EXPERIMENT 4: CALIBRATION AND HANDLING OF VOLUMETRIC GLASSWARE: CALIBRATING A 50 ML BURET.

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OBJECTIVES

There are three main objectives: 1) to develop basic skills in cleaning, handling and calibrating volumetric glassware; 2) to estimate the systematic error[1] in the volume delivered by a 50-mL buret and construct a graph to convert the measured volume to the true volume delivered; 3) to obtain a good estimate of the standard deviation of the method by applying the statistical treatment referred to as "pooling".

THEORY

Volumetric glassware e.g. burets, pipets, and flasks, are typically calibrated by determining the mass of a fixed volume of distilled water that they contain or deliver. It is good practice to inspect volumetric glassware for damage before use, paying particular attention to pipette and burette tips. Any damaged volumetric glassware should be disposed of. Class A glassware must be used for all quantitative analysis where volumes are critical. Quality volumetric glassware is labeled with the dispensing volume and temperature at which that particular volume will be delivered. For accurate calibration, buoyancy as well as temperature corrections must be applied to the mass weighed. To simplify the calculations, **Table 1** provides the appropriate correction factors to convert the mass of water to the corresponding volume at a given temperature T.

Table 1: Correction Factors (CF) for Volumetric Calibration

Temperature (^{o}C)	Correction Factor***** (mL/g)	Temperature (^{o}C)	Correction Factor***** (mL/g)
20	1.0028	25	1.0040
21	1.0030	26	1.0043
22	1.0033	27	1.0045
23	1.0035	28	1.0048
24	1.0037	29	1.0051
		30	1.0054

NOTE: Correction factors are based on the volume occupied by 1.000 g of water and all are corrected for buoyancy. (See class text for details)

All volumetric glassware must be carefully cleaned before being calibrated, and no droplets should stick to the walls (there should be no water breaks). Burettes should be thoroughly cleaned by hand. It is inadvisable to wash burettes in automatic glass washing machines. Burets and pipets do not need to be dry; volumetric flasks must be drained and dried at room temperature. Water used for calibration purposes should be in thermal equilibrium with its surroundings, so you should draw the water well in advance, measure its temperature at frequent intervals, and wait until no further changes occur. According to USP <31> most of the volumetric apparatus available in the US are calibrated at 20°C despite temperatures prevailing in most analytical laboratories typically approaches 25°C, which is the temperature specified for the majority of the Pharmacopeial assays. In most USP assays this discrepancy is inconsequential provided that the room temperature is reasonably constant.

[1] ***Systematic or determinate errors*** are those that, in principle, can be identified and corrected. A common systematic error is the use of an uncalibrated buret.

DIRECTIONS FOR THE USE OF A BURET:

Burets allow the analyst to deliver any volume of liquid up to their maximum capacity. A buret consists of a calibrated tube plus a valve, or stopcock, by which the flow of liquid is controlled. There are four types of burettes: glass graduated, auto-zero, dispensing or automatic. A typical Class A buret is shown in **Figure 1**. Burets are available in sizes from 1 mL to 1000 mL. It is convenient to use burets fitted with PTFE stopcocks. Glass stopcocks, which require lubrication, should be avoided. Some solutions, particularly bases, may cause a glass stopcock to freeze upon long contact; therefore, thorough cleaning is required after each use. On the other hand, Teflon valves are unaffected by most common reagents and require no lubricant. **According to USP <741>,** the buret should be chosen so that its delivered volume represents not less than 30% of its nominal volume. Where less than 10 mL of titrant is to be delivered a suitable micro-buret must be employed.

A buret must be carefully cleaned and its valve tested for leaks. Follow the instructions provided by your instructor. Washing is best achieved by filling the burette, over a sink, with water containing an appropriate cleaning solution. Clean the buret thoroughly with detergent and a long brush. After this, it should be rinsed well, first with tap water and then with distilled water unless specified otherwise in the analytical method. Check that the buret drains without droplets sticking on its walls. If droplets remainst, repeat the procedure. On occasions, it may be necessary to use a strong cleaning solution[2] to soak the buret for a few seconds. Rinse promptly and repeat the treatment if needed. Consult your instructor for details. The buret should be clamped upside down to drain. If stubborn residues remain on the stopcock they can be removed by placing the stopcock in a beaker containing a cleaning agent and sonicating in a sonic bath. With the burette reassembled and the stopcock closed, it should be rinsed with the solution to be used. This is best achieved by removing the burette from its stand, tilting it and adding the solution to say one-fifth of its capacity. The burette should then be tilted and rotated so that all of the internal surfaces are rinsed. This solution should then be allowed to drain out through the stopcock. This rinsing process should then be repeated at least 3 times. This cleaning procedure applies to all volumetric glassware.

To test for leaks, simply fill the buret with water and check that the reading does not change with time. For a 50.00 mL Buret, readings should be estimated to the nearest 0.01 mL. A piece of white card or paper held behind the burette will facilitate reading of the graduated scale. Always read the volume that corresponds to the bottom of the meniscus. To avoid parallax[3] *error*, keep your eye at the same height as the liquid surface. See Figure 2.

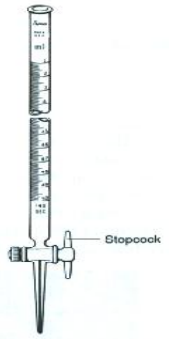

Figure 1: Typical Class A Buret

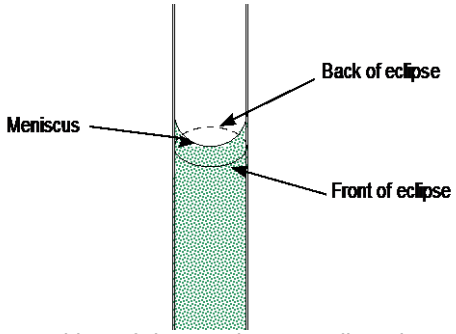

Figure 2: Correct position of the meniscus. Adjust the meniscus so it is in the center of the ellipse formed by the rear and front areas of the 0.00 mL mark.

CALIBRATION OF A 50-ML BURET:

[2] **Caution:** Strong cleaning solutions (e.g. peroxydisulfate-sulfuric acid solution) eat dirt, grease, clothing and your skin, if not handled properly. **Use them with extreme care**. Use a 75-100 mL portion of the solution. Do not leave the solution in the buret for more than 15 seconds. Rinse immediately with tap water, followed by distilled water.

[3] ***Parallax*** is a phenomenon that causes the volume delivered to appear smaller if the meniscus is viewed from above, and larger if it is viewed from below.

Laboratory records must maintained with sufficient detail, so that other equally qualified analysts can reconstruct the experimental conditions and review the results obtained. When collecting data, the data should generally be obtained with as is (with all corresponding decimal places) and rounded only after final calculations are completed.

The Analytical Data- Interpretation and Treatment chapter of the USP (Chapter <1010>), states that all measurements are, at best, estimates of the actual ("true" or "accepted") value for they contain random variability (also referred to as random error) and may also contain systematic variation (bias). Thus, a measured value differs from the actual value because of variability inherent in the measurement. In order to determine the experimental error on a volume delivered by a 50-mL buret, the exact volume will be measured together with its corresponding weight. The manufacturer's tolerance for a Class A[4] 50-mL buret is ± 0.05 mL. This means that if for example 10 mL of liquid are delivered, the real volume could be 10.04 mL, and still be within that tolerance. See Table 2. If an array of measurements consists of individual results that are representative of the whole, statistical methods can be used to estimate informative properties of the analysis, and statistical tests can be employed to investigate whether it is likely that these properties comply with given analytical requirements.

A way to correct for ***systematic errors*** of this type is to construct an experimental calibration curve, as the one illustrated in **Figure 3**. Fill the buret to the mark with distilled water, previously equilibrated to room temperature, and transfer 10 mL portions to a previously weighed flask. Weigh the flask and its contents. From the difference in these masses, calculate the mass of water delivered. Calculate the exact volume delivered (corrected for temperature and buoyancy) with the aid of **Table 1**. Repeat the procedure several times. Refer to **Box 1** for an example of the required data and calculations.

Table 2: Tolerances of Class-A volumetric glassware:

Burets

Volume (mL)	Smallest graduation (mL)	Tolerance (mL)
5	0.01	± 0.01
10	0.05 or 0.02	± 0.02
25	0.1	± 0.03
50	0.1	**± 0.05**
100	0.2	± 0.10

Tolerances of Class A volumetric flasks

Flask capacity (mL)	Tolerance (mL)
1	±0.02
2	±0.02
5	±0.02
10	±0.02
25	±0.03
50	±0.05
100	±0.08
200	±0.10
250	±0.12
500	±0.20
1 000	±0.30
2 000	±0.50

Tolerances of Class A transfer pipets

Volume (mL)	Tolerance (mL)
0.5	±0.006
1	±0.006
2	±0.006
3	±0.01
4	±0.01
5	±0.01
10	±0.02
15	±0.03
20	±0.03
25	±0.03
50	±0.05
100	±0.08

[4] The National Institute of Standards and Technology (NIST) have prescribed certain tolerances, or absolute errors, for different kinds of volumetric glassware. The letter "A" stamped on the side of a buret and other volumetric glassware indicates that they comply with Class A tolerance values.

Box 1
EXAMPLE OF DATA AND CALCULATIONS USED TO PREPARE THE CALIBRATION CURVE: GRAPH OF CORRECTION (mL) VS. VOLUME DELIVERED.

A buret was drained to the 10.00 mL mark at a temperature of 24°C. The following results were obtained.

DATA	CALCULATION	TRIAL 1	TRIAL 2
Final reading	V_f (mL)	10.01	10.08
Initial reading	V_i (mL)	0.03	0.04
Difference (D)	$D= V_f-V_i$	9.98	10.04
Mass (M)		9.9840	10.0560
Actual volume delivered (A)	$A= M*CF^{(Table\ 1)}$	10.02	10.09
Correction	C=A-D	+0.04	+0.05
Average Correction		+0.045	

To calculate the actual volume of water delivered when 9.9840 g are dispensed at 24°C, use the correction factor in Table 1: volume = 9.984 g (1.0037 mL/g) = 10.02 mL.

To obtain the correction for a volume greater than 10 mL, add the masses of water successively collected in the flask. Given the following masses, estimate the correction for a 30-mL volume:

	Volume interval (mL):	Mass delivered (g)
Sum:	0.03-30.06 mL	79.890 - 50.000=29.890 g

The actual volume of water delivered is (29.890 g) (1.0038 mL/g) = 30.00 mL. Since the volume reading on the buret is 30.03 mL, the buret correction at 30 mL is -0.03 mL.

What information does the calibration curve provide?

For example, if you begin a titration at 0.04 mL and end at 29.00 mL, you would deliver 28.96 *mL, provided that the buret is perfect*. However, **Figure 3** shows that the buret delivers 0.03 mL less than the indicated amount, so only 28.93 mL is really dispensed. To use the calibration curve in the future, you should begin all titrations near the 0.00 mL mark.

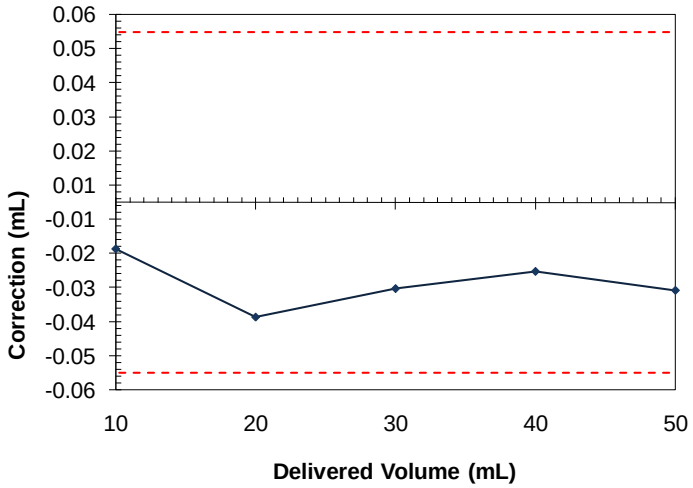

Figure 3: Correction curve for a 50-mL buret.

Box 2 Statistical Treatment of Data
(Pooled Standard Deviation)

Experimental measurements always have some degree of random error. Therefore, no conclusion can be established with complete certainty. Statistics provide us tools to accept conclusions that have a high probability of being correct and to reject those that do not.

Populations and samples:

In the statistical treatment of data, it is assumed that the small number of replicate experimental results obtained in the laboratory is a minute fraction of an infinite number that could be obtained, in principle, if an infinite period of time and an infinite amount of sample were available. Statisticians call this small set of data a **sample,** and regard it as a subset of an infinite **population.** Rules in statistics apply in principle only to populations. In order to apply these rules, it must be assumed that the sample is really representative of the population.

If an infinite number of measurements are performed, and their distribution is plotted, the result is a bell-shaped curve, or Gaussian distribution, characterized by a **mean** and a **standard deviation**. The **population mean**, μ, is the value at the center of such a distribution and the **population standard deviation, σ,** is a measurement of its width. The term population indicates that the number of measurements, N, approaches infinite. The population standard deviation is defined as:

$$\sigma = \sqrt{((\Sigma(x_i - \mu)/N)} \qquad \sqrt{} = \text{square root}$$

Where N corresponds to the number of measurements and μ is the population mean.

If a large number of experimental measurements is done and their distribution plotted, the resultant curve will also approach a bell-shaped or Gaussian distribution. The mean of the distribution is now referred to as the **sample mean, $\bar{x}$,** and the standard deviation as the **sample standard deviation, s**. These are defined as:

Sample Mean: $\bar{x} = \Sigma x_i / N$

Where N= number of measurements and Σx_i = sum of the measured values.

Sample Standard deviation: $s = \sqrt{((\Sigma(x_i - \bar{x})^2)/(N-1))}$

Where N-1 correspond to the number of degrees of freedom[5].

The smaller the sample standard deviation, the narrower the distribution, and more precise or reproducible the results are. The sample standard deviation (**s**) is always equal or larger than the population standard deviation (σ).
The reliability of s as a measure of precision improves as N, the number of measurements, increases. Typically, if N is larger than 20, **s** can be considered a good approximation of the population standard deviation σ.

NOTE: Refer to Appendix of this manual: "Analytical errors and statistical treatment of data"

[5] The degrees of freedom indicate the number of independent results that are really used to calculate the standard deviation.

POOLING DATA TO IMPROVE THE RELIABILITY OF S:

For analyses that are time consuming or for which the sample supply is limited, it is impossible or impractical to use more than 20 replicates to get a value of **s** that approximates that of **σ**. In situations like this, data accumulated over a period of time from a series of similar samples, can be **pooled** to provide an estimate of s that is more reliable than the value for any individual subset. However, we must assume the same sources of random error for all the measurements. These assumptions are usually valid if the samples have similar compositions and have been analyzed using exactly the same experimental procedure.

To obtain a pooled estimate of the standard deviation s_{pooled}, the squares of the deviations from the mean for each subset are added and divided by the proper number of degrees of freedom. Since one degree of freedom is lost for each subset, so the total number of degrees of freedom is equal to the total number of measurements minus the number of subsets.

The equation for computing a pooled standard deviation from t sets of data is:

$$s_{\text{pooled}} = \sqrt{\frac{\sum_{i=1}^{N_1}(x_i - \bar{x}_1)^2 + \sum_{j=1}^{N_2}(x_j - \bar{x}_2)^2 + \sum_{k=1}^{N_3}(x_k - \bar{x}_3)^2 + \ldots + \sum_{p=1}^{N_{n_t}}(x_p - \bar{x}_{n_t})^2}{N_1 + N_2 + N_3 + \ldots - n_t}}$$

where N_1 is the number of data in set 1, N_2 is the number in set 2, and so forth. The term n_t refers to the number of data sets that are pooled. The example that follows illustrates the calculations that should be performed.

EXAMPLE:

Samples of seven fish taken from the Mississippi River were analyzed, using atomic absorption spectroscopy, to determine their concentration of mercury. Given the following data, calculate the pooled estimate of the standard deviation of the method:

Specimen Number	Number of samples measured	Hg Content, ppm	Mean, ppm	Squares of the deviations from the Means $\left(\Sigma(x_i - \bar{x})^2\right)$
1	3	1.80, 1.58, 1.64	1.673	0.0259
2	4	0.96, 0.98, 1.02, 1.10	1.015	0.0115
3	2	3.13, 3.35	3.240	0.0242
4	6	2.06, 1.93, 2.12, 2.16, 1.89, 1.95	2.018	0.0611
5	4	0.57, 0.58, 0.64, 0.49	0.570	0.0114
6	5	2.35, 2.44, 2.70, 2.48, 2.44	2.482	0.0685
7	4	1.11, 1.15, 1.22, 1.04	1.130	0.0170
No. of measurements	28	**Sum of squares =**		**0.2196**

The values in columns 4 and 5 for specimen number 1 were calculated as follows:

x_i	$\lvert(x_i - \bar{x})\rvert$	$(x_i - \bar{x})^2$
1.80	0.127	0.0161
1.58	0.093	0.0087
1.64	0.033	0.0011
5.02	**Sum of squares**	**0.0259**

Mean: $\bar{x} = 5.02 \div 3 = 1.673$

Similar calculations were performed for the rest of the specimens.

$$\mathbf{S_{pooled}} = \sqrt{\mathbf{(0.2196 \div (28\text{-}7))}} = 0.100 \text{ ppm} \approx 0.1 \text{ ppm Hg}$$

Remember that $\mathbf{S}_{pooled}$ is a good approximation of the population standard deviation if the number of degrees of freedom ($N\text{-}N_T$) for the pooled analysis is larger than 20.

CONTROL CHARTS:

Control charts are a mean to assess the variability of a process. These charts are currently known as statistical process control (SPC) tools. These charts work under the assumption that measurements follow normal distributions. Therefore sample average and variability must be carefully controlled. Measurements will fall out of specification if one or more of the following move beyond the target limits: sample average, and sample variability. Therefore one control chart per variable is needed in process control. Two common control charts used in the chemical industry are the mean and variability charts. These are measurement control charts built under the assumption that the measurements follow a normal distribution. Normally the average and variability charts are originated from the same samples and used together in process control since both variables are statistically independent. The results are plotted on charts where their limits have been calculated from statistically tested parameters. The use of control charts allows buret manufacturers to ensure customers that their products meets their specifications.

PRELABORATORY EXERCISE

1. What do the symbols “TD” and “TC” means on volumetric glassware?
2. Which is more accurate a transfer pipet or a measuring pipet?
3. An empty 10 mL volumetric flask weighs 10.2634 g. When the flask is filled to the mark with distilled water and weighed again in the air at 20°C, the mass is 20.2144 g. What is the true volume of the flask at 20°C?
4. Explain the term “parallax”
5. Explain the term tolerance and why it is important in the validation of volumetric glassware?

APPRATUS AND MATERIALS

- 50-mL buret
- two 50 mL Erlenmeyer flasks with rubber stoppers
- thermometer
- distilled water
- analytical balance

EXPERIMENTAL

1. Thoroughly clean the buret, as previously described, until it drains without leaving any drops on the walls.
2. Obtain about 300 mL of distilled water. Allow it to equilibrate to room temperature before filling the buret. Record its actual temperature.
3. Fill the buret and force out any air bubbles on the tip. Adjust the meniscus to 0.00 mL and touch the beaker wall with the buret tip to remove any suspended drop of water. Allow the buret to stand for 5 minutes while you weigh a 50 mL Erlenmayer flask fitted with a rubber stopper. Hold the flask with a tissue or paper towel, not with your hands, to avoid changing its mass with fingerprint residues. If the water level on the buret has changed, tighten the stopcock and recheck for leaks.
4. Drain ~10 mL of water, at a rate of less than 20 mL/min, into the weighed flask. Cap it tightly to prevent evaporation. Before reading the buret, wait for about 30 seconds for the film of liquid on the walls to descend. **ESTIMATE ALL READINGS TO 0.01 ML.** Weigh the flask again to determine the mass of water delivered.
5. Now drain the buret from 10 to ~ 20 mL and measure the mass of water delivered.
6. Drain the buret from 20 to ~ 30 mL and measure the mass of water delivered.
7. Drain the buret from 30 to ~ 40 mL and measure the mass of water delivered.
8. Drain the buret from 40 to ~ 50 mL and measure the mass of water delivered.

9. Repeat the entire process (10, 20, 30, 40 and 50 mL runs) for a second and third time.
10. Use Table 1 to convert the mass of water to volume delivered.
11. Record the 50 mL data from each student in your section in the pooling data tables from the spreadsheet available on the laboratory website.

CALCULATIONS

1. Calculate the difference among the final and initial readings of the buret for each of the 10.00 mL intervals. Refer to Example in Box 1. Note: Remember to add volumes and masses for volumes greater than 10.00 mL.
2. Calculate the actual volume delivered by the buret for each of the 10.00 mL intervals e.g. 10, 20, 30, 40, and 50 mL.
3. Calculate the volume correction for each of the 10.00 mL intervals.
4. Repeat the calculations in steps 1 and 2 for the second and third trials.
5. Calculate the average correction for each of the 10.00 mL intervals.
6. Prepare a CORRECTION GRAPH (AVERAGE CORRECTION VS VOLUME DELIVERED), as the one illustrated in Figure 3.
7. Estimate the standard deviation and the relative standard deviation, RSD (ppt) of the actual volume at each of the 10.00 mL intervals
8. Perform the pooling exercise using your section data set. **(Refer to Box 2- Statistical Treatment of Data)**
9. Construct a mean control chart by plotting the average actual volume of each replicate set vs. the buret number.
10. Construct a variability control chart by plotting the standard deviation for average actual volume of each replicate set vs. the buret number.
11. Given that the upper and lower control limits for the volume and deviation are of 50.00 (±0.05), and 0.00 (±0.05) mL respectively. Determine if the buret lot in your laboratory meets the manufacturer's specifications. The lot is within the acceptance parameters if both the mean and variability charts fall within the specified range. If that is not the case explain the sources of variability and propose a plausible course of action to correct the problem.

QUESTIONS

1. What are systematic errors?
2. Describe at least 3 ways in which a systematic error might occur while using a buret to deliver a known volume of liquid.
3. Explain the term "buoyancy" and how it can affect the calibration of a volumetric device.
4. What is the protocol (GLP) for the use of a buret?
5. What is the confidence level of your experiment?
6. Describe what a control chart is and how can it be used to calibrate your buret.