EXPERIMENT 1: Multi-Techniques: "INTRODUCTION TO STANDARD OPERATING PROCEDURES (SOP's), AND GOOD LABORATORY PRACTICES (GLP's) IN THE ANALYTICAL LABORATORY"

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PURPOSE

Introduce the student to the fundamental techniques that will be used throughout the rest of this course. In later experiments your grade will depend on your mastery of these skills. For some experiments, a perfect score will require that your answers be well within 0.2% of the "true" composition of your "unknown" sample. On some experiments, an error of as much as 1 or 2% may result in very low scores on accuracy, often the biggest portion of the ***experiment grade***. Your performance in this experiment is very important for your future success!

THEORY

Standard operating procedures stating what steps will be taken and how will be carried out during any experimental or industrial process, are the bulwark of quality assurance. Adhering to these procedures, guards against the normal human desire to take shortcuts based on assumptions that could be false. The **detection limit**, also called the **lower limit of detection (LOD),** is the smallest quantity of analyte that is "significantly different" from the blank. The standard deviation is a measure of the *noise* (random variation) in a blank or a small signal. It is assumed that the standard deviation of the signal from samples with concentrations near the detection limit is similar to the standard deviation from the blanks. When the analytical response is 3 times as great as the noise, it is ready detectable but still too small for accurate measurement.

$$\mathbf{LOD = 3s_b / m}$$

A response that is 10 times as great as the noise is defined as the **lower limit of quantification (LOQ),** or the smallest amount that can be measured with reasonable accuracy.

$$\mathbf{LOQ = 10s_b / m}$$

PRELAB EXERCISE:

1. Read Chapter 2 (Harris, D.C. *Quantitative Chemical Analysis,* 8th ed.; W.H. Freeman: New York, 2010), a .pdf copy of this chapter can be found in Moodle.
2. What is the importance of the USP in analytical chemistry?
3. Which is more efficient a transfer pipet or a measuring pipet? Justify your answer.
4. Define the terms random error, systematic error, and bias.
5. Mention two advantages for the technique of "weighing by difference".

NOTES FROM THE INSTRUCTOR:

1. If this is your first day in the laboratory you must talk with the instructor before you begin this experiment.
2. Contact him via e-mail beforehand.
3. Did you experience any problems accessing our web page? These should have been addressed by now.
4. ***Do not forget to bring proper laboratory attire: closed shoes, lab coat, safety glasses or goggles.***
5. ***Students must also bring the following items for their personal use:***
   - ✓ ***Combination lock***
   - ✓ ***Paper towel roll***
   - ✓ ***Ivory clear or any odors, colors & phosphate free detergent***
   - ✓ ***Nitrile gloves (available at Walgreens and other retail stores)***
   - ✓ ***Thermometer***

6. Today is your day to ask questions: Take advantage of it. Get used to interact with your laboratory instructor and the coordinator. Learn how to perform the techniques properly. Things will get serious next week. You will use some of today's results to work with, later in the semester. If you are casual and careless today, you will develop bad habits, collect incorrect data or make bad solutions, and this will impact your overall course grade.
7. Planning and efficiency are critical on this course.
8. There is a lot of relevant reading. Probably, you will not be able to read and understand all of it before each experiment. What must you do then?
    - ✓ Read the experiment. Note anything that you do not understand or are unfamiliar with.
    - ✓ Find discussions of aspects you are unfamiliar within the textbook. Use the index and the suggested reading lists as a guide for finding what you need.
    - ✓ Look at the heading of the suggested reading sections. Read anything you think will be important or you think is interesting.
    - ✓ Perform the experiment. Again use the textbook, index and suggested reading to help complete the write-ups and calculations for the experiments.
9. This is a chemical engineering laboratory. The first thing in any chemical engineer's mind when starting an experiment should be: What process and chemical reactions are taking place? So, you must plan ahead and read today's experiment, considering the reactions involved, before coming to the laboratory.

EXERCISE #1 : Using the Analytical Balance

A. Calibration of an Analytical Balance

This is a simple gravimetric exercise to give you practice in using the balance, its calibration methods, and the recording of data in your laboratory notebook. This exercise will also give you a clearer idea of the performing reliability of gravimetric equipment.

For convenience, balances are classified according to their readability, typically from 100 mg to 0.1 μg, as shown in Table 1:

Table 1 Balance classification by readability

Balance classification	Balance readability	Typical capacity	Other details
Precision	100 mg to 1 mg (1 to 3-place)	1 – 2 kg	Typically, top pan balances
Analytical	0.1 mg (4-place)	200 g	General laboratory balances
Semi-micro	0.01 mg (5-place)	50 – 200 g	Useful for improved accuracy in relatively heavy objects
Micro	1 μg or 2 μg (6-place)	2 – 5 g	For accurate weighing of relatively small quantities
Ultra micro	0.1 μg or 0.2 μg (7-place)	~ 1 g	For accurate weighing of small quantities

Some balances have more than one readability range. The readability to which a particular mass is displayed is then a function of the design of the balance's ranges, the magnitude of the load and the configuration settings of the balance.

According to the manufacturer and the United States Pharmacopeia (USP), calibration of a balance requires to:

- Keep the balance away from pressure and temperature gradients
- Check that the balance is clean and the weighing pan is free of corrosion. If there are any

problems, report them to your laboratory instructor.

- Verify the balance is properly level and on a balance table
- Tare the mass of the empty pan (with all the windows closed)
- Calibrate the balance using its internal calibration method, according to the manufacturer's manual and your instructor's instructions.
- Prior to weighing, be certain that samples are at room temperature.
- Perform an external calibration of the balance (according to the manufacturer's manual or as outlined by your instructor for a set of weighing standards).

Procedure 1**: Basics of USP <41> (Weights and Balances)**

Exercise 1A and 1B
Using a series of standard weights:

1A Perform a replicate analysis (**7** replicates in triplicate ea.) of a 100 g (or 50g) standard mass. Calculate the uncertainty (standard deviation) of your results.

1B Measure the mass (in triplicate) of a weighing dish (or weighing paper), four standard weights ranging from 0-10 g, and an unknown sample (provided by your instructor). The weighing dish will be used to weight the standards and unknown.

Beware: Your unknown mass should be in the range of your standard weights.

ALWAYS Record all data in your notebook.

Note: Data from 1A and 1B will be used for the next Experiment ("Using the Computer for Data Analysis").

Practical Considerations:

- Use tongs, or finger pads to prevent moisture uptake while handling the sample.
- Avoid using gloves since traces of oils can be transferred to the sample.
- Place the object to be weighted on the center of the balance pan.
- Always weigh by difference (not by tarring).

According to USP <41>:

1. An accurate weighing is performed with a weighing device whose measurement uncertainty (random + systematic error) does not exceed 1% of the reading.
2. Measurement uncertainty is satisfactory if three times the standard deviation on the mass of not less than 10 replicates divided by the mass weighed do not exceed 0.001.

Exercise 1C Weighing by Difference

"Weighing by difference is the act of determining the weight of a sample by obtaining two weighing bottle weights, one before and one after dispensing the sample, and then subtracting the two weights. The used of utensils should be avoid to reduce the loss of a portion of the sample, which might adhere to the utensil or paper. In the process of weighing by difference, NO UTENSIL OR WEIGHING PAPER contacts the sample because the sample is shaken into the receiving vessel directly from the weighing bottle. If the two weights are determined on an analytical balance, it is desirable to avoid touching the weighing bottle with fingers from the time the first weight is obtained until the second weight is obtained. This avoids putting fingerprints on the weighing bottle that would add measurable weight to the weighing before the second weight is determined. One can conveniently avoid fingerprints by wearing clean gloves or by using a rolled-up paper towel (1)."

Ref (1): Analytical Chemistry for Technicians, 3rd Edition, 2003, John Kenkel, Chapter3, pg. 52.

Procedure:

1. Obtain from your instructor a weighing bottle containing your sample
2. Record directly in your lab notebook, the weigh of the weighing bottle containing your sample (this will be your initial weigh).
3. Applying the technique of "Weighing by difference" remove a small portion of the sample to a clean beaker (100mL).
4. Weigh your weighing bottle (this will be your second weigh).
5. Determine the weigh of your sample and record in your lab notebook.

- **NOTE**: A weighing bottle can be dried in an oven because it is not pre-calibrated. **It i*s a standard operation procedure (SOP) that volumetric glassware available in the laboratory can not be washed with steam or hot water, nor dried with blowers or in glass drying ovens.***
- **Y**ou may dry the ***weighing bottle*** using Kimwipes however, ***in future experiments it should be oven dried***. If you have time and want to gain experience, dry in an oven until constant weight is attained.

EXERCISE #2: BASICS OF USP <31> (VOLUMETRIC APPARATUS)

This exercise will give you practice in the right used of pipets, using the balance, and using your laboratory notebook. It may also give you a clearer idea of the calibration reliability of volumetric glassware.

This calibration is done by: Accurately weighing the volume of the liquid delivered by the pipet. If the density of the liquid is accurately known at the temperature you are working, the volume delivered can be accurately determined (see Experiment 3 for details). Always use distilled water.

a. **Volumetric (Class A)**
b. **Mohr (Class B)**
c. **Serological (Class B)**
d. **Micropipet**
e. **Ostwald Foling (Class A/B)**
f. **Lambda (N/A)**
g. **Pasteur (N/A)**

- *Note: Class A glassware must be used for all quantitative analysis where volumes are critical.*

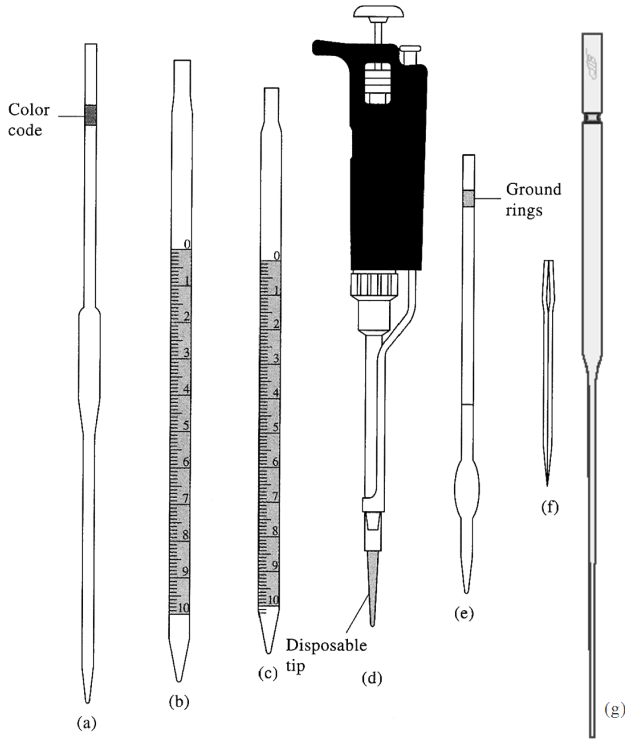

Figure 1: Apparatus for precisely measuring volume (volumetric pipets have better precision)

Procedure:

1. Clean your 10 mL pipet so that no droplets of distilled water remain on its inside surface as it drains. Clean and dry a **50 mL Erlenmeyer flask.**
2. Weigh the flask and its cap to the nearest tenth of a milligram.
3. Use finger pads, or their equivalent to handle the bottle. Fingerprints often generate significant weighing errors!
4. Using a filling bulb (See Figure 1), fill the pipet above the etched line with distilled water from a 250 mL beaker. **MOUTH PIPETTING IS FORBIDDEN IN THIS COURSE!**
5. Measure and record the temperature of water using the thermometer you brought to the laboratory. Dry the outside surface of the pipet with a Kimwipe and carefully reduce your finger pressure to allow the liquid level to reach exactly the etched line and transfer the water into the flask.

6. Cover the Erlenmeyer flask with its cap. Weigh the flask and its contents.
7. Repeat the procedure two more times (triplicate analysis). The three weight values should agree at least within 1 part per thousand.

Practical Considerations:

- The pipet should be held in a vertical position with the etched line at eye level.
- The bottom of the meniscus should coincide with the etched line.
- Touch the inside wall of the beaker with the tip of the pipet in order to remove any drop that may have formed.
- This manipulation is a bit tricky and may have to be repeated several times until you can achieve the conditions you want. Once accomplished, touch the inside wall of the receiving flask with the tip of the pipet and drain its contents. It is better if the tip touches the inner wall of the container near the bottom, but not so the liquid.
- Allow the pipet to drain for 10 or 20 seconds. **DO NOT** blow out the remaining portion of water in the tip! Remove the pipet.

Common errors:

- warming of the pipet by holding the bulb portion in your hand
- failure to allow sufficient drainage time
- disturbing the residual liquid that should remain in the tip
- general carelessness in handling the weighing bottle
- loss of water before draining its contents (a sudden movement may squirt some water from the tip; slanting the pipet toward the horizontal before moving reduces this error).

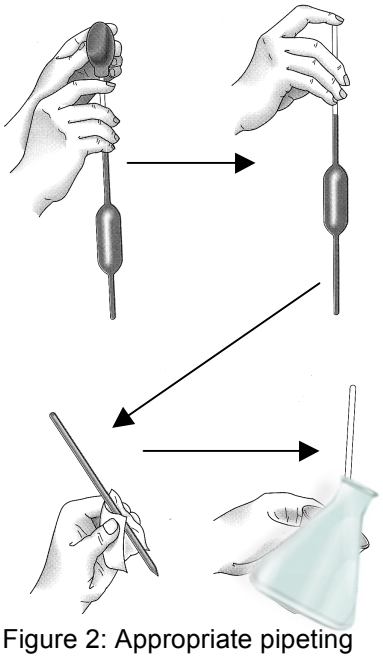

Figure 2: Appropriate pipeting procedure.

7. Use the density table (Table 1), to determine the appropriate density of water at the experimental temperature.
8. Calculate the actual volume delivered by the pipet on each trial. Then perform the following calculations:
   a. the average volume
   b. the standard deviation of those volumes (Is this value within the specifications or tolerance of the pipet?)
   c. the percent relative error
   d. the precision of your method, expressed as relative standard deviation in parts per thousand.

Table 1. Relative density of water at different temperatures

Temp (°C)	Density (g/mL)	Temp (°C)	Density (g/mL)
15	0.9980	25	0.9960
16	0.9979	26	0.9957
17	0.9977	27	0.9954
18	0.9975	28	0.9952
19	0.9973	29	0.9949
20	0.9971	30	0.9946
21	0.9969		
22	0.9967		
23	0.9965		
24	0.9962		

EXERCISE #3: QUANTITATIVE TRANSFER & CLEANING VALIDATION

In this exercise, each student will prepare 100 mL of a 5% v/v HCl cleaning solution. ***USP<1051> states that "SUCCESS IN CONDUCTING MANY ANALYTICAL ASSAYS DEPENDS UPON THE UTMOST CLEANINGNESS OF THE APPARATUS USED".***

Procedure:

1. Wash and scrub a 150-250 mL beaker. Rinse with tap water.
2. Rinse the inside of the clean beaker with at least three small portions of distilled water, until no droplets remain on its walls. DO NOT DRY! This is the standard analytical technique or procedure for cleaning glassware.
3. Add about 25 mL of distilled water into the clean, rinsed beaker.
4. Wash and rinse clean a 10 mL graduated cylinder, using the procedure described above.
5. Working in the hood where acids are stored, pour 15-20 mL of reagent grade 50% ^{V}/v hydrochloric acid to an acid-dispensing flask.
6. Add 10 mL of 50% ^{V}/v hydrochloric acid into the graduated cylinder and poured into the beaker with distilled water.

Note: Add acid to water, one way to remember it is by knowing that the order is like the "A & W Root Beer".

7. Rinse thoroughly the graduated cylinder with 3 very small portions (~ 1 mL) of distilled water. Add rinses to the acid solution in the beaker. Homogenize the solution by carefully mixing it with a glass stirring rod.
8. After quantitatively and carefully transferring these rinses, place about 3 mL of water in the graduated cylinder. Add one or two drops of 0.5 M AgNO3. If the solution turns white or cloudy, your transfer was not quantitative. Discard the contents of the graduated cylinder down the drain.
   a. What is this white precipitate, and what is the reaction that produces it?
9. Once you complete your laboratory you can discard the solution down the drain after diluting it with plenty of tap water.
10. Note that it is a standard procedure to rinse volumetric glassware before and after use. A second set of rinses using the analytical solution is also required when preparing analytical solutions.

Exercise #4: Locker Check in and Cleaning Glassware

Your instructor will assign you a locker. It will contain the glassware required for the experiments. You are responsible for its appropriate use, care and cleanliness.

- Clean all your glassware thoroughly and organize the locker as indicated by the instructor.
- Wash and scrub all glassware. Rinse with tap water. Then rinse with distilled water.