aid

#### Procedure

**Part I**

Make sure to keep careful written notes of observations and data. As stated earlier, colored beads are used to represent different chemicals. The beads are harmless. The beads all have the same size and volume. Thus, percent by number and percent by volume are all identical. White (or colors other than red) beads represent a sample matrix (soil), while the red beads represent a poison which is harmful at concentrations above a fractional composition of 0.10.

1. Working as a team of two students, obtain a sample of mixed beads from the TA, a flat tray, and the three beakers. Qualitatively describe the sample.

a. Mix the beads up.

b. Take a random sample using your 50 mL beaker. Fill the beaker to the top, but don’t overfill.

c. Dump the sample into the tray. Record the count of red and non-red beads.

d. Return the beads to the original container.

e. Repeat steps **a** to **d** four more times.

f. Repeat steps **a** to **e** twice more, once with a 25 mL beaker and once with a 10 mL beaker.

g. Put all the beads back in the original container and return it to the TA.

**Part II**

1. Work as a team of two students. Each student should obtain a bulk sample of ~ 1-2 g of Kool Aid from the instructor. Each student should individually complete the following steps.

2. Pour your sample into a large watch glass, mix it, and shape it into a cone. Divide the cone into four equal quadrants. This process is called “piling and quartering” and is designed to separate a sample without biasing the particle size distribution.

2. Using the analytical balance, weigh out (by difference) three different 0.2 gram samples of the mixture from different quadrants of the pile. Record the mass accurately to four places after the decimal. Transfer each sample quantitatively into a labeled 50-mL volumetric flask and dilute to the mark with DI water. Mix by inverting the flask several times. You may store your prepared solutions in beakers.

3. Measure the absorbance of each solution at a wavelength of 504 nm. See instructions on how to use the LabQuest and Ocean Optics spectrophotometer, below. For the first solution only: measure the absorbance three times in a row. To do this, simply remove the cuvette from the spectrometer and replace it. This helps us understand the uncertainty in making the absorbance measurement. Just measure the absorbances of the second and third solutions once each.

4. Rinse out your volumetric flasks and beakers to prepare them for the next step.

5. Take a 1-2 gram portion of your original bulk sample and put it into a mortar. Use the pestle to grind the sample really well. This may take several minutes of hard grinding activity. Get the powder as fine and homogeneous as you can. Pile the sample into a cone shape, as above.

6. Weigh out (by difference) three different 0.2 gram samples of the mixture from different quadrants of the pile. Record the mass accurately. Transfer each sample quantitatively into a labeled 50-mL volumetric flask and dilute to the mark with DI water. Mix by inverting the flask several times. You may store your prepared solutions in beakers.

7. Measure the absorbance of each solution at a wavelength of 504 nm. These measurements should be made once each. When done, clean up your area.

**Instructions for using LabQuest with the Ocean Optics Spectrophotometer.**

*Note: use the stylus, not your finger to touch the LabQuest screen. Make sure you place the cuvette in the spectrometer so that the light shines through the clear portion of the cuvette! Use the same cuvette for all your measurements.*

1) Place a cuvette containing DI water in the cell compartment of your Ocean Optics instrument and push “sensors”, go to ” calibrate” and then “USB spectrometer”. Wait for the lamp to warm up. Push “Finish calibration”, then “ok” on LabQuest. This is not a true calibration, but merely zeros the spectrometer using DI water.

2) Discard the DI water and rinse the cuvette with one of your samples. Fill the cuvette with your sample and place in the cell compartment of your Ocean Optics spectrometer. Push “play” on your LabQuest screen.

3) Push stop. The LabQuest registers the wavelength associated with the highest absorbance value. Double check to ensure that the wavelength registered on your LabQuest is the same, or very close, to as the wavelength of maximum absorption of Allura Red (504 nm).

4) Measure the absorbances of your samples at 504 nm.

*Data Analysis and Calculations*

**Part I**

1. Calculate the fractional composition of red beads (fr) for each individual sampling experiment. See Equation 2.

2. Combine the five individual measurements of fractional red bead composition (fr) for each individual *sample size* (beaker size). Calculate the mean, sample standard deviation, relative standard deviation (RSD), percent relative standard deviation (%RSD), and a 95% confidence interval for the fractional read bead composition.

3. Using Equation 3, calculate the theoretical RSD for each of your three sample sizes. Use the mean fractional red bead composition (fr) for each sample size. You will need to calculate the average number of beads sampled for each beaker size. Compare the theoretical RSD to the RSD you calculated in step 2. *Does your experimental data agree with the overall trend predicted for the RSD as a function of sample size? If not, discuss a potential reason for the discrepancy.*

4. Use equation 4 to calculate the Ingamells’ sampling constant for each sampling size. Average the three values to get an overall estimate of the constant. *Use the constant to predict the sample size (number of beads) that should be used to obtain a %RSD of 1%.*

**Part II**—work as a team.

1. For each of your measured absorbances, calculate the *weight percent* Allura Red in the Kool Aid samples. Hint: use Equation 5, and the information given, to find the concentration (in moles/L) of Allura Red in each 50 mL sample. Then calculate the number of grams of Allura Red in each 50 mL sample. Divide by the number of grams of Kool Aid used to prepare each sample and multiply by 100. Use these calculated values in all calculations below. Watch your significant figures.

2. Calculate the mean, standard deviation, RSD, and %RSD for the one set of values (weight percent Allura Red) that were measured three times in a row in the spectrometer. The standard deviation here represents s*meas*, the uncertainty of making absorbance measurements in the Ocean Optics spectrophotometer.

3. Calculate the mean, standard deviation, RSD, and %RSD for the two different sets of samples (weight percent Allura Red). Since you and your partner are working together, you should have six measurements for each set of samples to pool. The set of six samples (three from each partner) prepared from the unground bulk Kool Aid can be called “as obtained” and the set of six samples (three from each partner) prepared from the ground Kool Aid can be called “ground before analysis”. For the single sample measured three times, simply choose one of the three measurements to use. The standard deviations you obtain in this step are s*sampling*, the uncertainty of sampling.

4. Discuss the three different %RSD values you obtained. Did grinding the sample improve the %RSD?

5. Test to see if grinding changed the measured mean. Use a t-test as described on page 76-77 (Case 2) of your text. Based on the t-test for comparison of means, are the two means significantly different at the 95% confidence level?

6. Test to determine whether grinding the sample significantly reduces the uncertainty of sampling. Use the F-test (page 80-81 of your text) to determine whether the two standard deviations (“ground before analysis” and “as obtained”) are significantly different from each other at the 95% confidence level. Does grinding the sample significantly reduce the uncertainty of sampling?

7. Calculate the variance for sampling, s2*sampling*, for each set of samples: “as obtained” and “ground before analysis”. Calculate the variance for the measurement, s2*meas*, using the standard deviation calculated in step 2. Variances should be reported to one significant figure.

8. Calculate the total variance, s2*total*, for each set of samples.

Part III—work as a team.

1. A performance evaluation sample of allura red will be provided to you. Measure its absorbance and determine the concentration of this sample by using Beer’s Law.

*Discussion Questions*

1. The bead sampling experiment in Part I was very simple. Identify and discuss two factors that would make sampling of a real soil sample more complicated.

2. In Part II we looked at three different types of variance:

 a. The variance in making a spectrophotometric measurement.

 b. The variance in sampling from a bulk, heterogeneous, sample.

 c. The variance in sampling from a ground-up, more homogenous, sample.

 Discuss the relative sizes of the different types of variance in Part II of this experiment. How did grinding the Kool Aid affect the sampling variance? How did it affect the measured mean? How did it affect the total variance? What would be a good way to determine the variance due to sample preparation in this experiment?

3. What are some possible sources of error in Part II? How can these be corrected?

*Lab Report*

Each team member must submit the following:

1. Statistical values calculated in the Data Analysis section. Tabulate this data and present it in a logical, easy to follow, way.

2. Answers to all questions in the Data Analysis and Calculations section and all the Discussion Questions.

3. Copies of the appropriate pages from your lab notebook (Experiment 2). See page 11 of this manual for requirements.

The lab report should have a title page that lists the title of the experiment, the date, your name in bold or underlined, and the names of all team members.

*References*

1. University of Tennessee Knoxville, Department of Chemistry. Chemistry 319 Laboratory Manual. <http://www.chem.utk.edu/~chem319/> Accessed July 8, 2011.
2. An Experiment in the Sampling of Solids for Chemical Analysis. Guy, R. E.; Ramble, L.; Wentzell, P. D. *J. Chem. Educ.* **1998**, *75*, 1028-1033.
3. Effect of Sample Size on Sampling Error. Vitt, J. E.; Engstrom, R. C.; *J. Chem. Educ.* **1999**, *76*, 99-100.
4. A Classroom Exercise in Sampling Technique. Ross, M. R.; Bacon, D. W.; Wolsey, W. C. *J. Chem. Educ.* **2000**,*77*, 1015-1016.
5. Two Experiments illustrating the Importance of Sampling in Quantitative Chemical Analysis. *J. Chem. Educ.* **2002**,*79*, 360-363.