



1

chapter

Laboratory Standard Operating Procedures

Andrew P. Neilson (✉)

*Department of Food Science and Technology,
Virginia Polytechnic Institute and State University,
Blacksburg, VA, USA
e-mail: andrewn@vt.edu*

Dennis A. Lonergan

*The Vista Institute,
Eden Prairie, MN, USA
e-mail: dennis@thevistainstitute.com*

S. Suzanne Nielsen

*Department of Food Science, Purdue University,
West Lafayette, IN, USA
e-mail: niensens@purdue.edu*

- 1.1 Introduction
- 1.2 Precision and Accuracy
- 1.3 Balances
 - 1.3.1 Types of Balances
 - 1.3.2 Choice of Balance
 - 1.3.3 Use of Top Loading Balances
 - 1.3.4 Use of Analytical Balances
 - 1.3.5 Additional Information
- 1.4 Mechanical Pipettes
 - 1.4.1 Operation
 - 1.4.2 Pre-rinsing
 - 1.4.3 Pipetting Solutions of Varying Density or Viscosity
 - 1.4.4 Performance Specifications
 - 1.4.5 Selecting the Correct Pipette
- 1.5 Glassware
 - 1.5.1 Types of Glassware/Plasticware
 - 1.5.2 Choosing Glassware/Plasticware
 - 1.5.3 Volumetric Glassware
 - 1.5.4 Using Volumetric Glassware to Perform Dilutions and Concentrations
 - 1.5.5 Conventions and Terminology
 - 1.5.6 Burets
 - 1.5.7 Cleaning of Glass and Porcelain
- 1.6 Reagents
 - 1.6.1 Acids
 - 1.6.2 Distilled Water
 - 1.6.3 Water Purity
 - 1.6.4 Carbon Dioxide-Free Water
 - 1.6.5 Preparing Solutions and Reagents
- 1.7 Data Handling and Reporting
 - 1.7.1 Significant Figures
 - 1.7.2 Rounding Off Numbers
 - 1.7.3 Rounding Off Single Arithmetic Operations
 - 1.7.4 Rounding Off the Results of a Series of Arithmetic Operations
- 1.8 Basic Laboratory Safety
 - 1.8.1 Safety Data Sheets
 - 1.8.2 Hazardous Chemicals
 - 1.8.3 Personal Protective Equipment and Safety Equipment
 - 1.8.4 Eating, Drinking, Etc.
 - 1.8.5 Miscellaneous Information

1.1 INTRODUCTION

This chapter is designed to cover “standard operating procedures” (SOPs), or best practices, for a general food analysis laboratory. The topics covered in this chapter include balances, mechanical pipettes, glassware, reagents, precision and accuracy, data handling, data reporting, and safety. These procedures apply to all the laboratory experiments in this manual, and therefore a thorough review of general procedures will be invaluable for successful completion of these laboratory exercises.

This manual covers many of the basic skills and information that are necessary for one to be a good analytical food chemist. Much of this material is the type that one “picks up” from experience. Nothing can replace actual lab experience as a learning tool, but hopefully this manual will help students learn proper lab techniques early rather than having to correct improper habits later. When one reads this manual, your reaction may be “is all of this attention to detail necessary?” Admittedly, the answer is “not always.” This brings to mind an old Irish proverb that “the best person for a job is the one that knows what to ignore.” There is much truth to this proverb, but a necessary corollary is that one must know what they are ignoring. The decision to use something other than the “best” technique must be conscious decision and not one made from ignorance. This decision must be based not only upon knowledge of the analytical method being used but also on how the resulting data will be used. Much of the information in this manual has been obtained from an excellent publication by the US Environmental Protection Agency entitled *Handbook for Analytical Quality Control in Water and Wastewater Laboratories*.

1.2 PRECISION AND ACCURACY

To understand many of the concepts in this chapter, a rigorous definition of the terms “precision” and “accuracy” is required here. Precision refers to the **reproducibility** of replicate observations, typically measured as **standard deviation (SD)**, **standard error (SE)**, or **coefficient of variation (CV)**. Refer to Chap. 4 in this laboratory manual and Smith, 2017, for a more complete discussion of precision and accuracy. The smaller these values are, the more reproducible or precise the measurement is. Precision is determined not on reference standards, but by the use of actual food samples, which cover a range of concentrations and a variety of interfering materials usually encountered by the analyst. Obviously, such data should not be collected until the analyst is familiar with the method and has obtained a reproducible standard curve (a mathemati-

cal relationship between the analyte concentration and the analytical response). There are a number of different methods available for the determination of precision. One method follows:

1. Three separate concentration levels should be studied, including a low concentration near the sensitivity level of the method, an intermediate concentration, and a concentration near the upper limit of application of the method.
2. Seven replicate determinations should be made at each of the concentrations tested.
3. To allow for changes in instrument conditions, the precision study should cover at least 2 h of normal laboratory operation.
4. To permit the maximum interferences in sequential operation, it is suggested that the samples be run in the following order: high, low, and intermediate. This series is then repeated seven times to obtain the desired replication.
5. The precision statement should include a range of standard deviations over the tested range of concentration. Thus, three standard deviations will be obtained over a range of three concentrations.

Accuracy refers to the degree (absolute or relative) of difference between observed and “actual” values. The “actual” value is often difficult to ascertain. It may be the value obtained by a standard reference method (the accepted manner of performing a measurement). Another means of evaluating accuracy is by the addition of a known amount of the material being analyzed for the food sample and then calculation of % **recovery**. This latter approach entails the following steps:

1. Known amounts of the particular constituent are added to actual samples at concentrations for which the precision of the method is satisfactory. It is suggested that amounts be added to the low-concentration sample, sufficient to double that concentration, and that an amount be added to the intermediate concentration, sufficient to bring the final concentration in the sample to approximately 75% of the upper limit of application of the method.
2. Seven replicate determinations at each concentration are made.
3. Accuracy is reported as the percent recovery at the final concentration of the spiked sample. Percent recovery at each concentration is the mean of the seven replicate results.

A fast, less rigorous means to evaluate precision and accuracy is to analyze a food sample and replicate a spiked food sample, and then calculate the recovery of the amount spiked. An example is shown in Table 1.1.

1.1
 table

Measured calcium content (g/L) of milk and spiked milk

| Replicate | Milk | Milk + 0.75 g Ca/L |
|-----------|--------|--------------------|
| 1 | 1.29 | 2.15 |
| 2 | 1.40 | 2.12 |
| 3 | 1.33 | 2.20 |
| 4 | 1.24 | 2.27 |
| 5 | 1.23 | 2.07 |
| 6 | 1.40 | 2.10 |
| 7 | 1.24 | 2.20 |
| 8 | 1.27 | 2.07 |
| 9 | 1.24 | 1.74 |
| 10 | 1.28 | 2.01 |
| 11 | 1.33 | 2.12 |
| Mean | 1.2955 | 2.0955 |
| SD | 0.062 | 0.138 |
| %CV | 4.8 | 6.6 |

The accuracy can then be measured by calculating the % of the spike (0.75 g/L) detected by comparing the measured values from the unspiked and spiked samples:

$$\text{accuracy} \approx \% \text{ recovery} = \quad (1.1)$$

$$\frac{\text{measured spiked sample}}{\text{measured sample} + \text{amount of spike}} \times 100\%$$

$$\text{accuracy} \approx \% \text{ recovery} = \frac{2.0955 \text{ g/L}}{1.2955 \text{ g/L} + 0.75 \text{ g/L}} \times 100\% = 102.44\%$$

The method measured the spike to within 2.44%. By adding 0.75 g/L Ca to a sample that was measured to have 1.2955 g/L Ca, a perfectly accurate method would result in a spiked sample concentration of 1.2955 g/L + 0.75 g/L = 2.0455 g/L. The method actually measured the spiked sample at 2.0955 g/L, which is 2.44% greater than it should be. Therefore, the accuracy is estimated at ~2.44% relative error.

1.3 BALANCES

1.3.1 Types of Balances

Two general types of balances are used in most laboratories. These are **top loading balances** and **analytical balances**. Top loading balances usually are sensitive to 0.1–0.001 g, depending on the specific model in use (this means that they can measure differences in the mass of a sample to within 0.1–0.001 g). In general, as the **capacity** (largest mass that can be measured) increases, the sensitivity decreases. In other words, balances that can measure larger masses generally measure differences in those masses to fewer decimal places. Analytical

balances are usually sensitive to 0.001–0.00001 g, depending on the specific model. It should be remembered, however, that **sensitivity** (ability to detect small differences in mass) is not necessarily equal to **accuracy** (the degree to which the balances correctly report the actual mass). The fact that a balance can be read to 0.01 mg does not necessarily mean it is accurate to 0.01 mg. What this means is that the balance can distinguish between masses that differ by 0.01 mg, but may not accurately measure those masses to within 0.01 mg of the actual masses (because the last digit is often rounded). The accuracy of a balance is independent of its sensitivity.

1.3.2 Choice of Balance

Which type of balance to use depends on “how much accuracy” is needed in a given measurement. One way to determine this is by calculating how much **relative (%) error** would be introduced by a given type of balance. For instance, if 0.1 g of a reagent was needed, weighing it on a top loading balance accurate to within only ±0.02 g of the actual mass would introduce approximately 20% error:

$$\begin{aligned} \% \text{ error in measured mass} &= \\ \frac{\text{absolute error in measured mass}}{\text{measured mass}} \times 100\% &\quad (1.2) \end{aligned}$$

$$\% \text{ error in measured mass} = \frac{0.02 \text{ g}}{0.1 \text{ g}} \times 100\% = 20\%$$

This would clearly be unacceptable in most situations. Therefore, a more accurate balance would be needed. However, the same balance (with accuracy to within ±0.02 g) would probably be acceptable for weighing out 100 g of reagent, as the error would be approximately 0.02%:

$$\% \text{ error in measured mass} = \frac{0.02 \text{ g}}{100 \text{ g}} \times 100\% = 0.02\%$$

The decision on “how much accuracy” is needed can only be answered when one knows the function of the reagent in the analytical method. This is one reason why it is necessary to understand the chemistry involved in an analytical method, and not to simply approach an analytical method in a cookbook fashion. Therefore, a general guideline regarding which balance to use is hard to define.

Another situation in which care must be exercised in determining what type of balance to use is when a difference in masses is to be calculated. For instance, a dried crucible to be used in a total ash determination may weigh 20.05 g on a top loading balance, crucible plus sample = 25.05 g, and the ashed crucible 20.25 g. It may appear that the use of the top loading balance

with its accuracy of ± 0.02 g would introduce approximately 0.1% error, which would often be acceptable. Actually, since a difference in weight (0.20 g) is being determined, the error would be approximately 10% and thus unacceptable. In this case, an analytical balance is definitely required because sensitivity is required in addition to accuracy.

1.3.3 Use of Top Loading Balances

These instructions are generalized but apply to the use of most models of top loading balances:

1. Level the balance using the bubble level and the adjustable feet (leveling is required so that the balance performs correctly).
2. Either *zero* the balance (so the balance reads 0 with nothing on the pan) or *tare* the balance so that the balance reads 0 with a container that will hold the sample (empty beaker, weighing boat, etc.) on the weighing pan. The tare function is conveniently used for “subtracting” the weight of the beaker or weighing boat into which the sample is added.
3. Weigh the sample.

1.3.4 Use of Analytical Balances

It is always wise to consult the specific instruction manual for an analytical balance before using it. Speed and accuracy are both dependent on one being familiar with the operation of an analytical balance. If it has been a while since you have used a specific type of analytical balance, it may be helpful to “practice” before actually weighing a sample by weighing a spatula or other convenient article. The following general rules apply to most analytical balances and should be followed to ensure that accurate results are obtained and that the balance is not damaged by improper use:

1. Analytical balances are expensive precision instruments; treat them as such.
2. Make sure that the balance is level and is on a sturdy table or bench free of vibrations.
3. Once these conditions are met, the same procedure specified above for top loading balances is used to weigh the sample on an analytical balance.
4. Always leave the balance clean.

1.3.5 Additional Information

Other points to be aware of regarding the use of balances are the following:

1. Many analyses (moisture, ash, etc.) require weighing of the final dried or ashed sample

with the vessel. The mass of the vessel must be known so that it can be subtracted from the final mass to get the mass of the dried sample or ash. Therefore, make sure to obtain the mass of the vessel before the analysis. This can be done by either weighing the vessel before taring the balance and then adding the sample or obtaining the mass of the vessel and then the mass of the vessel plus the sample.

2. The accumulation of moisture from the air or fingerprints on the surface of a vessel will add a small mass to the sample. This can introduce errors in mass that affect analytical results, particularly when using analytical balances. Therefore, beakers, weigh boats, and other weighing vessels should be handled with tongs or with gloved hands. For precise measurements (moisture, ash, and other measurements), weighing vessels should be pre-dried and stored in a desiccator before use, and then stored in a desiccator after drying, ashing, etc. prior to weighing the cooled sample.
3. Air currents or leaning on the bench can cause appreciable error in analytical balances. It is best to take the reading after closing the side doors of an analytical balance.
4. Most balances in modern laboratories are electric balances. Older lever-type balances are no longer in wide use, but they are extremely reliable.

1.4 MECHANICAL PIPETTES

Mechanical pipettes (i.e., **automatic pipettors**) are standard equipment in many analytical laboratories. This is due to their convenience, precision, and acceptable accuracy *when used properly and when calibrated*. Although these pipettes may be viewed by many as being easier to use than conventional glass volumetric pipettes, this does not mean that the necessary accuracy and precision can be obtained without attention to proper pipetting technique. Just the opposite is the case; if mechanical pipettes are used incorrectly, this will usually cause greater error than the misuse of glass volumetric pipettes. The proper use of glass volumetric pipettes is discussed in the section on glassware. The PIPETMAN mechanical pipette (Rainin Instrument Co., Inc.) is an example of a continuously adjustable design. The proper use of this type of pipette, as recommended by the manufacturer, will be described here. Other brands of mechanical pipettes are available, and although their specific instructions should be followed, their proper operation is usually very similar to that described here.

1.4.1 Operation

1. Set the desired volume on the digital micrometer/volumeter. For improved precision, always approach the desired volume by dialing downward from a larger volume setting. Make sure not to wind it up beyond its maximum capacity; this will break it beyond repair.
2. Attach a disposable tip to the shaft of the pipette and press on firmly with a slight twisting motion to ensure a positive, airtight seal.
3. Depress the **plunger** to the **first positive stop**. This part of the stroke is the calibrated volume displayed. Going past the first positive stop will cause inaccurate measurement.
4. Holding the mechanical pipette vertically, immerse the disposable tip into sample liquid to a depth indicated (Table 1.2), specific to the maximum volume of the pipette (P-20, 100, 200, 500, 1000, 5000, correspond to maximum volumes of 20, 100, 200, 500, 1000, and 5000 μL , respectively).
5. Allow plunger to *slowly* return to the “up” position. *Never permit it to snap up* (this will suck liquid up into the pipette mechanism, causing inaccurate measurement and damaging the pipette).
6. Wait 1–2 s to ensure that full volume of sample is drawn into the tip. If the solution is viscous such as glycerol, you need to allow more time.
7. Withdraw tip from sample liquid. Should any liquid remain on outside of the tip, wipe carefully with a lint-free cloth, taking care not to touch the tip opening.
8. To dispense sample, place tip end against side wall of vessel and depress plunger *slowly* past the first stop until the **second stop** (fully depressed position) is reached.
9. Wait (Table 1.3).

1.2

table

Appropriate pipette depth for automatic pipettors

| Pipette | Depth (mm) |
|-----------------------|------------|
| P-20D, P-100D, P-200D | 1–2 |
| P-500D, P-1000D | 2–4 |
| P-5000D | 3–6 |

1.3

table

Appropriate dispense wait time for automatic pipettors

| Pipette | Time (s) |
|-----------------------|----------|
| P-20D, P-100D, P-200D | 1 |
| P-500D, P-1000D | 1–2 |
| P-5000D | 2–3 |

10. With plunger fully depressed, withdraw mechanical pipette from vessel carefully with tip sliding along wall of vessel.
11. Allow plunger to return to top position.
12. Discard tip by depressing **tip-ejector button** smartly.
13. A fresh tip should be used for the next measurement if:
 - (a) A different solution or volume is to be pipetted.
 - (b) A significant residue exists in the tip (not to be confused with the visible “film” left by some viscous or organic solutions).

1.4.2 Pre-rinsing

Pipetting very viscous solutions or organic solvents will result in a significant film being retained on the inside wall of the tip. This will result in an error that will be larger than the tolerance specified if the tip is only filled once. Since this film remains relatively constant in successive pipettings with the same tip, accuracy may be improved by filling the tip, dispensing the volume into a waste container, refilling the tip a second time, and using this quantity as the sample. This procedure is recommended in all pipetting operations when critical reproducibility is required, whether or not tips are reused (same solution) or changed (different solutions/different volumes). Note that the “non-wettability” of the polypropylene tip is not absolute and that pre-rinsing will improve the precision and accuracy when pipetting any solution.

1.4.3 Pipetting Solutions of Varying Density or Viscosity

Compensation for solutions of varying viscosity or density is possible with any adjustable pipette by setting the digital micrometer slightly higher or lower than the required volume. The amount of compensation is determined empirically. Also, when dispensing viscous liquids, it will help to *wait* 1 s longer at the first stop before depressing to the second stop.

1.4.4 Performance Specifications

The manufacturer of PIPETMAN mechanical pipettes provides the information in Table 1.4, on the precision and accuracy of their mechanical pipettes.

1.4.5 Selecting the Correct Pipette

Although automatic pipettes can dispense a wide range of volumes, you may often have to choose the “best” pipette with the most accuracy/precision from among several choices. For example, a P5000

1.4

table

Accuracy and precision of PIPETMAN mechanical pipettes

| <i>Model</i> | <i>Accuracy^a</i> | <i>Reproducibility^a (standard deviation)</i> |
|--------------|--|---|
| P-20D | <0.1 μL @ 1–10 μL | <0.04 μL @ 2 μL |
| | <1 % @ 10–20 μL | <0.05 μL @ 10 μL |
| P-200D | <0.5 μL @ 20–60 μL | <0.15 μL @ 25 μL |
| | <0.8 % @ 60–200 μL | <0.25 μL @ 100 μL |
| P-1000D | <3 μL @ 100–375 μL | <0.3 μL @ 200 μL |
| | <0.8 % @ 375–1000 μL | <0.6 μL @ 250 μL |
| | | <1.0 μL @ 500 μL |
| P-5000D | <12 μL @ 0.5–2 mL | <1.3 μL @ 1000 μL |
| | <0.6 % @ 2.0–5.0 mL | <3 μL @ 1.0 mL |
| | | <5 μL @ 2.5 mL |
| | | <8 μL @ 5.0 mL |

^aAqueous solutions, tips preprinsed once

(i.e., 5 mL) automatic pipettor could theoretically pipette anywhere between 0 and 5 mL. However, there are several limitations that dictate which pipettes to use. The first is a practical limitation: mechanical pipettes are limited by the graduations (the increments) of the pipette. The P5000 and P1000 are typically adjustable in increments of 0.01 mL (10 μL). Therefore, these pipettes cannot dispense volumes of <10 μL , nor can they dispense volumes with more precision than that of 10 μL . However, just because these pipettes can technically be adjusted to 10 μL does not mean that they should be used to measure volumes anywhere near this small. Most pipettes are labeled with a working range that lists the minimum and maximum volume, but this is not the range for ideal performance. Mechanical pipettes should be operated from 100% down to 10–20% of their maximum capacity (Table 1.5). Below 10–20% of their maximum capacity, performance (accuracy and precision) suffers. A good way of thinking of this is to use the largest pipette capable of dispensing the volume in a single aliquot.

Mechanical pipettes are invaluable pieces of laboratory equipment. If properly treated and maintained, they can last for decades. However, improper use can destroy them in seconds. Mechanical pipettes should be calibrated, lubricated, and maintained at least yearly by a knowledgeable pipette technician. Weighing dispensed water is often a good check to see if the pipette needs calibration.

1.5 GLASSWARE**1.5.1 Types of Glassware/Plasticware**

Glass is the most widely used material for construction of laboratory vessels. There are many grades and types of glassware to choose from, ranging from

1.5

table

Recommended volume ranges for mechanical pipettors

| <i>Maximum volume</i> | <i>Lowest recommended volume</i> |
|-----------------------------|-------------------------------------|
| 5 mL (5000 μL) | 1 mL (1000 μL) |
| 1 mL (1000 μL) | 0.1–0.2 mL (100–200 μL) |
| 0.2 mL (200 μL) | 0.02–0.04 mL (20–40 μL) |
| 0.1 mL (100 μL) | 0.01–0.02 mL (10–20 μL) |
| 0.05 mL (50 μL) | 0.005–0.01 mL (5–10 μL) |
| 0.02 mL (20 μL) | 0.002–0.004 mL (2–4 μL) |
| 0.01 mL (10 μL) | 0.001–0.002 mL (1–2 μL) |

student grade to others possessing specific properties such as resistance to thermal shock or alkali, low boron content, and super strength. The most common type is a highly resistant borosilicate glass, such as that manufactured by Corning Glass Works under the name “Pyrex” or by Kimble Glass Co. as “Kimax.” Brown/amber actinic glassware is available, which blocks UV and IR light to protect light-sensitive solutions and samples. The use of vessels, containers, and other apparatus made of Teflon, polyethylene, polystyrene, and polypropylene is common. Teflon stopcock plugs have practically replaced glass plugs in burets, separatory funnels, etc., because lubrication to avoid sticking (called “freezing”) is not required. Polypropylene, a methylpentene polymer, is available as laboratory bottles, graduated cylinders, beakers, and even volumetric flasks. It is crystal clear, shatterproof, autoclavable, chemically resistant, but relatively expensive as compared to glass. Teflon (polytetrafluoroethylene, PTFE) vessels are available, although they are very expensive. Finally, most glassware has a polar surface. Glassware can be treated to derivatize the surface (typically, tetramethylsilane, or TMS) to make it nonpolar, which is required for some assays. However, acid washing will remove this nonpolar layer.

1.5.2 Choosing Glassware/Plasticware

Some points to consider in choosing glassware and/or plasticware are the following:

1. Generally, special types of glass are not required to perform most analyses.
2. Reagents and standard solutions should be stored in borosilicate or polyethylene bottles.
3. Certain dilute metal solutions may plate out on glass container walls over long periods of storage. Thus, dilute metal standard solutions should be prepared fresh at the time of analysis.
4. Strong mineral acids (such as sulfuric acid) and organic solvents will readily attack polyethylene; these are best stored in glass or a resistant plastic.

5. Borosilicate glassware is not completely inert, particularly to alkalis; therefore, standard solutions of silica, boron, and the alkali metals (such as NaOH) are usually stored in polyethylene bottles.
6. Certain solvents dissolve some plastics, including plastics used for pipette tips, serological pipettes, etc. This is especially true for acetone and chloroform. When using solvents, check the compatibility with the plastics you are using. Plastics dissolved in solvents can cause various problems, including binding/precipitating the analyte of interest, interfering with the assay, clogging instruments, etc.
7. Ground-glass stoppers require care. Avoid using bases with any ground glass because the base can cause them to “freeze” (i.e., get stuck). Glassware with ground-glass connections (burets, volumetric flasks, separatory funnels, etc.) are very expensive and should be handled with extreme care.

For additional information, the reader is referred to the catalogs of the various glass and plastic manufacturers. These catalogs contain a wealth of information as to specific properties, uses, sizes, etc.

1.5.3 Volumetric Glassware

Accurately calibrated glassware for accurate and precise measurements of volume has become known as **volumetric glassware**. This group includes **volumetric flasks**, **volumetric pipettes**, and accurately **calibrated burets**. Less accurate types of glassware, including **graduated cylinders**, **serological pipettes**, and **measuring pipettes**, also have specific uses in the analytical laboratory when exact volumes are unnecessary. Volumetric flasks are to be used in preparing standard solutions, but not for storing reagents. The precision of an analytical method depends in part upon the accuracy with which volumes of solutions can be measured, due to the inherent parameters of the measurement instrument. For example, a 10 mL volumetric flask will typically be more precise (i.e., have smaller variations between repeated measurements) than a 1000 mL volumetric flask, because the neck on which the “fill to” line is located is narrower, and therefore smaller errors in liquid height above or below the neck result in smaller volume differences compared to the same errors in liquid height for the larger flask. However, accuracy and precision are often independent of each other for measurements on similar orders of magnitude. In other words, it is possible to have precise results that are relatively inaccurate and vice versa. There are certain sources of error, which must be carefully considered. The volumetric apparatus must be read correctly; the bottom of the

meniscus should be tangent to the calibration mark. There are other sources of error, however, such as changes in temperature, which result in changes in the actual capacity of glass apparatus and in the volume of the solutions. The volume capacity of an ordinary 100 mL glass flask increases by 0.025 mL for each 1° rise in temperature, but if made of borosilicate glass, the increase is much less. One thousand mL of water (and of most solutions that are ≤ 0.1 N) increases in volume by approximately 0.20 mL per 1 °C increase at room temperature. Thus, solutions must be measured at the temperature at which the apparatus was calibrated. This temperature (usually 20 °C) will be indicated on all volumetric ware. There may also be errors of calibration of the adjustable measurement apparatus (e.g., measuring pipettes), that is, the volume marked on the apparatus may not be the true volume. Such errors can be eliminated only by recalibrating the apparatus (if possible) or by replacing it.

A volumetric apparatus is calibrated “**to contain**” or “**to deliver**” a definite volume of liquid. This will be indicated on the apparatus with the letters “TC” (to contain) or “TD” (to deliver). Volumetric flasks are calibrated to contain a given volume, which means that the flask contains the specified volume \pm a defined tolerance (error). The certified TC volume only applies to the volume contained by the flask and it does not take into account the volume of solution that will stick to the walls of the flask if the liquid is poured out. Therefore, for example, a TC 250 mL volumetric flask will hold 250 mL \pm a defined tolerance; if the liquid is poured out, slightly less than 250 mL will be dispensed due to solution retained on the walls of the flask (this is the opposite of “to deliver” or TD, glassware discussed below). They are available in various shapes and sizes ranging from 1 to 2000 mL capacity. Graduated cylinders, on the other hand, can be either TC or TD. For accurate work the difference may be important.

Volumetric pipettes are typically calibrated to deliver a fixed volume. The usual capacities are 1–100 mL, although micro-volumetric pipettes are also available. The proper technique for using volumetric pipettes is as follows (this technique is for TD pipettes, which are much more common than TC pipettes):

1. Draw the liquid to be delivered into the pipette above the line on the pipette. Always use a pipette bulb or pipette aid to draw the liquid into the pipette. Never pipette by mouth.
2. Remove the bulb (when using the pipette aid, or bulbs with pressure release valves, you can deliver without having to remove it) and replace it with your index finger.
3. Withdraw the pipette from the liquid and wipe off the tip with tissue paper. Touch the tip of the pipette against the wall of the container from which the liquid was withdrawn (or a spare

beaker). Slowly release the pressure of your finger (or turn the scroll wheel to dispense) on the top of the pipette and allow the liquid level in the pipette to drop so that the bottom of the meniscus is even with the line on the pipette.

4. Move the pipette to the beaker or flask into which you wish to deliver the liquid. Do not wipe off the tip of the pipette at this time. Allow the pipette tip to touch the side of the beaker or flask. Holding the pipette in a vertical position, allow the liquid to drain from the pipette.
5. Allow the tip of the pipette to remain in contact with the side of the beaker or flask for several seconds. Remove the pipette. There will be a small amount of liquid remaining in the tip of the pipette. Do not blow out this liquid with the bulb, as TD pipettes are calibrated to account for this liquid that remains.

Note that some volumetric pipettes have calibration markings for both TC and TD measurements. Make sure to be aware which marking refers to which measurement (for transfers, use the TD marking). The TC marking will be closer to the dispensing end of the pipette (TC does not need to account for the volume retained on the glass surface, whereas TD does account for this).

Measuring and serological pipettes should also be held in a vertical position for dispensing liquids; however, the tip of the pipette is only touched to the wet surface of the receiving vessel *after* the outflow has ceased. Some pipettes are designed to have the small amount of liquid remaining in the tip blown out and added to the receiving container; such pipettes have a frosted band near the top. If there is no frosted band near the top of the pipette, do not blow out any remaining liquid.

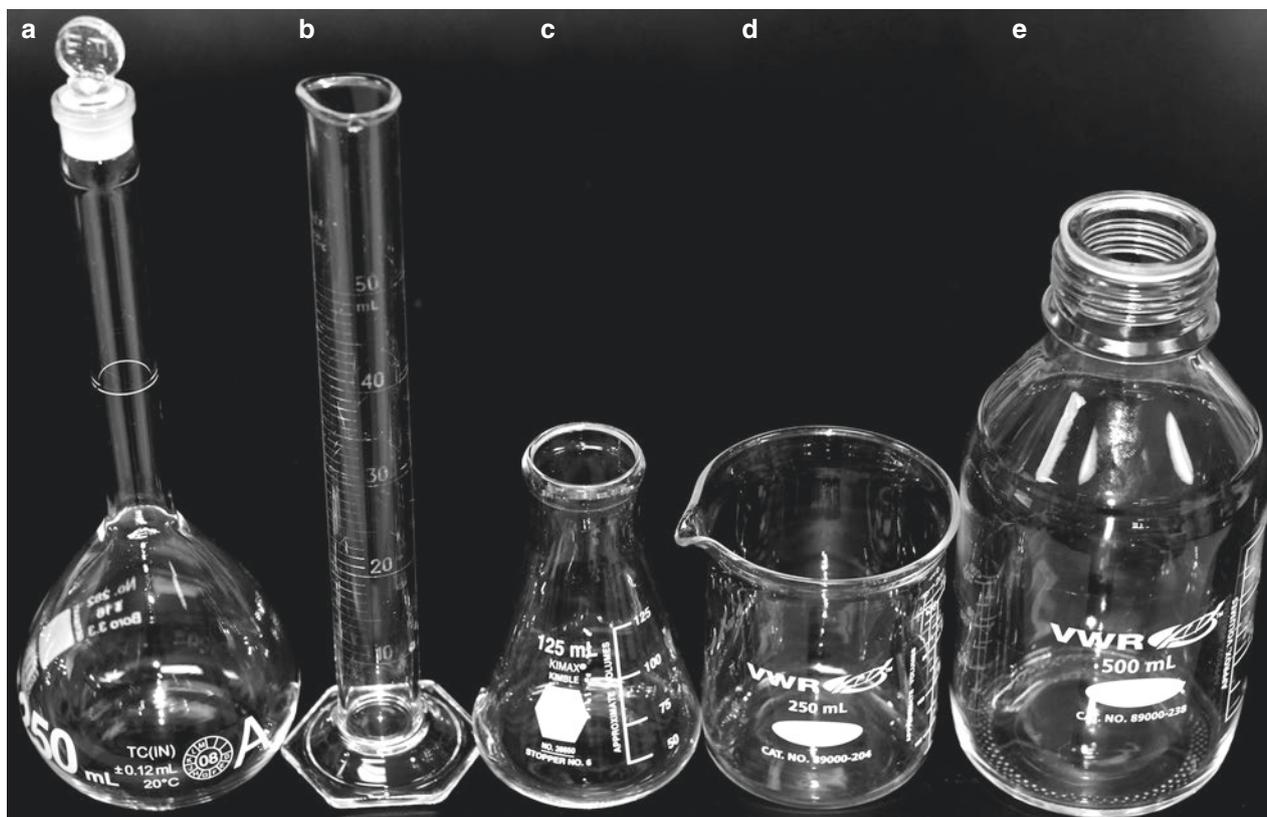
1.5.4 Using Volumetric Glassware to Perform Dilutions and Concentrations

Typically, dilutions are performed by adding a liquid (water or a solvent) to a sample or solution. Concentrations may be performed by a variety of methods, including rotary evaporation, shaking vacuum evaporation, vacuum centrifugation, boiling, oven drying, drying under N_2 gas, or freeze drying.

For bringing samples or solutions up to a known volume, the “gold standard” providing maximal accuracy and precision is a **Class A** glass volumetric flask (Fig. 1.1a). During manufacture, glassware to be certified as Class A is calibrated and tested to comply with tolerance specifications established by the American Society for Testing and Materials (ASTM, West Conshohocken, PA). These specifications are the

standard for laboratory glassware. Class A glassware has the tightest tolerances and therefore the best accuracy and precision. These flasks are rated TC. Therefore, volumetric flasks are used to bring samples and solutions up to a defined volume. They are not used to quantitatively deliver or transfer samples because the delivery volume is not known. Other types of glassware (non-Class A flasks, graduated cylinders, Erlenmeyer flasks, round-bottomed flasks, beakers, bottles, etc., Fig. 1.1b) are less accurate and less precise. They should not be used for quantitative volume dilutions or concentrations if Class A volumetric flasks are available.

For transferring a known volume of a liquid sample for a dilution or concentration, the “gold standard” providing maximal accuracy and precision is a Class A glass volumetric pipette (Fig. 1.2a). These pipettes are rated “to deliver” (TD), which means that the pipette will deliver the specified volume \pm a defined tolerance (error). The certified TD volume takes into account the volume of solution that will stick to the walls of the pipette as well as the volume of the drop of solution that typically remains in the tip of the pipette after delivery (again, you should not attempt to get this drop out, as it is already accounted for). Therefore, for example, a TD 5 mL pipette will hold slightly more than 5 mL but will deliver (dispense) $5 \text{ mL} \pm$ a defined tolerance (the opposite of TC glassware). It is important to note that volumetric pipettes are used only to deliver a known amount of solution. Typically they should not be used to determine the final volume of the solution unless the liquids dispensed are the only components of the final solution. For example, if a sample is dried down and then liquid from a volumetric pipette is used to resolubilize the solutes, it is unknown if the solutes significantly affect the volume of the resulting solution, unless the final volume is measured, which may be difficult to do. Although the effect is usually negligible, it is best to use volumetric glassware to assure that the final volume of the resulting solution is known (the dried solutes could be dissolved in a few mL of solvent and then transferred to a volumetric flask for final dilution). However, it is acceptable to add several solutions together using volumetric pipettes and then add the individual volumes together to calculate the final volume. However, using a single volumetric flask to dilute to a final volume is still the favored approach, as using one measurement for the final volume reduces the uncertainty. (The errors, or tolerances, of the amounts added are also added together; therefore, using fewer pieces of glassware lowers the uncertainty of the measurement even if the tolerances of the glassware are the same.) For example, suppose you need to measure out 50 mL of solution. You have access to a 50 mL volumetric flask and a 25 mL volumetric pipette, both of which have tolerances of $\pm 0.06 \text{ mL}$. If you obtain 50 mL by filling



1.1

figure

Class A volumetric flask (a) and other types of non-Class A volume measuring glassware: graduated cylinder (b), Erlenmeyer flask (c), beaker (d), and bottle (e)

the volumetric flask, the measured volume is $50 \text{ mL} \pm 0.06 \text{ mL}$ (or somewhere between 49.94 and 50.06 mL). If you pipette 25 mL twice into a beaker, the tolerance of each measurement is $25 \text{ mL} \pm 0.06 \text{ mL}$, and the tolerance of the combined volume is the sum of the means and the errors:

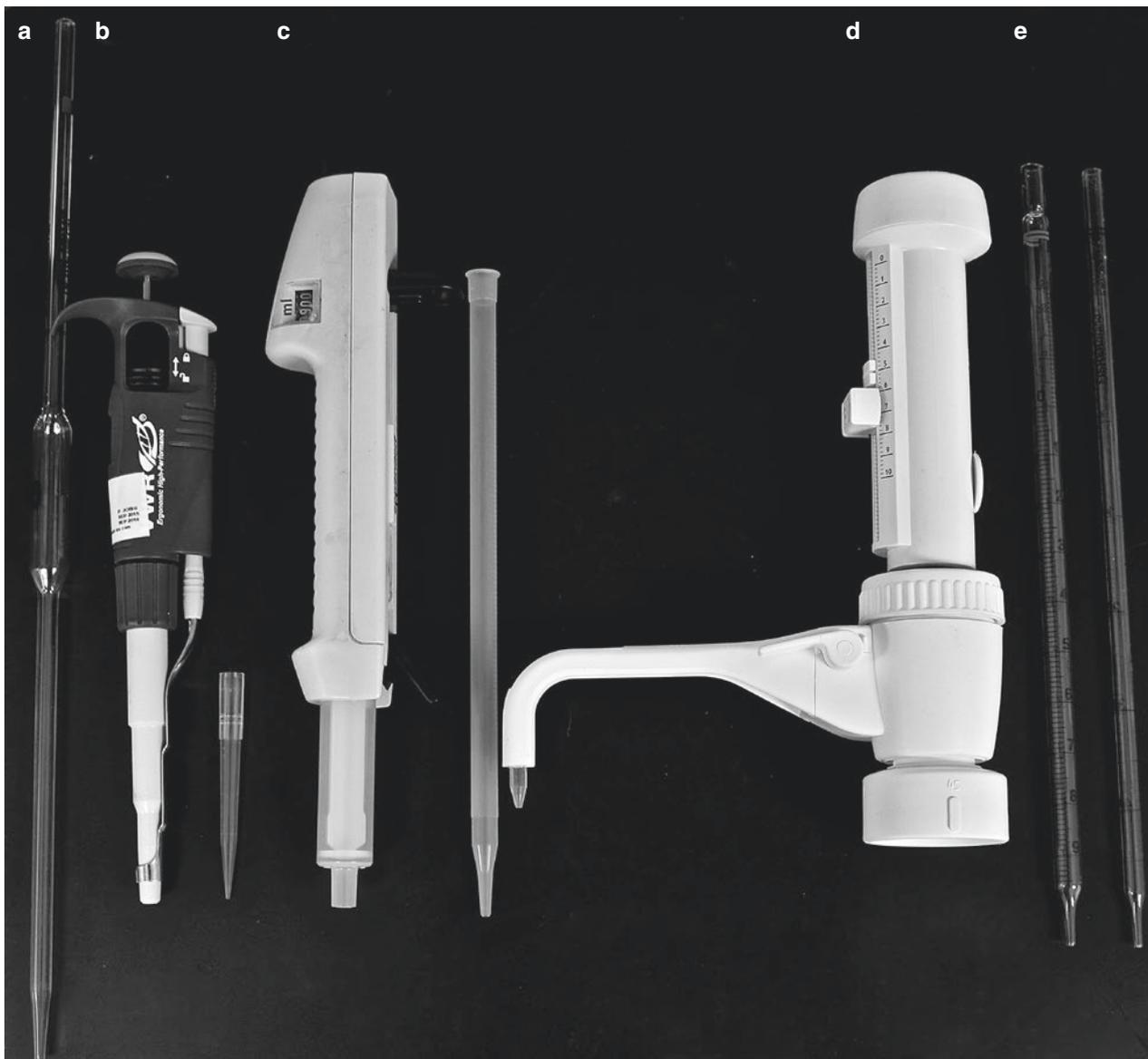
$$(25 \text{ mL} \pm 0.06 \text{ mL}) + (25 \text{ mL} \pm 0.06 \text{ mL}) = \\ 50 \text{ mL} \pm 0.12 \text{ mL} = 49.88\text{--}50.12 \text{ mL}$$

This additive property of tolerances, or errors, compounds further as more measurements are combined; conversely, when the solution is brought to volume using a volumetric flask, only a single tolerance factors into the error of the measurement.

Other types of pipettes (non-Class A volumetric glass pipettes, adjustable pipettors, automatic pipettors, reed pipettors, serological pipettes, etc., Fig. 1.2b) and other glassware (graduated cylinders, etc.) are less accurate and less precise. They should not be used for quantitative volume transfers. Pipettes are available (but rare) that are marked with lines for both TC and TD. For these pipettes, the TD line would represent the volume delivered when the drop at the tip is dispensed and TC when the drop remains in the pipette.

Information typically printed on the side of the pipette or flask includes the class of the pipette or flask, whether the glassware is TD or TC, the TC or TD volume, and the defined tolerance (error) (Fig. 1.3). Note that the specifications are typically valid at a specified temperature, typically 20 °C. Although it is rare that scientists equilibrate solutions to exactly 20 °C before volume measurement, this temperature is assumed to be approximate room temperature. Be aware that the greater the deviation from room temperature, the greater the error in volume measurement. The specific gravity (density) of water at 4, 20, 60, and 80 °C relative to 4 °C is 1.000, 0.998, 0.983, and 0.972. This means that a given mass of water has lower density (greater volume for given mass) at temperatures above 20 °C. This is sometimes seen when a volumetric flask is brought exactly to volume at room temperature and then is placed in an ultrasonic bath to help dissolve the chemicals, warming the solution. A solution that was exactly at the volume marker at room temperature will be above the volume when the solution is warmer. To minimize this error, volumes should be measured at room temperature.

Volumetric glassware (flasks and pipettes) should be used for quantitative volume measurements during



1.2
figure

Class A volumetric pipette (a) and non-volumetric pipettes: adjustable pipettors (b), reed pipettor (c), serological pipettes (d)



1.3
figure

Image of the label on a Class A volumetric flask pipette (a) and Class A volumetric pipette (b)



1.4
figure

Image of a liquid meniscus at the line for a Class A volumetric flask

dilutions and concentrations whenever possible to maximize the accuracy and precision of the procedure. For both volumetric flasks and pipettes, the level of the liquid providing the defined volume is indicated by a line (usually white or red) etched or printed on the neck of the glassware. To achieve the TD or TC volume, the bottom of the meniscus of the liquid should be at the line as shown in Fig. 1.4.

For a volumetric flask, the proper technique for achieving the correct volume is to pour the liquid into the flask until the meniscus is close to the marking line, and then add additional liquid dropwise (with a manual pipette or Pasteur pipette) until the bottom (NOT the top or middle) of the meniscus is at the line with your eye level to the line. (If you do not look straight at the line, occur, making it appear that so that your eye and the line are at the same level, a phenomenon known as “parallax” can occur, making it appear that the bottom of the meniscus is at the line when in fact it is not, resulting in errors in volume measurement.) If the level of the liquid is too high, liquid can be removed using a clean pipette (or the liquid poured out and start again). However, be aware that this cannot be done when preparing a reagent for which the solutes were accurately measured into the flask and you are adding liquid to make up to volume. In this case, you must start over. For this reason, the best practice is to add liquid slowly, and then use a pipette to add liquid dropwise when approaching the desired volume.

For a volumetric pipette, the proper technique for achieving the correct volume is to draw liquid into the pipette until the meniscus is above the line, and then withdraw the pipette from the liquid and dispense the excess liquid from the pipette until the bottom of the meniscus is at the line. It is critical that the pipette be withdrawn from the solution for this step. If the level of the liquid goes below the line, additional liquid is drawn up, and the process is repeated. Proper volumetric measurements require practice and should be repeated until they are performed correctly. Improper

volumetric measurements can result in significant error being introduced into the measurement.

Typical tolerances for lab glassware are presented in Tables 1.6 and 1.7. References for ASTM specifications are found at <http://www.astm.org/>.

A comparison of Tables 1.6 and 1.7 reveals some important points. First, even for Class A glassware, the tolerances for volumetric transfer pipettes (pipettes with a single TD measurement) are much tighter than for graduated measuring pipettes (pipettes with graduations that can be used to measure a wide range of volumes) of the same volume. Second, even for Class A glassware, the tolerances for volumetric transfer pipettes and volumetric flasks are much tighter than

1.6
table

Volume tolerances of Class A glassware required by ASTM specifications

| Volume (mL) | Tolerance (\pm mL) | | | | |
|-------------|-----------------------|-------------------------------|--------------------------------|------------------|--------------------|
| | Buret | Volumetric (transfer) pipette | Measuring (graduated) pipettes | Volumetric flask | Graduated cylinder |
| 0.5 | | 0.006 | | | |
| 1 | | 0.006 | | 0.010 | |
| 2 | | 0.006 | 0.01 | 0.015 | |
| 3 | | 0.01 | 0.02 | 0.015 | |
| 4 | | 0.01 | 0.03 | 0.020 | |
| 5 | | 0.01 | 0.05 | 0.020 | 0.05 |
| 10 | 0.02 | 0.02 | 0.08 | 0.020 | 0.10 |
| 25 | 0.03 | 0.03 | 0.10 | 0.030 | 0.17 |
| 50 | 0.05 | 0.05 | | 0.050 | 0.25 |
| 100 | 0.10 | 0.08 | | 0.080 | 0.50 |
| 250 | | | | 0.012 | 1.00 |
| 500 | | | | 0.013 | 2.00 |
| 1000 | | | | 0.015 | 3.00 |

1.7
table

Volume tolerances of non-Class A glassware required by ASTM specifications

| Volume (mL) | Tolerance (\pm mL) | | | |
|-------------|-----------------------|-------------------------------|------------------|--------------------|
| | Buret | Volumetric (transfer) pipette | Volumetric flask | Graduated cylinder |
| 0.5 | | 0.012 | | |
| 1 | | 0.012 | | |
| 2 | | 0.012 | | |
| 3 | | 0.02 | | |
| 4 | | 0.02 | | |
| 5 | | 0.02 | | 0.10 |
| 10 | 0.04 | 0.04 | 0.04 | 0.20 |
| 25 | 0.06 | 0.06 | 0.06 | 0.34 |
| 50 | 0.10 | 0.10 | 0.24 | 0.50 |
| 100 | 0.20 | 0.16 | 0.40 | 1.00 |
| 250 | | | 0.60 | 2.00 |
| 500 | | | | 4.00 |
| 1000 | | | | 6.00 |

for graduated cylinders of the same volume. Therefore, volumetric transfer pipettes and volumetric flasks are preferred for dilutions and concentrations. For example, a 1000 mL Class A volumetric flask has a tolerance of ± 0.015 mL (the actual TC volume is somewhere between 999.985 and 1000.015 mL), while a 1000 mL graduated cylinder has a tolerance of ± 3.00 mL (the actual TC volume is somewhere between 997 and 1003 mL). This is a 200-fold larger potential error in the measurement of 1000 mL! Finally, tolerances for non-Class A glassware are much broader than for Class A, and thus Class A should be used if available.

1.5.5 Conventions and Terminology

To follow the analytical procedures described in this manual and perform calculations correctly, common terminology and conventions (a convention is a standard or generally accepted way of doing or naming something) must be understood. A common phrase in dilutions and concentrations is “**diluted to**” or “**diluted to a final volume of**.” This means that the sample or solution is placed in a volumetric flask, and the final volume is adjusted to the specified value. In contrast, the phrase “**diluted with**” means that the specified amount is added to the sample or solution. In this latter case, the final mass/volume must be calculated by adding the sample mass/volume and the amount of liquid added. For example, suppose you take a 1.7 mL volume and either (1) dilute to 5 mL with methanol or (2) dilute with 5 mL methanol. In the first case, this means that the sample (1.7 mL) is placed in a volumetric flask and methanol (~3.3 mL) is added so that the final volume is 5 mL total. In the second case, the sample (1.7 mL) is combined with 5 mL methanol, and the final volume is 6.7 mL. As you can see, these are very different values. This will always be the case except when one of the volumes is much larger than the other. For example, if you were working with a 10 μ L sample, diluting it “to 1 L” or “with 1 L” would result in final volumes of 1 L and 1.00001 L, respectively. It is important to understand the differences between these two conventions to perform procedures correctly and interpret data accurately.

Another common term in dilutions/concentrations is the term “**fold**” or “**X**.” This refers to the ratio of the final and initial concentrations (or volumes and masses) of the sample or solution during each step. An “X-fold dilution” means that the concentration of a sample decreases (and typically the volume increases) by a given factor. For example, if 5 mL of an 18.9% NaCl solution is diluted tenfold (or 10X) with water, 45 mL water is added so that the final volume is 50 mL (tenfold or 10X greater than 5 mL) and the final concentration is 1.89% NaCl (tenfold or 10X less than 18.9%). Conversely, an “X-fold concentration” means that the concentration of a sample increases (and typically the volume decreases) by the stated factor. For example, if 90 mL of a 0.31 ppm

salt solution is concentrated tenfold (10X), the volume is decreased to 9 mL (either by reducing to 9 mL or drying completely and reconstituting to 9 mL, tenfold or 10X lower than 90 mL), and the final concentration is 3.1 ppm salt (tenfold or 10X more than 0.31 ppm). Although tenfold or 10X was used for these examples, any value can be used. In microbiology, values of 10X, 100X, 1000X, etc. are commonly used due to the log scale used in that field. However, less standard dilutions of any value are routinely used in analytical chemistry.

The last terminology system for dilutions and concentrations involves **ratios**. This system is somewhat ambiguous and is not used in the *Food Analysis* text or lab manual. This system refers to dilutions as “X:Y,” where X and Y are the masses or volumes of the initial and final solutions/samples. For example, it may be stated that “the solution was diluted 1:8.” This system is ambiguous for the following reasons:

1. The first and last numbers typically refer to the initial and final samples, respectively (therefore, a 1:8 dilution would mean 1 part initial sample and 8 parts final sample). However, there is no standard convention. Therefore, an “X:Y” dilution could be interpreted either way.
2. There is no standard convention as to whether this system describes the “diluted to” or “diluted with” (as described above) approach. Therefore, diluting a sample 1:5 could be interpreted as either (1) diluting 1 mL sample with 4 mL for a final volume of 5 mL (“diluted to”) or (2) diluting 1 mL sample with 5 mL for a final volume of 6 mL (“diluted with”).

Because of these ambiguities, the ratio system is discouraged in favor of the “X-fold” terminology. However, ratio dilutions still appear in some literature. If possible, it is recommended that you investigate to clarify what is meant by this terminology.

Another factor to consider is that liquid volumes are often not strictly additive. For example, exactly 500 mL 95% v/v ethanol aq. added to 500 mL distilled water will not equal 1000 mL; in fact, the new volume will be closer to 970 mL. Where did the missing 30 mL go? Polar molecules such as water undergo different three-dimensional intermolecular bonding in a pure solution versus in a mixture with other solute or chemicals such as ethanol. The difference in bonding causes an apparent contraction in this case. As well, addition of solute to an exact volume of water will change the volume after dissolved. To account for this effect, volumetric glassware is used to bring mixed solutions up to a final volume after initial mixing. When two liquids are mixed, the first liquid is volumetrically transferred into a volumetric flask, and then the second liquid is added to volume, with intermittent swirling or vortexing to mix the liquids as they are being combined. For mixing

solids into solvents, the chemicals are first placed in a volumetric flask, dissolved in a partial volume, and then brought to exact volume with additional solvent.

1.5.6 Burets

Burets are used to deliver definite volumes. The more common types are usually of 25 or 50 ml capacity, graduated to tenths of a milliliter, and are provided with stopcocks. For precise analytical methods in microchemistry, microburets are also used. Microburets generally are of 5 or 10 ml capacity, graduated in hundredths of a milliliter division. General rules in regard to the manipulation of a buret are as follows:

1. Do not attempt to dry a buret that has been cleaned for use, but rather rinse it two or three times with a small volume of the solution with which it is to be filled.
2. Do not allow alkaline solutions to stand in a buret, because the glass will be attacked, and the stopcock, unless made of Teflon, will tend to freeze.
3. A 50 ml buret should not be emptied faster than 0.7 ml per second; otherwise, too much liquid will adhere to the walls; as the solution drains down, the meniscus will gradually rise, giving a high false reading.

It should be emphasized that improper use of and/or reading of burets can result in serious calculation errors.

1.5.7 Cleaning of Glass and Porcelain

In the case of all apparatus for delivering liquids, the glass must be absolutely clean so that the film of liquid never breaks at any point. Careful attention must be paid to this fact or the required amount of solution will not be delivered. The method of cleaning should be adapted to both the substances that are to be removed and the determination to be performed. Water-soluble substances are simply washed out with hot or cold water, and the vessel is finally rinsed with successive small amounts of distilled water. Other substances more difficult to remove, such as lipid residues or burned material, may require the use of a detergent, organic solvent, nitric acid, or aqua regia (25% v/v conc. HNO₃ in conc. HCl). In all cases it is good practice to rinse a vessel with tap water as soon as possible after use. Material allowed to dry on glassware is much more difficult to remove.

1.6 REAGENTS

Chemical reagents, solvents, and gases are available in a variety of **grades of purity**, including technical grade, analytical reagent grade, and various

“ultrapure” grades. The purity of these materials required in analytical chemistry varies with the type of analysis. The parameter being measured and the sensitivity and specificity of the detection system are important factors in determining the purity of the reagents required. **Technical grade** is useful for making cleaning solutions, such as the nitric acid and alcoholic potassium hydroxide solutions mentioned previously. For many analyses, **analytical reagent grade** is satisfactory. Other analyses, e.g., trace organic and HPLC, frequently require special “ultrapure” reagents and solvents. In methods for which the purity of reagents is not specified, it is intended that analytical reagent grade be used. Reagents of lesser purity than that specified by the method should not be used.

There is some confusion as to the definition of the terms **analytical reagent grade**, **reagent grade**, and **ACS analytical reagent grade**. A review of the literature and chemical supply catalogs indicates that the three terms are synonymous. National Formulary (NF), US Pharmaceutical (USP), and Food Chemicals Codex (FCC) are grades of chemicals certified for use as food ingredients. It is important that only NF, USP, or FCC grades be used as food additives if the product is intended for consumption by humans, rather than for chemical analysis.

1.6.1 Acids

The concentration of common commercially available acids is given in Table 1.8.

1.6.2 Distilled Water

Distilled or demineralized water is used in the laboratory for dilution, preparation of reagent solutions, and final rinsing of washed glassware.

1.8

table

Concentration of common commercial strength acids

| Acid | Molecular weight (g/mol) | Concentration (M) | Specific gravity |
|----------------------|--------------------------|-------------------|------------------|
| Acetic acid, glacial | 60.05 | 17.4 | 1.05 |
| Formic acid | 46.02 | 23.4 | 1.20 |
| Hydriodic acid | 127.9 | 7.57 | 1.70 |
| Hydrochloric acid | 36.5 | 11.6 | 1.18 |
| Hydrofluoric acid | 20.01 | 32.1 | 1.167 |
| Hypophosphorous acid | 66.0 | 9.47 | 1.25 |
| Lactic acid | 90.1 | 11.3 | 1.2 |
| Nitric acid | 63.02 | 15.99 | 1.42 |
| Perchloric acid | 100.5 | 11.65 | 1.67 |
| Phosphoric acid | 98.0 | 14.7 | 1.70 |
| Sulfuric acid | 98.0 | 18.0 | 1.84 |
| Sulfurous acid | 82.1 | 0.74 | 1.02 |

Ordinary distilled water is usually not pure. It may be contaminated by dissolved gases and by materials leached from the container in which it has been stored. Volatile organics distilled over from the original source feed water may be present, and nonvolatile impurities may occasionally be carried over by the steam, in the form of a spray. The concentration of these contaminants is usually quite small, and distilled water is used for many analyses without further purification. There are a variety of methods for purifying water, such as distillation, filtration, and ion exchange. Distillation employs boiling of water and condensation of the resulting steam, to eliminate nonvolatile impurities (such as minerals). Ion exchange employs cartridges packed with ionic residues (typically negatively charged) to remove charged contaminants (typically positively charged minerals) when water is passed through the cartridge. Finally, filtration and reverse osmosis remove insoluble particulate matter above a specific size.

1.6.3 Water Purity

Water purity has been defined in many different ways, but one generally accepted definition states that high purity water is water that has been distilled and/or deionized so that it will have a specific resistance of 500,000 Ω (2.0 $\mu\Omega$ /cm conductivity) or greater. This definition is satisfactory as a base to work from, but for more critical requirements, the breakdown shown in Table 1.9 has been suggested to express degrees of purity.

Distilled water is usually produced in a steam-heated metal still. The feed water is (or should be) softened to remove calcium and magnesium to prevent scale (Ca or Mg carbonate) formation. Several companies produce ion-exchange systems that use resin-packed cartridges for producing “distilled water.” The lifespan of an ion-exchange cartridge is very much a function of the mineral content of the feed water. Thus, the lifespan of the cartridge is greatly extended by using distilled or reverse osmosis-treated water as the incoming stream. This procedure can also be used for preparing ultrapure water, especially if a low flow rate is used and the ion-exchange cartridge is of “research” grade.

1.9

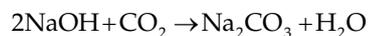
table

Classification of water purity

| Degree of purity | Maximum conductivity ($\mu\Omega$ /cm) | Approximate concentration of electrolytes (mg/L) |
|--------------------|---|--|
| Pure | 10 | 2–5 |
| Very pure | 1 | 0.2–0.5 |
| Ultrapure | 0.1 | 0.01–0.02 |
| Theoretically pure | 0.055 | 0.00 |

1.6.4 Carbon Dioxide-Free Water

Carbon dioxide (CO_2) dissolved in water can interfere with many chemical measurements. Thus, CO_2 -free water may need to be produced. CO_2 -free water may be prepared by boiling distilled water for 15 min and cooling to room temperature. As an alternative, distilled water may be vigorously aerated with a stream of inert gas (e.g., N_2 or He_2) for a period sufficient to achieve CO_2 removal. The final pH of the water should lie between 6.2 and 7.2. It is not advisable to store CO_2 -free water for extended periods. To ensure that CO_2 -free water remains that way, an **ascarite trap** should be fitted to the container such that air entering the container (as boiled water cools) is CO_2 -free. Ascarite is silica coated with NaOH, and it removes CO_2 by the following reaction:



Ascarite should be sealed from air except when water is being removed from the container.

1.6.5 Preparing Solutions and Reagents

The accurate and reproducible preparation of laboratory reagents is essential to good laboratory practice. Liquid reagents are prepared using volumetric glassware (pipettes and flasks) as appropriate.

To prepare solutions from solid reagents (such as sodium hydroxide):

1. Determine the amount of solid reagent needed.
2. Fill the TC volumetric flask $\sim\frac{1}{4}$ – $\frac{1}{2}$ full with the solvent.
3. Add the solid reagent (it is best to pre-dissolve solids in a beaker with a small amount of liquid, and then add this to the flask; rinse the smaller beaker thoroughly and also put the rinses into flask).
4. Swirl to mix until essentially dissolved.
5. Fill the flask to volume with the solvent.
6. Cap and invert the flask ~ 10 – 20 times to completely mix the solution.

Note that it is not appropriate to simply combine the solid reagent with the final volume and assume that the final volume does not change. This is particularly true for high % concentrations. For example, 1 L of a 10% aqueous NaOH solution is correctly made by filling a 1 L flask with ~ 25 – 500 mL water, adding 100 g NaOH, mixing until dissolved, and diluting to 1 L. It would be incorrect to simply combine 100 g NaOH with 1 L water, as the dissolved solid will take up some volume in solution. (Note that solid NaOH is difficult to dissolve, requires a stir bar, and is exothermic, releasing heat upon dissolution; therefore, do not handle the glass with bare hands.) Additionally, if a stir bar is used, make sure to remove this after the solution

is dissolved but BEFORE diluting to volume. Note that sonication is preferred to using a stir bar in a volumetric flask.

The following similar procedures are used to prepare reagents from two or more liquids:

1. Determine the total volume of the final reagent.
2. Obtain a TC volumetric flask (if possible) equal to the final volume.
3. Use TD volumetric glassware to add the correct amount of the liquids with the smallest volumes.
4. Dilute to volume with the liquid with the largest volume, gently swirling during addition.
5. Cap and invert the flask ~10–20 times to completely mix the solution.

Note that a TC volumetric flask should be used whenever possible to bring the solution to final volume. For example, the correct way to prepare 1 L of a 5% ethanol in water solution is to use a 50 mL TD pipette to dispense 50 mL ethanol into a 1L TC flask and then fill the flask to volume with water. It would be incorrect to simply combine 50 mL ethanol and 950 mL water, since complex physical properties govern the volume of a mixture of liquids, and it cannot be assumed that two liquids of different densities and polarities will combine to form a volume equal to the sum of their individual volumes. If the final volume is not a commonly available TC flask size, then use TD glassware to deliver all reagents.

The use of graduated cylinders and beakers should be avoided for measuring volumes for reagent preparation.

1.7 DATA HANDLING AND REPORTING

1.7.1 Significant Figures

The term **significant figure** is used rather loosely to describe some judgment of the number of reportable digits in a result. Often the judgment is not soundly based and meaningful digits are lost or meaningless digits are accepted. Proper use of significant figures gives an indication of the reliability of the analytical method used. Thus, reported values should contain only significant figures. A value is made up of significant figures when it contains all digits known to be true and one last digit in doubt. For example, if a value is reported at 18.8 mg/l, the "18" must be a firm value, while the "0.8" is somewhat uncertain and may be between "0.7" or "0.9." The number zero may or may not be a significant figure:

1. Final zeros after a decimal point are always significant figures. For example, 9.8 g to the nearest mg is reported as 9.800 g.

2. Zeros before a decimal point with other preceding digits are significant. With no preceding digit, a zero before the decimal point is not significant.
3. If there are no digits preceding a decimal point, the zeros after the decimal point but preceding other digits are not significant. These zeros only indicate the position of the decimal point.
4. Final zeros in a whole number may or may not be significant. In a conductivity measurement of 1000 $\mu\Omega/\text{cm}$, there is no implication that the conductivity is $1000 \pm 1 \mu\Omega/\text{cm}$. Rather, the zeros only indicate the magnitude of the number.

A good measure of the significance of one or more zeros before or after another digit is to determine whether the zeros can be dropped by expressing the number in exponential form. If they can, the zeros are not significant. For example, no zeros can be dropped when expressing a weight of 100.08 g in exponential form; therefore the zeros are significant. However, a weight of 0.0008 g can be expressed in exponential form as 8×10^{-4} g, and the zeros are not significant. Significant figures reflect the limits of the particular method of analysis. If more significant figures are needed, selection of another method will be required to produce an increase in significant figures.

Once the number of significant figures is established for a type of analysis, data resulting from such analyses are reduced according to the set rules for rounding off.

1.7.2 Rounding Off Numbers

Rounding off numbers is a necessary operation in all analytical areas. However, it is often applied in chemical calculations incorrectly by blind rule or prematurely and, in these instances, can seriously affect the final results. Rounding off should normally be applied only as follows:

1. If the figure following those to be retained is less than 5, the figure is dropped, and the retained figures are kept unchanged. As an example, 11.443 is rounded off to 11.44.
2. If the figure following those to be retained is greater than 5, the figure is dropped, and the last retained figure is raised by 1. As an example, 11.446 is rounded off to 11.45.
3. When the figure following those to be retained is 5 and there are no figures other than zeros beyond the 5, the figure is dropped, and the last place figure retained is increased by 1 if it is an odd number, or it is kept unchanged if an even number. As an example, 11.435 is rounded off to 11.44, while 11.425 is rounded off to 11.42.

1.7.3 Rounding Off Single Arithmetic Operations

Addition: When adding a series of numbers, the sum should be rounded off to the same numbers of decimal places as the addend with the smallest number of places. However, the operation is completed with all decimal places intact and rounding off is done afterward. As an example:

$$11.1 + 11.12 + 11.13 = 33.35$$

The sum is rounded off to 33.4

Multiplication: When two numbers of unequal digits are to be multiplied, all digits are carried through the operation, and then the product is rounded off to the number of significant digits of the less accurate number.

Division: When two numbers of unequal digits are to be divided, the division is carried out on the two numbers using all digits. Then the quotient is rounded off to the lower number of significant digits between the two values.

Powers and roots: When a number contains n significant digits, its root can be relied on for n digits, but its power can rarely be relied on for n digits.

1.7.4 Rounding Off the Results of a Series of Arithmetic Operations

The rules for rounding off are reasonable for simple calculations. However, when dealing with two nearly equal numbers, there is a danger of loss of all significance when applied to a series of computations that rely on a relatively small difference in two values. Examples are calculation of variance and standard deviation. The recommended procedure is to carry several extra figures through the calculation and then to round off the final answer to the proper number of significant figures. This operation is simplified by using the memory function on calculators, which for most calculators is a large number, often 10 or more, digits.

1.8 BASIC LABORATORY SAFETY

1.8.1 Safety Data Sheets

Safety Data Sheets (SDSs), formerly called Material Safety Data Sheets (MSDSs), are informational packets that are “intended to provide workers and emergency personnel with procedures for handling or working with that substance in a safe manner and include information such as physical data (melting point, boiling point, flash point, etc.), toxicity, health effects, first aid,

reactivity, storage, disposal, protective equipment, and spill-handling procedures” (http://en.wikipedia.org/wiki/Material_safety_data_sheet#United_States).

SDSs are available for all reagents, chemicals, solvents, gases, etc. used in your laboratory. You can consult these documents if you have questions regarding how to safely handle a material, the potential risks of the material, how to properly clean up a spill, etc. They should be available to you in a centralized location (typically, a binder) in the lab. If not available, you may request these from your instructor or find them online. Generally, the following information is available on a MSDS or SDS in a 16-section format:

1. Identification of the substance/mixture
2. Hazard identification
3. Composition/information on ingredients
4. First aid measures
5. Firefighting measures
6. Accidental release measures
7. Handling and storage
8. Exposure controls/personal protection
9. Physical and chemical properties
10. Stability and reactivity
11. Toxicological information
12. Ecological information
13. Disposal considerations
14. Transport information
15. Regulatory information
16. Other information

1.8.2 Hazardous Chemicals

Food analysis laboratories, like any chemical laboratory, often contain hazardous compounds, including:

1. Acids (hydrochloric acid, sulfuric acid, etc.)
2. Bases (e.g., sodium hydroxide)
3. Corrosives and oxidizers (sulfuric acid, nitric acid, perchloric acid, etc.)
4. Flammables (organic solvents such as hexane, ether, alcohols)

1.8.3 Personal Protective Equipment and Safety Equipment

It is important to understand the location and use of lab safety equipment. The purpose of this is threefold:

1. To prevent accidents and/or injuries in the lab
2. To quickly and effectively respond to any accident and/or injury in the lab
3. Be able to perform laboratory procedures without excessive worrying about lab hazards

Your laboratory instructor should provide instruction regarding basic laboratory safety equipment. You should be aware of these general rules and the existence of this equipment.

Proper clothing is required to work in any chemical laboratory. The following standards and rules regarding dress are generally applicable, although standards may vary between laboratories:

1. Close-toed shoes (no flip-flops, sandals, or other "open" footwear).
2. Long pants (dresses, skirts, and shorts may be allowed in some laboratories).
3. No excessively loose clothing or accessories.
4. Long hair should be pulled back from the face into a ponytail or otherwise restrained.

You should be able to obtain and wear the following personal protective equipment (PPE) and understand their proper use:

1. Safety glasses, goggles, and face shields
2. Lab coat or apron
3. Shoe covers
4. Latex or acetonitrile gloves
5. Puncture-resistant gloves
6. Heat-resistant gloves

You should be aware of the locations of the following safety equipment items and their proper use:

1. First aid kit
2. Bodily fluids cleanup kit
3. Acid, base, and solvent spill kits
4. Fire extinguisher and fire blanket
5. Safety shower and eyewash station
6. Solid, liquid, chlorinated, and biohazard waste disposal containers, if applicable
7. Sharps and broken glass disposal containers, if applicable

1.8.4 Eating, Drinking, Etc.

Your hands may become contaminated with substances used in the lab simply by touching lab benches, glassware, etc. This may happen even without your knowledge. Even if you are not handling hazardous substances, previous lab occupants may not have cleaned benches and glassware, leaving behind hazardous substances that you are unaware of. To avoid spreading potentially harmful substances from your hands to your face, eyes, nose, and mouth (where they may irritate sensitive or be introduced to circulation by mucus membranes, ingestion, or inhalation), the following activities are prohibited in chemical laboratories: eating, drinking, smoking, chewing tobacco or snuff, and applying cosmetics (e.g., lip balm). The following should not even be brought into a chemical

laboratory: food, water, beverages, tobacco, and cosmetics. Some unconscious activities (e.g., touching your face and eyes) are difficult to avoid. However, wearing gloves in the laboratory may minimize these actions.

1.8.5 Miscellaneous Information

The following general rules and guidelines apply to working in the laboratory:

1. When combining acid and water, always add the acid to water (instead of adding water to the acid). When acid dissolves in water, heat is released. This can cause splattering of the solution. By adding the acid to water, the heat is dissipated, and splattering is reduced or eliminated.
2. Be aware that dissolving sodium hydroxide in water generates heat. Making high concentrations of aqueous sodium hydroxide can lead to very hot solutions that can burn bare hands. Allow these solutions to cool, or handle with heat-resistant gloves.
3. Broken glass and other sharps (razor blades, scalpel blades, needles, etc.) should be disposed of in puncture-resistant sharps containers.
4. Do not pour waste or chemicals down the drain. This practice can damage the building's plumbing and harm the environment. Dispose of liquid, solid, chlorinated, radioactive, and biohazard wastes into the appropriate containers provided by the lab instructor. If you are unsure how to properly dispose of waste, ask your instructor or teaching assistant.
5. Handle volatile, noxious, or corrosive compounds in the fume hood with appropriate PPE.

RESOURCE MATERIALS

- Analytical Quality Control Laboratory. 2010. Handbook for analytical quality control in water and wastewater laboratories. U.S. Environmental Protection Agency, Technology Transfer.
- Anonymous. 2010. Instructions for Gilson Pipetman. Rainin Instrument Co., Inc., Washburn, MA.
- Applebaum, S.B. and Crits, G.J. 1964. "Producing High Purity Water". Industrial Water Engineering.
- Smith JS. 2017. Evaluation of analytical data, Ch. 4, In: Nielsen SS (ed.) Food analysis, 5th edn. Springer, New York.
- Willare, H.H. and Furman, W.H. 1947. Elementary Quantitative Analysis – Theory and Practice. Van Nostrand Co., Inc., New York.