measuring or **graduated pipet** (see Figures 2.9 and 2.10). Variations of the latter are also called **clinical**, or **serological**, **pipets**.

Pipets are designed **to deliver** a specified volume at a given temperature, and they are marked "**TD**." Again, the volume can be considered to be constant with small changes in temperature. Pipets are calibrated to account for the drainage film remaining on the glass walls. This drainage film will vary somewhat with the time taken to deliver, and usually the solution is allowed to drain under the force of gravity and the pipet is removed shortly after the solution is delivered. A uniform drainage time should be adopted.

The volumetric pipet is used for accurate measurements since it is designed to deliver only one volume and is calibrated at that volume. Accuracy to four significant figures is generally achieved, although with proper calibration, five figures may be obtained if necessary. See the table on the *back cover* for tolerances of class A transfer pipets. Measuring pipets are straight-bore pipets that are marked at different volume intervals. These are not as accurate because nonuniformity of the internal diameter of the device will have a relatively larger effect on total volume than is the case for pipets with a bulb shape. Also, the drainage film will vary with the volume delivered. At best, accuracy to three significant figures can be expected from these pipets, unless you make the effort to calibrate the pipet for a given volume delivered.

Most volumetric pipets are calibrated to deliver with a certain small volume remaining in the tip. This should not be shaken or blown out. In delivering, the pipet is held vertically and the tip is touched on the side of the vessel to allow smooth delivery without splashing and so that the proper volume will be left in the tip. The forces of attraction of the liquid on the wall of the vessel will draw out a part of this.

Fig. 2.9. Transfer or volumetric pipets.

Some pipets are **blowout** types (including measuring pipets calibrated to the entire tip volume). The final volume of solution must be blown out from the tip to deliver the calibrated amount. These pipets are easy to identify, as they will always have one or two **ground bands or rings** around the top. (These are not to be confused with a colored ring that is used only as a color coding for the volume of the pipet.) The solution is not blown out until it has been completely drained by gravity. Blowing to increase the rate of delivery will change the volume of the drainage film.

Volumetric pipets are available in sizes of 100 to 0.5 mL or less. Measuring and serological pipets range from a total capacity of 25 to 0.1 mL. Measuring pipets can be used for accurate measurements, especially for small volumes, if they are calibrated at the particular volume wanted. The larger measuring pipets usually deliver too quickly to allow drainage as fast as the delivery, and they have too large a bore for accurate reading.

In using a pipet, one should always wipe the outside of the tip dry after filling. If a solvent other than water is used, or if the solution is viscous, pipets must be recalibrated for the new solvent or solution to account for difference in drainage rate.

Pipets are filled by suction, using a rubber pipet bulb, a pipet pump, or other such pipetting device. Before using a pipet, practice filling it with water. No solution should be pipetted by mouth.

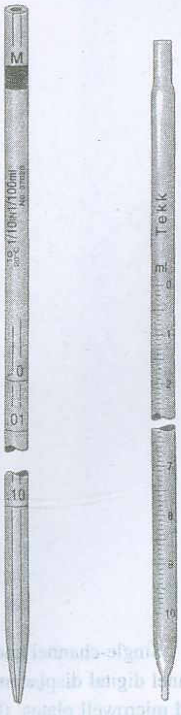


Fig. 2.10. Measuring pipets.

SYRINGE PIPETS

These can be used for both macro and micro volume measurements. The calibration marks on the syringes may not be very accurate, but the reproducibility can be excellent if an automatic deliverer is used, such as a spring-load device that

Fig. 2.11. Hamilton microliter syringe.

Syringe pipets are useful for delivering microliter volumes.

draws the plunger up to the same preset level each time. The volume delivered in this manner is free from drainage errors because the solution is forced out by the plunger. The volume delivered can be accurately calibrated. Microliter syringe pipets are used for introduction of samples into gas chromatographs. A typical syringe is illustrated in Figure 2.11. They are fitted with a needle tip, and the tolerances are as good as those found for other micropipets. In addition, any desired volume throughout the range of the syringe can be delivered.

The above syringe pipets are useful for accurate delivery of viscous solutions or volatile solvents; with these materials the drainage film would be a problem in conventional pipets. Syringe pipets are well suited to rapid delivery and also for thorough mixing of the delivered solution with another solution as a result of the rapid delivery.

A second type of syringe pipet is that shown in Figure 2.12. This type is convenient for rapid, one-hand dispensing of fixed volumes in routine procedures and is widely used in the clinical chemistry laboratory. It contains a disposable nonwetting plastic tip (e.g., polypropylene) to reduce both film error and contamination. A thumb button operates a spring-loaded plunger, which stops at an intake or a discharge stop; the latter stop is beyond the former to ensure complete delivery. The sample never contacts the plunger and is contained entirely in the plastic tip. These pipets are available in volumes of 1 to 1000 μL and are reproducible to 1 to 2% better, depending on the volume.

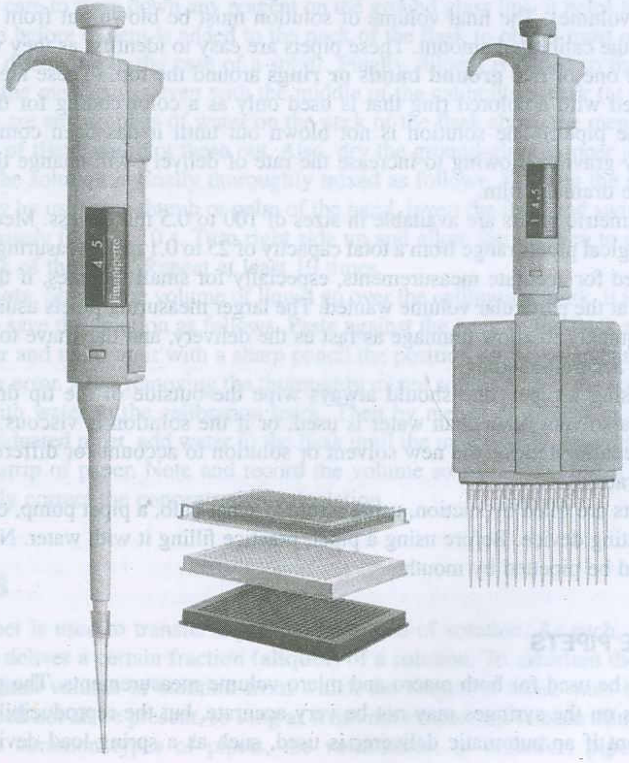


Fig. 2.12. Single-channel and multichannel digital displacement pipets and microwell plates. (Courtesy of Thermo Labsystems.)

The actual volume delivered by these and other micropipets frequently does not need to be known because they are used in relative measurements. For example, the same pipet may be used to deliver a sample and an equal volume of a standard solution for calibrating the instrument used for the measurement. Precision in delivery is usually more important than the absolute volume delivered. The standard for pipet calibration use in Europe (where many of these pipets are manufactured) is the German DIN 126650 (or a similar international standard ISO 8655). Calibrations are based on gravimetric testing (weighing of water). The DIN standard does not give separate limits for accuracy and precision, but rather uses a combined error limit equal to percent accuracy plus 2 times the standard deviation, that is, it gives a range in which we are 95% confident the delivery will fall (see Chapter 3 for a discussion of standard deviation and confidence limits). Table 2.2 lists the DIN error limits for single-channel displacement pipets. Table 2.3 lists accuracies and precisions for a typical model single-channel pipet.

Besides the manually operated syringes, there are electronically controlled and variable-volume motor-driven syringes available for automated repetitive deliveries. Also, you may purchase pipets with multiple syringes for simultaneous delivery, with for example, 12 or 16 channels. These are useful for delivering solutions into microwell plates used in biotechnology or clinical chemistry laboratories that process thousands of samples (Figure 2.12). You may find more information on displacement pipets from representative manufacturers, for example, www.finnpipette.com and www.eppendorf.com.

The volume may not be accurately known, but it is reproducible.

BURETS

A buret is used for the accurate delivery of a variable amount of solution. Its principal use is in **titrations**, where a standard solution is added to the sample solution until the **end point** (the detection of the completion of the reaction) is reached. The conventional buret for macrotitrations is marked in 0.1-mL increments from 0 to 50 mL; one is illustrated in Figure 2.13. The volume delivered can be read to the nearest 0.01 mL by interpolation (good to about ±0.02 or ±0.03 mL). Burets

Table 2.2

DIN 12650 Error Limits for Single Channel Air Displacement Pipets[a]

Nominal Volume (μL)	Maximum Error (μL)	Relative Error (%)
1	±0.15	±15.0
2	±0.20	±10.0
5	±0.30	±6.0
10	±0.30	±3.0
20	±0.40	±2.0
50	±0.80	±1.6
100	±1.50	±1.5
200	±2.00	±1.0
500	±5.00	±1.0
1000	±10.00	±1.0
2000	±20.00	±1.0
5000	±50.00	±1.0
10000	±100.00	±1.0

[a]These limits apply to manufacturers with a controlled environment. If the tests are performed by a user in a normal laboratory environment, the limits in the table may be doubled.
Courtesy of Thermo Labsystems Oy, Finland.

Table 2.3

Accuracy and Precision for Single Channel Digital Finnpipettes

Range (μL)	Increment (μL)	Volume (μL)	Accuracy (μL)	Accuracy (%)	Precision[a] s.d. (μL)	Precision[a] CV (%)
0.2–2	0.01	2	±0.050	±2.5	0.040	2.0
		0.2	±0.024	±12.0	0.020	10.0
0.5–10	0.1	10	±0.100	±1.0	0.050	0.5
		1	±0.025	±2.5	0.020	2.0
0.5–10	0.1	10	±0.100	±1.0	0.080	0.8
		1	±0.035	±3.5	0.030	3.0
2–20	0.1	20	±0.200	±1.0	0.080	0.4
		2	±0.060	±3.0	0.030	1.5
5–40	0.5	40	±0.240	±0.6	0.120	0.3
		5	±0.100	±2.0	0.100	2.0
10–100	1.0	100	±0.80	±0.8	0.20	0.2
		10	±0.30	±3.0	0.10	1.0
20–200	1.0	200	±1.20	±0.6	0.40	0.2
		20	±0.36	±1.8	0.14	0.7
200–1000	5.0	1000	±6.00	±0.6	2.00	0.2
		200	±1.80	±0.9	0.60	0.3
100–1000	5.0	1000	±6.00	±0.6	2.00	0.2
		100	±1.00	±1.0	0.60	0.6

[a]s.d. = standard deviation, CV = coefficient of variation.
Courtesy of Thermo Labsystems Oy, Finland.

Fig. 2.13. Typical buret.

are also obtainable in 10-, 25-, and 100-ml capacities, and microburets are available in capacities of down to 2 mL, where the volume is marked in 0.01-mL increments and can be estimated to the nearest 0.001 mL. Ultramicroburets of 0.1-mL capacity graduated in 0.001-mL (1-μL) intervals are used for microliter titrations.

Drainage film is a factor with conventional burets, as with pipets, and this can be a variable if the delivery rate is not constant. The usual practice is to deliver at a fairly slow rate, about 15 to 20 mL per minute, and then to wait several seconds after delivery to allow the drainage to "catch up." In actual practice, the rate of delivery is only a few drops per minute near the end point, and there will be no time lag between the flow rate and the drainage rate. As the end point is approached, fractions of a drop are delivered by just opening, or "cracking," the stopcock and then touching the tip of the buret to the wall of the titration vessel. The fraction of the drop is then washed down into the solution with distilled water.

CARE AND USE OF VOLUMETRIC GLASSWARE

We have mentioned a few precautions in the use of volumetric flasks, pipets, and burets. Your laboratory instructor will supply you with detailed instructions in the use of each of these tools. A discussion of some general precautions and good laboratory technique follows.

Cleanliness of glassware is of the utmost importance. If films of dirt or grease are present, liquids will not drain uniformly and will leave water breaks or droplets on the walls. Under such conditions the calibration will be in error. Initial cleaning should be by repeated rinses with water. Then try cleaning with dilute nitric

acid and rinse with more water. Use of a buret or test tube brush aids the cleaning of burets and necks of volumetric flasks—but be careful of scratching the interior walls. Pipets should be rotated to coat the entire surface with detergent. There are commercial cleaning solutions available that are very effective.

Rinse pipets and burets with the solution to be measured.

Pipets and burets should be rinsed at least twice with the solution with which they are to be filled. If they are wet, they should be rinsed first with water, if they have not been already, and then a minimum of *three* times with the solution to be used; about one-fifth the volume of the pipet or buret is adequate for each rinsing. A volumetric flask, if it is wet from a previously contained solution, is rinsed with three portions of water only since later it will be filled to the mark with water. It need not be dry.

Note that analytical glassware should not be subjected to the common practice employed in organic chemistry laboratories of drying either in an oven (this can affect the volume of calibrated glassware) or by drying with a towel or by rinsing with a volatile organic solvent such as acetone (which can cause contamination). The glassware usually does not have to be dried. The preferred procedure is to rinse it with the solution that will fill it.

Avoid parallax error in reading buret or pipet volumes.

Care in reading the volume will avoid parallax error, that is, error due to incorrect alignment of the observer's eye, the meniscus, and the scale. This applies in the reading of any scale, such as the pointer scale of an analytical balance. Correct position is with your eye at the same level as the menicus. If the eye level is above the meniscus, the volume read will be smaller than that taken; the opposite will be true if the eye level is too low.

After glassware is used, it can usually be cleaned sufficiently by immediate rinsing with water. If the glassware has been allowed to dry, it should be cleaned with detergent. Volumetric flasks should be stored with the stopper inserted, and preferably filled with distilled water. Burets should be filled with distilled water and stoppered with a rubber stopper when not in use.

GENERAL TIPS FOR ACCURATE AND PRECISE TITRATING

Your buret probably has a Teflon stopcock, and this will not require lubrication. Make sure it is secured tightly enough to prevent leakage, but not so tight as to make rotation hard. If your buret has a ground-glass stopcock, you may have to grease the stopcock. A thin layer of stopcock grease (not silicone lubricant) is applied uniformly to the stopcock, using very little near the hole and taking care not to get any grease in the hole. The stopcock is inserted and rotated. There should be a uniform and transparent layer of grease, and the stopcock should not leak. If there is too much lubricant, it will be forced into the barrel or may work into the buret tip and clog it. Grease can be removed from the buret tip and the hole of the stopcock by using a fine wire. If the buret contains a Teflon stopcock, it does not require lubrication. The buret is filled above the zero mark and the stopcock is opened to fill the tip. Check the tip for air bubbles. If any are present, they may work out of the tip during the titration, causing an error in reading. Work air bubbles out by rapid opening and closing of the stopcock to squirt the titrant through the tip or tapping the tip while solution is flowing. No bubbles should be in the barrel of the buret. If there are, the buret is probably dirty.

The initial reading of the buret is taken by allowing it to drain slowly to the zero mark. Wait a few seconds to make certain the drainage film has caught up to the meniscus. Read the buret to the nearest 0.02 mL (for a 50-mL buret). The initial reading may be 0.00 mL or greater. The reading is best taken by placing your finger just in back of the meniscus or by using a meniscus illuminator (Figure 2.14). The meniscus illuminator has a white and a black field, and the black field is

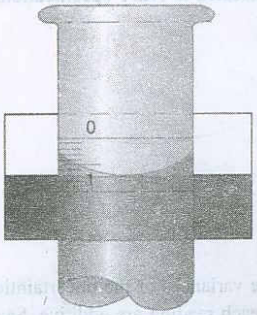


Fig. 2.14. Meniscus illuminator for buret.

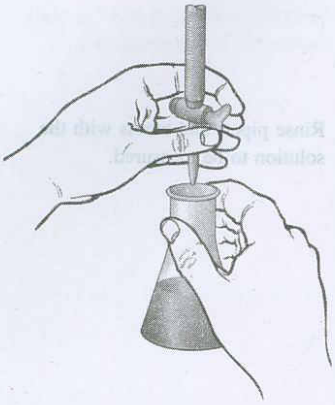


Fig. 2.15. Proper technique for titration.

positioned just below the meniscus. Avoid parallax error by making the reading at eye level.

The titration is performed with the sample solution in an Erlenmeyer flask. The flask is placed on a white background, and the buret tip is positioned within the neck of the flask. The flask is swirled with the right hand while the stopcock is manipulated with the left hand (Figure 2.15), or whatever is comfortable. This grip on the buret maintains a slight inward pressure on the stopcock to ensure that leakage will not occur. The solution can be more efficiently stirred by means of a magnetic stirrer and stirring bar.

As the titration proceeds, the indicator changes color in the vicinity where the titrant is added, owing to local excesses; but it rapidly reverts to the original color as the titrant is dispersed through the solution to react with the sample. As the end point is approached, the return to the original color occurs more slowly, since the dilute solution must be mixed more thoroughly to consume all the titrant. At this point, the titration should be stopped and the sides of the flask washed down with distilled water from the wash bottle. A drop from the buret is about 0.02 to 0.05 mL, and the volume is read to the nearest 0.02 mL. It is therefore necessary to split drops near the end point. This can be done by slowly turning the stopcock until a fraction of a drop emerges from the buret tip and then closing it. The fraction of drop is touched off onto the wall of the flask and is washed into the flask with the wash bottle, or it is transferred with a glass stirring rod. There will be a sudden and "permanent" (lasting at least 30 s) change in the color at the end point when a fraction of a drop is added.

Subsequent titrations can be speeded up by using the first to *approximate* the end-point volumes.

The titration is usually performed in triplicate. After performing the first titration, you can calculate the approximate volume for the replicate titrations from the weights of the samples and the molarity of the titrant. This will save time in the titrations. The volume should not be calculated to nearer than 0.1 mL in order to avoid bias in the reading.

After a titration is complete, unused titrant should never be returned to the original bottle but should be discarded.

If a physical property of the solution, such as potential, is measured to detect the end point, the titration is performed in a beaker with magnetic stirring so electrodes can be placed in the solution.

TOLERANCES AND PRECISION OF GLASSWARE

Class A glassware is accurate enough for most analyses. It can be calibrated to NIST specifications.

The National Institute of Standards and Technology (NIST) has prescribed certain *tolerances*, or absolute errors, for different volumetric glassware, and some of these are listed on the *back cover* of the text. For volumes of greater than about 25 mL, the tolerance is within 1 part per thousand (ppt) relative, but it is larger for smaller volumes. The letter "A" stamped on the side of a volumetric flask, buret, or pipet indicates that it complies with class A tolerances. This says nothing about the precision of delivery. Volumetric glassware that meets NIST specifications or that is certified by NIST can be purchased, but at a significantly higher price than uncertified glassware. Less expensive glassware may have tolerances double those specified by NIST. It is a simple matter, however, to calibrate this glassware to an accuracy as good as or exceeding the NIST specifications (see Experiment 2).

The variances or the uncertainties in each reading are additive. See propagation of error, Chapter 3.

The precision of reading a 50-mL buret is about ± 0.02 mL. Since a buret is always read twice, the total absolute uncertainty may be as much as ± 0.04 mL. The relative uncertainty will vary inversely with the total volume delivered. It becomes apparent that a titration with a 50-mL buret should involve about 40 mL

titrant to achieve a precision of 1 ppt. Smaller burets can be used for increased precision at smaller volumes. Pipets will also have a certain precision of reading, but only one reading is required for volumetric pipets.

CALIBRATION OF GLASSWARE—FOR THE ULTIMATE ACCURACY

Example 2.1 illustrated how Equation 2.1 may be used in the calibration of glassware, to correct for buoyancy of the water used for calibration, that is, to correct to weight in vacuum. Dividing the weight of the water in vacuum by its density at the given temperature will convert it to volume.

Table 2.4 lists the calculated volumes for a gram of water in air at atmospheric pressure for different temperatures, corrected for buoyancy with stainless steel weights of density 7.8 g/cm^3. These are used to give the volume of the glassware

Table 2.4

Glassware Calibration

	A	B	C	D	E	F	G	H
1	**Table 2.4 Glassware Calibration**							
2	Weight in vacuum assuming stainless steel weights, density 7.8 g/mL.							
3	Glass expansion for borosilicate glass, 0.000025 mL/mL/ °C.							
4								
5	Save this spreadsheet contained in your CD, Chapter 2, to your desktop, and use it to calculate							
6	calibrated volumes of glassware. Substitute into the appropriate Cell B, at temperature T, the							
7	weight of water in air, obtained from the glassware at the temperature of the measurement (Cell A).							
8	The calibration volume at the temperature, T (Cell D), and at 20°C (Cell F), is calculated.							
9	Round the calculated values to the appropriate number of significant figures, usually							
10	four or five.							
11								
12	**T, °C**	**Wt. H_2O**	**Wt. in**	**Vol. at T,**	**Glass expansion,**	**Vol. at 20°,**	**Density,**	
13		**in air, g**	**vacuum, g**	**mL**	**at 20°C, mL**	**mL**	**g/mL**	
14	10	1.0000	**1.0010**	**1.0013**	**-0.000250**	**1.0016**	0.9997026	
15	11	1.0000	1.0010	1.0014	-0.000225	1.0017	0.9996081	
16	12	1.0000	1.0010	1.0015	-0.000200	1.0017	0.9995004	
17	13	1.0000	1.0010	1.0017	-0.000175	1.0018	0.9993801	
18	14	1.0000	1.0010	1.0018	-0.000150	1.0020	0.9992474	
19	15	1.0000	1.0010	1.0019	-0.000125	1.0021	0.9991026	
20	16	1.0000	1.0010	1.0021	-0.000100	1.0022	0.9989460	
21	17	1.0000	1.0010	1.0023	-0.000075	1.0023	0.9987779	
22	18	1.0000	1.0010	1.0025	-0.000050	1.0025	0.9985896	
23	19	1.0000	1.0010	1.0026	-0.000025	1.0027	0.9984082	
24	20	1.0000	1.0010	1.0028	0.000000	1.0028	0.9982071	
25	21	1.0000	1.0010	1.0031	0.000025	1.0030	0.9979955	
26	22	1.0000	1.0010	1.0033	0.000050	1.0032	0.9977735	
27	23	1.0000	1.0010	1.0035	0.000075	1.0034	0.9975415	
28	24	1.0000	1.0010	1.0038	0.000100	1.0037	0.9972995	
29	25	1.0000	1.0010	1.0040	0.000125	1.0039	0.9970479	
30	26	1.0000	1.0010	1.0043	0.000150	1.0041	0.9967867	
31	27	1.0000	1.0010	1.0045	0.000175	1.0044	0.9965162	
32	28	1.0000	1.0010	1.0048	0.000200	1.0046	0.9962365	
33	29	1.0000	1.0010	1.0051	0.000225	1.0049	0.9959478	
34	30	1.0000	1.0010	1.0054	0.000250	1.0052	0.9956502	
35								
36	Formulas are entered into the boldface cells above as indicated below. They are							
37	copied down for all temperatures. See Chapter 3 for setting up a spreadsheet.							
38	**Cell C14:** $W_{vac} = W_{air} + W_{air}(0.0012/D_o - 0.0012/D_w) = W_{air}(0.0012/1.0 + 0.0012/7.8)$							
39			=B14+B14*(0.0012/1.0-0.0012/7.8)			Copy down		
40	**Cell D14:** V_T (mL)= $W_{vac,T}$(g)/D_T(g/mL)							
41			=C14/G14			Copy down		
42	**Cell E14:** Glass expans. = (T - 20) (deg) x 0.000025 (mL/mL/deg) x W_{air} (g)							
43			=(A14-20)*0.000025*B14			Copy down		
44	**Cell F14:** $V_{20o} = V_T$ - $Glass_{exp}$ =		D14-E14			Copy down		

being calibrated, from the weight of water contained or delivered by the glassware. (The values are not significantly different for brass weights of 8.4 g/cm^3 density. See Example 2.2.) The glass volumes calculated for the standard temperature of 20°C include slight adjustments for borosilicate glass (Pyrex or Kimax) container expansion or contraction with temperature changes (volumetric glassware has a cubical coefficient of expansion of about 0.000025 per degree centigrade, resulting in changes of about 0.0025% per degree; for 1 mL, this is 0.000025 mL per degree.). Water expands about 0.02% per degree around 20°C. Volume (concentration) corrections may be made using the water density data in Table 2.4, taking the ratios of the relative densities.

Your CD has the Table 2.4 spreadsheet, with formulas as indicated in the table. You can substitute specific weights of water in air, obtained from a flask, pipet, or buret, in cell B at the temperature of measurement to obtain the calculated calibration volume at temperature, T, and for 20°C. We describe the use of spreadsheets in Chapter 3. The CD also has a table and figure of the percent error for weight in vacuum as a function of sample density.

Example 2.3

(a) Use Table 2.4 to calculate the volume of the 20-mL pipet in Example 2.1, from its weight in air. Assume the temperature is 23°C. (b) Give the corresponding volume at 20°C as a result of glass contraction. (c) Compare with the volume calculated using the weight in air with that calculated using the weight in vacuum and the density in water (Example 2.1).

Solution

(a) From Table 2.4, the volume per gram in air is 1.0035 mL at 23°C:

$$19.994 \text{ g} \times 1.0035 \text{ mL/g} = 20.064 \text{ mL}$$

(b) The glass contraction at 20°C relative to 23°C is 0.0015 mL (0.000025 mL/mL/°C × 20 mL × 3°C), so the pipet volume at 20°C is 20.062 mL.

(c) The density of water at 23°C is 0.99754 g/mL, so from the weight in vacuum:

$$20.015 \text{ g}/0.99754 \text{ g/mL} = 20.064 \text{ mL}$$

The same value is obtained.

Example 2.4

You prepared a solution of hydrochloric acid and standardized it by titration of primary standard sodium carbonate. The temperature during the standardization was 23°C, and the concentration was determined to be 0.1127_2 M. The heating system in the laboratory malfunctioned when you used the acid to titrate an unknown, and the temperature of the solution was 18°C. What was the concentration of the titrant?

Solution

$$M_{18°} = M_{23°} \times (D_{18°}/D_{23°})$$
$$= 0.1127_2 \times (0.99859/0.99754)$$
$$= 0.1128_4\ M$$

(See Chapter 3 for significant figures and the meaning of the subscript numbers.)

TECHNIQUES FOR CALIBRATING GLASSWARE

You generally calibrate glassware to five significant figures, the maximum precision you are likely to attain in filling or delivering solutions. Hence, your net weight of water needs to be five figures. If the glassware exceeds 10 mL, this means weighing to 1 mg is all that is needed. This can be readily and conveniently accomplished using a top-loading balance, rather than a more sensitive analytical balance. (Note: If the volume number is large without regard to the decimal, e.g., 99, then four figures will suffice—see Chapter 3 discussion on significant figures. A 10-mL pipet, e.g., may calibrate to 9.997 mL, which is as accurate as 10.003 mL, i.e., the last figure is 1 part in 10,000.)

1. Volumetric Flask Calibration. To calibrate a volumetric flask, first weigh the clean, dry flask and stopper. Then fill it to the mark with distilled water. There should be no droplets on the neck. If there are, blot them with tissue paper. The flask and water should be equilibrated to room temperature. Weigh the filled flask, and then record the temperature of the water to 0.1°C. The increase in weight represents the weight in air of the water contained by the flask.

2. Pipet Calibration. To calibrate a pipet, weigh a dry Erlenmeyer flask with a rubber stopper or a weighing bottle with a glass stopper or cap, depending on the volume of water to be weighed. Fill the pipet with distilled water (whose temperature you have recorded) and deliver the water into the flask or bottle, using proper pipetting technique, and quickly stopper the container to avoid evaporation loss. Reweigh to obtain the weight in air of water delivered by the pipet.

3. Buret Calibration. Calibrating a buret is similar to the procedure for a pipet, except that several volumes will be delivered. The internal bore of the buret is not perfectly cylindrical, and it will be a bit "wavy," so the actual volume delivered will vary both plus and minus from the nominal volumes marked on the buret, as increased volumes are delivered. You will ascertain the volume at 20% full-volume increments (e.g., each 10 mL, for a 50-mL buret) by filling the buret each time and then delivering the nominal volume into a dry flask. (The buret is filled each time to minimize evaporation errors. You may also make successive deliveries into the same flask, i.e., fill the buret only once. Make rapid deliveries.) Since the delivered volume does not have to be exact, but close to the nominal volume, you can make fairly fast deliveries, but wait about 10 to 20 s for film drainage. Prepare a plot of volume correction versus nominal volume and draw straight lines between each point. Interpolation is made at intermediate volumes from the lines. Typical volume corrections for a 50-mL buret may range up to ca. 0.05 mL, plus or minus.

Example 2.5

You calibrate a 50-mL buret at 10-mL increments, filling the buret each time and delivering the nominal volume, with the following results:

Buret Reading (mL)	*Weight H_2O Delivered (g)*
10.02	10.03
20.08	20.03
29.99	29.85
40.06	39.90
49.98	49.86

Construct a plot of volume correction versus volume delivered. The temperature of the water is 20°C and stainless steel weights are used.

Solution

From Table 2.4 (or use the CD Table 2.4 for automatic calculation of volumes):

$$W_{vac} = 10.03 + 10.03(0.00105) = 10.03 + 0.01 = 10.04 \text{ g}$$
$$\text{Vol.} = 10.04 \text{ g}/0.9982 \text{ g/mL} = 10.06 \text{ mL}$$

Likewise, for the others, we construct the table:

Nominal Volume (mL)	*Actual Volume (mL)*	*Correction (mL)*
10.02	10.06	+0.04
20.08	20.09	+0.01
29.99	29.93	−0.04
40.06	40.01	−0.05
49.98	50.00	0.00

Prepare a graph of nominal volume (y axis) versus correction volume. Use 10, 20, 30, 40, and 50 mL as the nominal volumes.

SELECTION OF GLASSWARE—HOW ACCURATE DOES IT HAVE TO BE?

Only certain volumes need to be measured accurately, those involved in the quantitative calculations.

As in weighing operations, there will be situations where you need to accurately know volumes of reagents or samples measured or transferred (accurate measurements), and others in which only approximate measurements are required (rough measurements). If you wish to prepare a standard solution of 0.1 *M* hydrochloric acid, it can't be done by measuring an accurate volume of concentrated acid and diluting to a known volume because the concentration of the commercial acid is not known adequately. Hence, an approximate solution is prepared that is then standardized. We see in the table on the inside *back cover* that the commercial acid is about 12.4 *M*. To prepare 1 L of a 0.1 *M* solution, about 8.1 mL needs to be taken and diluted. It would be a waste of time to measure this (or the water used for dilution) accurately. A 10-mL graduated cylinder or 10-mL measuring pipet will

suffice, and the acid can be diluted in an ungraduated 1-L bottle. If, on the other hand, you wish to dilute a stock standard solution accurately, then a transfer pipet must be used and the dilution must be done in a volumetric flask. Any volumetric measurement that is a part of the actual analytical measurement must be done with the accuracy required of the analytical measurement. This generally means four-significant-figure accuracy, and transfer pipets and volumetric flasks are required. This includes taking an accurate portion of a sample, preparation of a standard solution from an accurately weighed reagent, and accurate dilutions. Burets are used for accurate measurement of variable volumes, as in a titration. Preparation of reagents that are to be used in an analysis just to provide proper solution conditions (e.g., buffers for pH control) need not be prepared highly accurately, and less accurate glassware can be used, for example, graduated cylinders.

2.5 Preparation of Standard Base Solutions

Sodium hydroxide usually used as the titrant when a base is required. It contains significant amounts of water and sodium carbonate, and so it cannot be used as a primary standard. For accurate work, the sodium carbonate must be removed from the NaOH because it reacts to form a buffer that decreases the sharpness of the end point. In addition, an error will result if the NaOH is standardized using a phenolphthalein end point (in which case the CO_3^{2-} is titrated only to HCO_3^-), and then a methyl orange end point is used in the titration of a sample (in which case the CO_3^{2-} is titrated to CO_2). In other words, the effective molarity of the base has been increased, owing to further reaction of the HCO_3^-.

Remove Na_2CO_3 by preparing a saturated solution of NaOH.

Sodium carbonate is essentially insoluble in nearly saturated sodium hydroxide. It is conveniently removed by dissolving the weighed NaOH in a volume (milliliters) of water equal to its weight in grams. The insoluble Na_2CO_3 can be allowed to settle for several days, and then the clear supernatant liquid can be carefully decanted,[1] or it can be filtered in a Gooch crucible with an asbestos mat (do not wash the filtered Na_2CO_3). The former procedure is preferred because of the carcinogenic nature of asbestos. This procedure does not work with KOH because K_2CO_3 remains soluble.

See Experiment 6 for preparing and standardizing sodium hydroxide.

Water dissolves CO_2 from the air. In many routine determinations not requiring the highest degree of accuracy, carbonate or CO_2 impurities in the water will result in an error that is small enough to be considered negligible. For the highest accuracy, however, CO_2 should be removed from all water used to prepare solutions for acid–base titrations, particularly the alkaline solutions. This is conveniently done by boiling and then cooling under the cold-water tap.

Sodium hydroxide is usually standardized by titrating a weighed quantity of primary standard potassium acid phthalate (KHP), which is a moderately weak acid ($K_a = 4 \times 10^{-6}$), approximately like acetic acid; a phenolphthalein end point is used. The sodium hydroxide solution should be stored in a plastic bottle to prevent absorption of CO_2 from the air. If the bottle must be open (e.g., a siphon bottle), the opening is protected with an **Ascarite** (asbestos impregnated with NaOH) tube.

[1]The solution must be kept in a test tube stoppered with a material other than glass, or other appropriate vessel, to keep out atmospheric carbon dioxide, which would continue to react with the sodium hydroxide to produce sodium carbonate. Use a rubber stopper since concentrated alkali causes glass joints to "freeze."

2.6 Preparation of Standard Acid Solutions

Hydrochloric acid is the usual titrant for the titration of bases. Most chlorides are soluble, and few side reactions are possible with this acid. It is convenient to handle. It is not a primary standard (although constant-boiling HCl, which is a primary standard, can be prepared), and an approximate concentration is prepared simply by diluting the concentrated acid. For most accurate work, the water used to prepare the solution should be boiled, although use of boiled water is not so critical as with NaOH; CO_2 will have a low solubility in strongly acidic solutions and will tend to escape during shaking of the solution.

See Experiment 7 for preparing and standardizing hydrochloric acid.

Primary standard sodium carbonate is usually used to standardize HCl solutions. Its disadvantage is that the end point is not sharp unless methyl red, methyl purple, and so forth is used as the indicator and the solution is boiled at the end point. A modified methyl orange end point may be used without boiling, but this is not so sharp. Another disadvantage is the low formula weight of Na_2CO_3. Tris-(hydroxymethyl)aminomethane (THAM), $(HOCH_2)_3CNH_2$, is another primary standard that is more convenient to use. It is nonhygroscopic, but it is still a fairly weak base ($K_b = 1.3 \times 10^{-6}$) with a low molecular weight. The end point is not complicated by released CO_2, and it is recommended as the primary standard unless the HCl is being used to titrate carbonate samples.

A secondary standard is less accurate than a primary standard.

If a standardized NaOH solution is available, the HCl can be standardized by titrating an aliquot with the NaOH. The end point is sharp and the titration is more rapid. The NaOH solution is a **secondary standard**. Any error in standardizing this will be reflected in the accuracy of the HCl solution. The HCl is titrated with the base, rather than the other way around, to minimize absorption of CO_2 in the titration flask. Phenolphthalein or bromothymol blue can be used as indicator.

2.7 Other Apparatus—Handling and Treating Samples

Besides apparatus for measuring mass and volume, there are a number of other items of equipment commonly used in analytical procedures.

BLOOD SAMPLERS

Syringes are used to collect blood samples.[2] Stainless steel or aluminum needles are generally used with glass or plastic syringes. These usually present no problem of contamination, although special precautions may be required for certain trace element analyses. **Vacutainers** or similar devices are often used in place of syringes. These are evacuated test tubes with a rubber cap. The needle is pushed through the cap after the other end has been inserted into the vein, and the blood is drawn into the evacuated tube. The tube may contain an anticoagulating agent to prevent clotting of the blood if plasma or whole blood samples are to be analyzed.

A finger puncture, instead of a venipuncture, is used when small quantities of blood are to be collected for microprocedures. Up to 0.5 mL or more blood can be squeezed from the finger into a small collection tube by puncturing the finger with a sterilized sharp-pointed knifelike object.

[2]You should *not* attempt to collect a blood sample unless you have been specifically trained to do so. A trained technician will generally be assigned to this job.

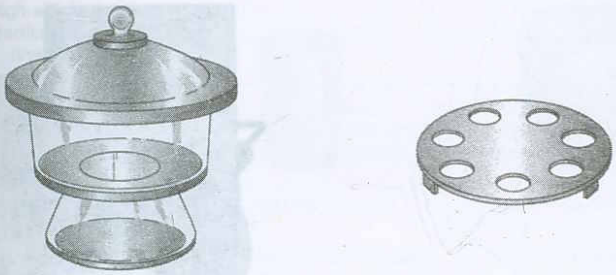


Fig. 2.16. Desiccator and desiccator plate.

DESICCATORS

Oven-dried samples or reagents are cooled in a desiccator before weighing.

A **desiccator** is used to keep samples dry while they are cooling and before they are weighed and, in some cases, to dry the wet sample. Dried or ignited samples and vessels are cooled in the desiccator. A typical glass desiccator is shown in Figure 2.16. A desiccator is an airtight container that maintains an atmosphere of low humidity. A desiccant such as calcium chloride is placed in the bottom to absorb the moisture. This desiccant will have to be changed periodically as it becomes "spent." It will usually become wet in appearance or caked from the moisture when it is time to be changed. A porcelain plate is usually placed in the desiccator to support weighing bottles, crucibles, and other vessels. An airtight seal is made by application of stopcock grease to the ground-glass rim on the top of the desiccator. A **vacuum desiccator** has a side arm on the top for evacuation so that the contents can be kept in a vacuum rather than just an atmosphere of dry air.

The top of a desiccator should not be removed any more than necessary since the removal of moisture from the air introduced is rather slow, and continued exposure will limit the lifetime of the desiccant. A red-hot crucible or other vessel should be allowed to cool in the air about 60 s before it is placed in the desiccator. Otherwise, the air in the desiccator will be heated appreciably before the desiccator is closed, and as the air cools, a partial vacuum will be created. This will result in a rapid inrush of air when the desiccator is opened and possible spilling or loss of sample as a consequence. A hot weighing bottle should not be stoppered when placed in a desiccator because on cooling, a partial vacuum is created and the stopper may seize. The stopper should be placed in the desiccator with the weighing bottle.

Table 2,5 lists some commonly used desiccants and their properties. Aluminum oxide, magnesium perchlorate, calcium oxide, calcium chloride, and silica gel can be regenerated by heating at 150, 240, 500, 275, and 150°C, respectively.

Table 2.5
Some Common Drying Agents

Agent	Capacity	Deliquescent[a]	Trade Name
$CaCl_2$ (anhydrous)	High	Yes	
$CaSO_4$	Moderate	No	Drierite (W. A. Hammond Drierite Co.)
CaO	Moderate	No	
$MgClO_4$ (anhydrous)	High	Yes	Anhydrone (J. T. Baker Chemical Co.); Dehydrite (Arthur H. Thomas Co.)
Silica gel	Low	No	
Al_2O_3	Low	No	
P_2O_5	Low	Yes	

[a]Becomes liquid by absorbing moisture.

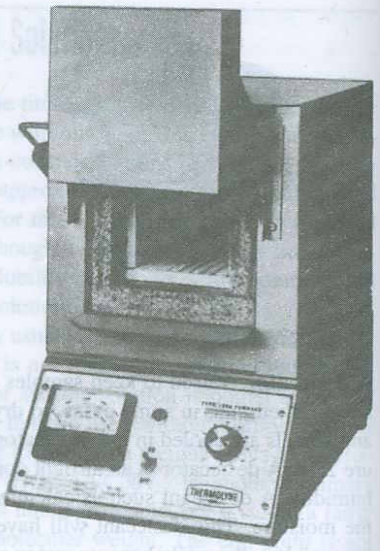


Fig. 2.17. Muffle furnace. (Courtesy of Arthur H. Thomas Company.)

Fig. 2.18. Drying oven. (Courtesy of Arthur H. Thomas Company.)

FURNACES AND OVENS

A **muffle furnace** (Figure 2.17) is used to ignite samples to high temperatures, either to convert precipitates to a weighable form or to burn organic materials prior to inorganic analysis. There should be some means of regulating the temperature since losses of some metals may occur at temperatures in excess of 500°C. Temperatures up to about 1200°C can be reached with muffle furnaces.

A **drying oven** is used to dry samples prior to weighing. A typical drying oven is shown in Figure 2.18. These ovens are well ventilated for uniform heating. The usual temperature employed is about 110°C, but temperatures of 200 to 300°C may be obtained.

HOODS

A **fume hood** is used when chemicals or solutions are to be evaporated. When perchloric acid or acid solutions of perchlorates are to be evaporated, the fumes should be collected, or the evaporation should be carried out in fume hoods specially designed for perchloric acid work (i.e., constructed from components resistant to attack by perchloric acid).

When performing trace analysis, as in trace metal analysis, care must be taken to prevent contamination. The conventional fume hood is one of the "dirtiest" areas of the laboratory since laboratory air is drawn into the hood and over the sample.

Laminar-flow hoods provide clean work areas.

Laminar-flow hoods or workstations are available for providing very clean work areas. Rather than drawing unfiltered laboratory air into the work area, the air is prefiltered and then flows over the work area and out into the room to create a positive pressure and prevent unfiltered air from flowing in. A typical laminar-flow workstation is shown in Figure 2.19. The high-efficiency particulate air (HEPA) filter removes all particles larger than 0.3 μm from the air. Vertical laminar-flow stations are preferred when fumes are generated that should not be blown over the operator. Facilities are available to exhaust noxious fumes.

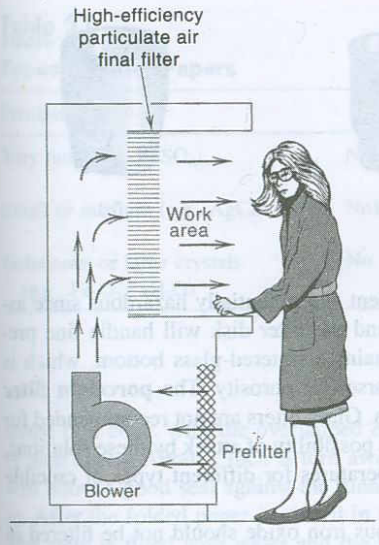


Fig. 2.19. Laminar-flow workstation. (Courtesy of Dexion, Inc., 344 Beltine Boulevard, Minneapolis, MN.)

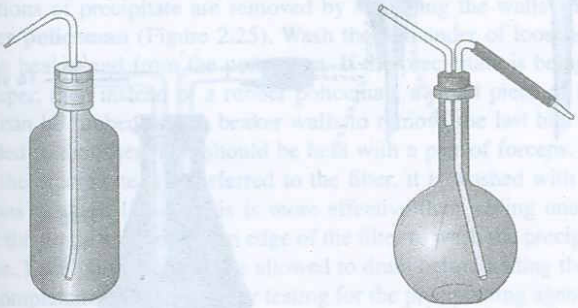
WASH BOTTLES

A **wash bottle** of some sort should be handy in any analytical laboratory, to be used for quantitative transfer of precipitates and solutions and to wash precipitates. These are commercially available in a variety of shapes and sizes, as seen in Figure 2.20. Alternatively they may be constructed from a Florence flask and glass tubing, as in Figure 2.20*b*.

CENTRIFUGES AND FILTERS

A **centrifuge** has many useful applications, particularly in the clinical laboratory, where blood may have to be separated into fractions such as serum or plasma, and proteins may have to be separated by precipitation followed by centrifuging.

Filters for filtering precipitates (e.g., in gravimetric analysis) are of various types. The Gooch crucible, sintered-glass crucible, and porcelain filter crucible are illustrated in Figure 2.21. The **Gooch crucible** is porcelain and has holes in the bottom; a glass filter disk is supported on top of it. In the past, an asbestos mat



(*a*) (*b*)

Fig. 2.20. Wash bottles: (*a*) polyethylene, squeeze type; (*b*) glass, blow type.

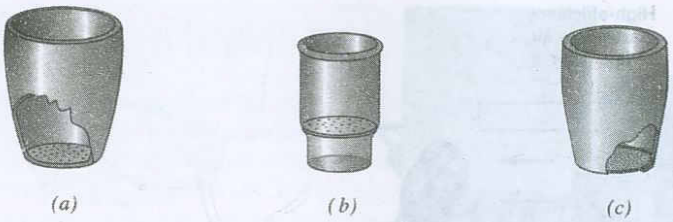


Fig. 2.21. Filtering crucibles: (*a*) Gooch crucible; (*b*) sintered-glass crucible; (*c*) porcelaine filter crucible.

was usually prepared, but this is inconvenient and potentially hazardous since asbestos fibers in the air are carcinogenic, and the filter disk will handle fine precipitates. The **sintered-glass crucible** contains a sintered-glass bottom, which is available in fine (F), medium (M), or coarse (C) porosity. The **porcelain filter crucible** contains a porous unglazed bottom. Glass filters are not recommended for concentrated alkali solutions because of the possibility of attack by these solutions. See Table 2.1 for maximum working temperatures for different types of crucible materials.

Gelatinous precipitates such as hydrous iron oxide should not be filtered in filter crucibles because they clog the pores. Even with filter paper, the filtration of the precipitates can be slow.

Filter crucibles are used with a **crucible holder** mounted on a filtering flask (Figure 2.22). A safety bottle is connected between the flask and the aspirator.

Ashless filter paper is generally used for quantitative work in which the paper is ignited away and leaves a precipitate suitable for weighing (see Chapter 10). There are various grades of filter papers for different types of precipitates. These are listed in Table 2.6 for Whatman (www.whatman.plc.uk) and for Schliecher and Schuell (www.s-und-s.de/english-index.html) papers.

TECHNIQUES OF FILTRATION

By proper fitting of the filter paper, the rate of filtration can be increased. A properly folded filter paper is illustrated in Figure 2.23. The filter paper is folded in the

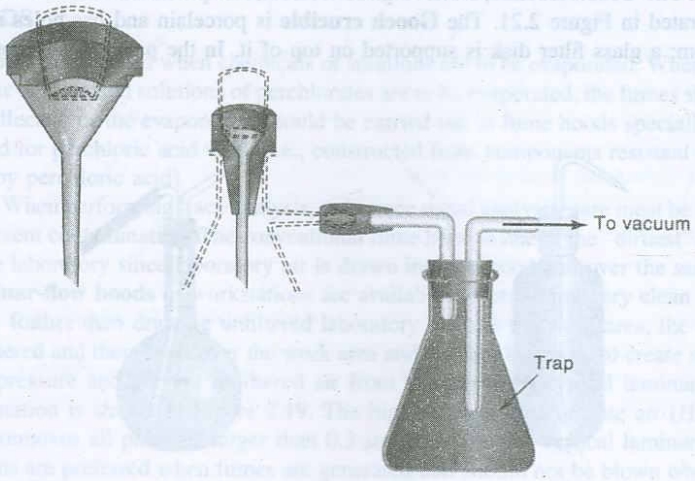


Fig. 2.22. Crucible holders.

Table 2.6
Types of Filter Papers

Precipitate	Whatman	Schliecher and Schuell
Very fine (e.g., $BaSO_4$)	No. 42 (2.5 μm)	No. 589/2 or 5, Blue or Red Band (2–4 μm)
Small or medium (e.g., AgCl)	No. 40 (8 μm)	No. 589/2, White Band (4–12 μm)
Gelatinous or large crystals (e.g., $Fe_2O_3 \cdot xH_2O$)	No. 41 (20–25 μm)	No. 589/1, Black Band (>12–25 μm)

shape of a cone, with the overlapped edges of the two quarters not quite meeting ($\frac{1}{8}$ inch apart). About $\frac{1}{4}$ inch is torn away from the corner of the inside edge. This will allow a good seal against the funnel to prevent air bubbles from being drawn in. After the folded paper is placed in the funnel, it is wetted with distilled water. The stem is filled with water and the top of the wet paper is pressed against the funnel to make a seal. With a proper fit, no air bubbles will be sucked into the funnel, and the suction supplied by the weight of the water in the stem will increase the rate of filtration. The filtration should be started immediately. The precipitate should occupy not more than one-third to one-half of the filter paper in the funnel because many precipitates tend to "creep." Do not allow the water level to go over the top of the paper.

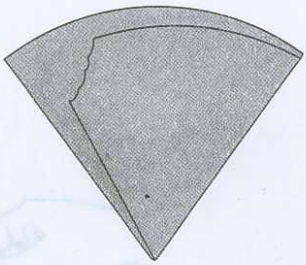


Fig. 2.23. Properly folded filter paper.

The precipitate should be allowed to settle in the beaker before filtration is begun. The bulk of the clear liquid can then be decanted and filtered at a rapid rate before the precipitate fills the pores of the filter paper.

Let the precipitate settle before filtering.

Care must be taken in the decanting and the transferring of the precipitate to avoid losses. This is properly done by use of a stirring rod and a wash bottle, as illustrated in Figure 2.24. The solution is decanted by pouring it down the glass rod, which guides it into the filter without splashing. The precipitate is most readily washed while still in the beaker. After the mother liquor has been decanted off, wash the sides of the beaker down with several milliliters of the wash liquid, and then allow the precipitate to settle as before. Decant the wash liquid into the filter and repeat the washing operation two or three times. The precipitate is then transferred to the filter by holding the glass rod and beaker in one hand, as illustrated, and washing it out of the beaker with wash liquid from the wash bottle.

Wash the precipitate while it is in the beaker.

If the precipitate must be collected quantitatively, as in gravimetric analysis, the last portions of precipitate are removed by scrubbing the walls with a moistened **rubber policeman** (Figure 2.25). Wash the remainder of loosened precipitate from the beaker and from the policeman. If the precipitate is being collected in a filter paper, then instead of a rubber policeman, a small piece of the ashless filter paper can be rubbed on the beaker walls to remove the last bits of precipitate and added to the filter. This should be held with a pair of forceps.

After the precipitate is transferred to the filter, it is washed with five or six small portions of wash liquid. This is more effective than adding one large volume. Divert the liquid around the top edge of the filter to wash the precipitate down into the cone. Each portion should be allowed to drain before adding the next one. Check for completeness of washing by testing for the precipitating agent in the last few drops of the washings.

Test for completeness of washing.

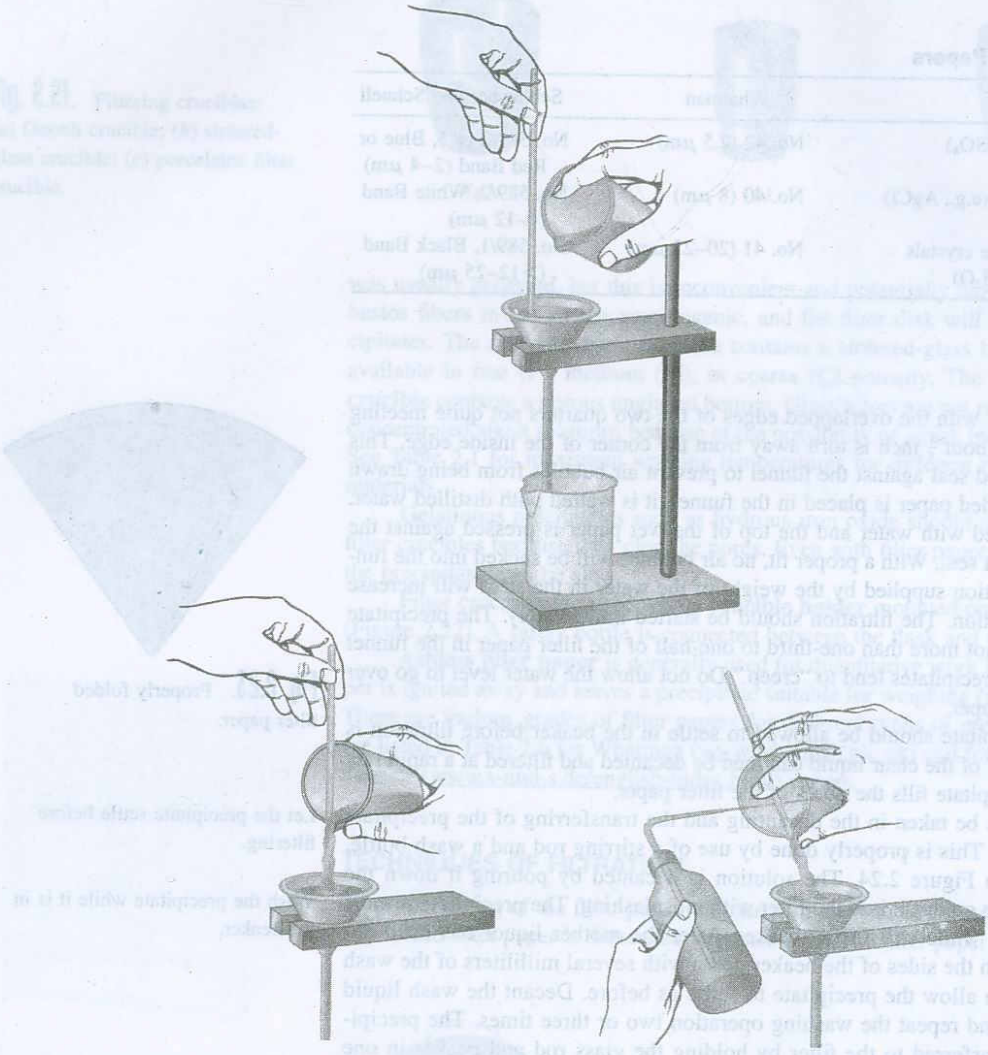


Fig. 2.24. Proper technique for transfer of a precipitate.

Fig. 2.25. Rubber policeman.

2.8 Igniting Precipitates—Gravimetric Analysis

If a precipitate is to be ignited in a porcelain filter crucible, the moisture should be driven off first at a low heat. The ignition may be done in a muffle furnace or by heating with a burner. If a burner is to be used, the filter crucible should be placed in a porcelain or platinum crucible to prevent reducing gases of the flame diffusing through the pores of the filter.

When precipitates are collected on filter paper, the cone-shaped filter containing the precipitate is removed from the funnel, the upper edge is flattened, and the corners are folded in. Then, the top is folded over and the paper and contents are placed in a crucible with the bulk of the precipitate on the bottom. The paper must now be dried and charred off. The crucible is placed at an angle on a triangle support with the crucible cover slightly ajar, as illustrated in Figure 2.26. The moisture is removed by low heat from the burner, with care taken to avoid splattering. The heating is gradually increased as moisture is evolved and the paper begins to char. Care should be taken to avoid directing the reducing portion of the flame into the crucible. A sudden increase in the volume of smoke evolved indicates that the paper is about to burst into flame, and the burner should be removed. If it does burst into flame, it should be smothered quickly by replacing the crucible cover. Carbon particles will undoubtedly appear on the cover, and these will ultimately have to be ignited. Finally, when no more smoke is detected, the charred paper is burned off by gradually increasing the flame temperature. The carbon residue should glow but should not flame. Continue heating until all the carbon and tars on the crucible and its cover are burned off. The crucible and precipitate are now ready for igniting. The ignition can be continued with the burner used at highest temperature or with the muffle furnace.

Do the initial ignition slowly.

Before a precipitate is collected in a filter crucible or transferred to a crucible, the crucible should be dried to constant weight (e.g., 1 h of heating, followed by $\frac{1}{2}$-h heatings) if the precipitate is to be dried, or it should be ignited to constant weight if the precipitate is to be ignited. Constant weight is considered to have been achieved with an analytical student balance when successive weighings agree within about 0.3 or 0.4 mg. The crucible plus the precipitate are heated to constant weight in a similar manner. After the first heating, the time of heating can be reduced by half. The crucible should be allowed to cool in a desiccator for at least $\frac{1}{2}$ h before weighing. Red-hot crucibles should be allowed to cool below redness before placing them in the desiccator (use crucible tongs—usually nickel plated or stainless steel to minimize contamination from rust). Before weighing a covered crucible, check for any radiating heat by placing your hand near it (don't touch).

Dry and weigh the crucible before adding the precipitate!

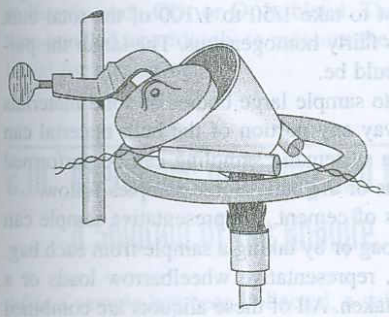


Fig. 2.26. Crucible and cover supported on a wire triangle for charring off paper.

2.9 Obtaining the Sample—Is It Solid, Liquid, or Gas?

See Chapter 3 for important statistical considerations in sampling.

Collecting a representative sample is an aspect of analytical chemistry that the beginning analytical student is often not concerned with because the samples handed to him or her are assumed to be homogeneous and representative. Yet this process can be the most critical aspect of an analysis. The significance and accuracy of measurements can be limited by the sampling process. Unless sampling is done properly, it becomes the weak link in the chain of the analysis. A life could sometimes depend on the proper handling of a blood sample during and after sampling. If the analyst is given a sample and does not actively participate in the sampling process, then the results obtained can only be attributed to the sample "as it was received." And the chain of custody as mentioned earlier must be documented.

Many professional societies have specified definite instructions for sampling given materials [e.g., the American Society for Testing and Materials (ASTM: www.astm.org), the Association of Official Analytical Chemists International (AOAC International: www.aoac.org), and the American Public Health Association (APHA: www.apha.org)]. By appropriate application of experience and statistics, these materials can be sampled as accurately as the analysis can be performed. Often, however, the matter is left up to the analyst. The ease or complexity of sampling will, of course, depend on the nature of the sample.

The problem involves obtaining a sample that is representative of the whole. This sample is called the **gross sample**. Its size may vary from a few grams or less to several pounds, depending on the type of bulk material. Once a representative gross sample is obtained, it may have to be reduced to a sufficiently small size to be handled. This is called the **sample**. Once the sample is obtained, an aliquot, or portion, of it will be analyzed. This aliquot is called the **analysis sample**. Several replicate analyses on the same sample may be performed by taking separate aliquots.

In the clinical laboratory, the gross sample is usually satisfactory for use as the sample because it is not large and it is homogeneous (e.g., blood and urine samples). The analysis sample will usually be from a few milliliters to a fraction of a drop (a few microliters) in quantity.

Some of the problems associated with obtaining gross samples of solids, liquids, and gases are considered below.

1. Solids. Inhomogeneity of the material, variation in particle size, and variation within the particle make sampling of solids more difficult than other materials. The easiest but usually most unreliable way to sample a material is the **grab sample**, which is one sample taken at random and assumed to be representative. The grab sample will be satisfactory only if the material from which it is taken is homogeneous. For most reliable results, it is best to take 1/50 to 1/100 of the total bulk for the gross sample, unless the sample is fairly homogeneous. The larger the particle size, the larger the gross sample should be.

Sampling (From the journals collection of the Chemical Heritage Foundations' Othmer Library.)

The easiest and most reliable time to sample large bodies of solid materials is while they are being moved. In this way any portion of the bulk material can usually be exposed for sampling. Thus, a systematic sampling can be performed to obtain aliquots representing all portions of the bulk. Some samples follow.

In the loading or unloading of bags of cement, a representative sample can be obtained by taking every fiftieth or so bag or by taking a sample from each bag. In the moving of grain by wheelbarrow, representative wheelbarrow loads or a shovelful from each wheelbarrow can be taken. All of these aliquots are combined to form the gross sample.

2. Liquids. These materials tend to be homogeneous and are much easier to sample. Liquids mix by diffusion only very slowly and must be shaken to obtain a homogeneous mixture. If the material is indeed homogeneous, a simple grab (single random) sample will suffice. For all practical purposes, this method is satisfactory for taking blood samples. Twenty-four-hour urine sample collections are generally more reliable than single specimens.

The timing of sampling of biological fluids is, however, very important. The composition of blood varies considerably before and after meals, and for many analyses a sample is collected after the patient has fasted for a number of hours. Preservatives such as sodium fluoride for glucose preservation and anticoagulants may be added to blood samples when they are collected.

Blood samples may be analyzed as *whole blood*, or they may be separated to yield *plasma* or *serum* according to the requirements of the particular analysis. Most commonly, the concentration of the substance external to the red cells (the extracellular concentration) will be a significant indication of physiological condition, and so serum or plasma is taken for analysis.

See Chapter 24 for more on sampling biological fluids.

If liquid samples are not homogeneous, and if they are small enough, they can be shaken and sampled immediately. For example, there may be particles in the liquid that have tended to settle. Large bodies of liquids are best sampled after a transfer or, if in a pipe, after passing through a pump when they have undergone the most thorough mixing. Large stationary liquids can be sampled with a "*thief*" *sampler*, which is a device for obtaining aliquots at different levels. It is best to take the sample at different depths at a diagonal, rather than straight down. The separate aliquots of liquids can be analyzed individually and the results combined, or the aliquots can be combined into one gross sample and replicate analyses performed. This latter procedure is probably preferred because the analyst will then have some idea of the precision of the analysis.

3. Gases. The usual method of sampling gases involves displacement of a liquid. The liquid must be one in which the sample has little solubility and with which it does not react. Mercury is the liquid employed most commonly. The mercury is allowed to trickle from the bottom of the container, whereupon the gas is pulled in at the top. Such a procedure allows collection of an average sample over a relatively long period of time. A grab-type sample is satisfactory in some cases. In the collecting of a breath sample, for example, the subject could blow into an evacuated bag. Auto exhaust could be collected in a large evacuated plastic bag.

See Chapter 26 for more on sampling environmental samples.

The volume of gross gas sample collected may or may not need to be known. Often, the *concentration* of a certain analyte in the gas sample is measured, rather than the *amount*. The temperature and pressure of the sample will, of course, be important in determining the volume and hence the concentration.

The gas sampling mentioned here does not concern gas constituents dissolved in liquids, such CO_2 or O_2 in blood. These are treated as liquid samples and are then handled accordingly to measure the gas in the liquid or to release it from the liquid for measuring.

2.10 Operations of Drying and Preparing a Solution of the Analyte

After a sample has been collected, a solution of the analyte must usually be prepared before the analysis can be continued. Drying of the sample may be required,

and it must be weighed or the volume measured. If the sample is already a solution (e.g., serum, urine, or water), then extraction, precipitation, or concentration of the analyte may be in order, and this may also be true with other samples.

In this section we describe common means for preparing solutions of inorganic and organic materials. Included are the dissolution of metals and inorganic compounds in various acids or in basic fluxes (fusion), the destruction of organic and biological materials for determination of inorganic constituents (using wet digestion or dry ashing), and the removal of proteins from biological materials so they do not interfere in the analysis of organic or inorganic constituents.

DRYING THE SAMPLE

Solid samples will usually contain variable amounts of adsorbed water. With inorganic materials, the sample will generally be dried before weighing. This is accomplished by placing it in a drying oven at 105 to 110°C for 1 or 2 h. Other nonessential water, such as that entrapped within the crystals, may require higher temperatures for removal.

Decomposition or side reactions of the sample must be considered during drying. Material unstable under conditions of heat can be dried by setting it in a desiccator; using a vacuum desiccator will hasten the drying process. If the sample is weighed without drying, the results will be on an "as is" basis and should be reported as such.

Plant and tissue samples can usually be dried by heating. See Chapter 1 for a discussion of the various weight bases (wet, dry, ash) used in connection with reporting analytical results for these samples.

SAMPLE DISSOLUTION

Before the analyte can be measured, some sort of sample alteration is generally necessary to get the analyte into solution or, for biological samples, to rid it of interfering organic substances, such as proteins. There are two types of sample preparation: those that totally destroy the sample matrix and those that are nondestructive or only partially destructive. The former type can generally be used only when the analyte is inorganic or can be converted to an inorganic derivative for measurement (e.g., Kjeldahl analysis, in which organic nitrogen is converted to ammonium ion—see below). The latter type must be used if the analyte to be measured is an organic substance.

DISSOLVING INORGANIC SOLIDS

Strong mineral acids are good solvents for many inorganics. *Hydrochloric acid* is a good general solvent for dissolving metals that are above hydrogen in the electromotive series. *Nitric acid* is a strong oxidizing acid and will dissolve most of the common metals, nonferrous alloys, and the "acid-insoluble" sulfides.

Perchloric acid, when heated to drive off water, becomes a very strong and efficient oxidizing acid in the dehydrated state. It dissolves most of the common metals and destroys traces of organic matter. It must be used with extreme caution because it will react explosively with many easily oxidizable substances, especially organic matter.

Fusions are used when acids do not dissolve the sample.

Some inorganic materials will not dissolve in acids, and **fusion** with an acidic or basic **flux** in the molten state must be employed to render them soluble. The sample is mixed with the flux in a sample-to-flux ratio of about 1 to 10 or 20, and the combination is heated in an appropriate crucible until the flux becomes molten.

When the melt becomes clear, usually in about 30 min, the reaction is complete. The cooled solid is then dissolved in dilute acid or in water. During the fusion process, insoluble materials react with the flux to form a soluble product. Sodium carbonate is one of the most useful basic fluxes, and acid-soluble carbonates are produced.

DESTRUCTION OF ORGANIC MATERIALS FOR INORGANIC ANALYSIS—BURNING OR ACID OXIDATION

Animal and plant tissue, biological fluids, and organic compounds are usually decomposed by **wet digestion** with a boiling oxidizing acid or mixture of acids, or by **dry ashing** at a high temperature (400 to 700°C) in a muffle furnace. In wet digestion, the acids oxidize organic matter to carbon dioxide, water, and other volatile products, which are driven off, leaving behind salts or acids of the inorganic constituents. In dry ashing, atmospheric oxygen serves as the oxidant; that is, the organic matter is burned off, leaving an inorganic residue. Oxidizing aids may be employed in dry ashing.

In dry ashing, the organic matter is burned off.

1. Dry Ashing. Although various types of dry ashing and wet digestion combinations are used with about equal frequency by analysts for organic and biological materials, simple dry ashing with no chemical aids is probably the most commonly employed technique. Lead, zinc, cobalt, antimony, chromium, molybdenum, strontium, and iron traces can be recovered with little loss by retention or volatilization. Usually a porcelain crucible can be used. Lead is volatilized at temperatures in excess of about 500°C, especially if chloride is present, as in blood or urine. Platinum crucibles are preferred for lead for minimal retention losses.

If an oxidizing material is added to the sample, the ashing efficiency is enhanced. Magnesium nitrate is one of the most useful aids, and with this it is possible to recover arsenic, copper, and silver, in addition to the above-listed elements.

Liquids and wet tissues are dried on a steam bath or by gentle heat before they are placed in a muffle furnace. The heat from the furnace should be applied gradually up to full temperature to prevent rapid combustion and foaming.

After dry ashing is complete, the residue is usually leached from the vessel with 1 or 2 mL hot concentrated or 6 *M* hydrochloric acid and transferred to a flask or beaker for further treatment.

Another dry technique is that of **low-temperature ashing**. A radio-frequency discharge is used to produce activated oxygen radicals, which are very reactive and will attack organic matter at low temperatures. Temperatures of less than 100°C can be maintained, and volatility losses are minimized. Introduction of elements from the container and the atmosphere is reduced, and so are retention losses. Radiotracer studies have demonstrated that 17 representative elements are quantitatively recovered after complete oxidation of organic substrate.

Elemental analysis in the case of organic compounds (e.g., for carbon or hydrogen) is usually performed by **oxygen combustion** in a tube, followed by an absorption train. Oxygen is passed over the sample in a platinum boat, which is heated and quantitatively converts carbon to CO_2 and hydrogen to H_2O. These combustion gases pass into the absorption train, where they are absorbed in preweighed tubes containing a suitable absorbent. For example, **Ascarite** (sodium hydroxide on asbestos) is used to absorb the CO_2, and **Dehydrite** (magnesium perchlorate) is used to absorb the H_2O. The gain in weight of the absorption tubes is a measure of the CO_2 and H_2O liberated from the sample. Details of this technique are important, and, should you have occasion to use it, you are referred to more comprehensive texts on elemental analysis.

In wet ashing, the organic matter is oxidized with an oxidizing acid.

2. Wet Digestion. Wet digestion with a mixture of nitric and sulfuric acids is the second most often used oxidation procedure. Usually a small amount (e.g., 5 mL) of sulfuric acid is used with larger volumes of nitric acid (20 to 30 mL). Wet digestions are usually performed in a Kjeldahl flask (Figure 2.28). The nitric acid destroys the bulk of the organic matter, but it does not get hot enough to destroy the last traces. It is boiled off during the digestion process until only sulfuric acid remains and dense, white SO_3 fumes are evolved and begin to reflux in the flask. At this point, the solution gets very hot, and the sulfuric acid acts on the remaining organic material. Charring may occur at this point if there is considerable or very resistant organic matter left. If the organic matter persists, more nitric acid may be added. Digestion is continued until the solution clears. All digestion procedures must be performed in a fume hood.

A much more efficient digestion mixture employs a mixture of nitric, perchloric, and sulfuric acids in a volume ratio of about 3:1:1. Ten milliliters of this mixture will usually suffice for 10 g fresh tissue or blood. The perchloric acid is an extremely efficient oxidizing agent when it is dehydrated and hot and will destroy the last traces of organic matter with relative ease. Samples are heated until nitric acid is boiled off and perchloric acid fumes, which are less dense than SO_3 but which fill the flask more readily, appear. The hot perchloric acid is boiled, usually until fumes of SO_3 appear, signaling the evaporation of all the perchloric acid. Sufficient nitric acid must be added at the beginning to dissolve and destroy the bulk of organic matter, and there must be sulfuric acid present to prevent the sample from going to dryness, or else there is danger of explosion from the perchloric acid. A hood specially designed for perchloric acid work should be used for all digestions incorporating perchloric acid.

This mixture is even more efficient if a small amount of molybdenum(VI) catalyst is added. As soon as water and nitric acid are evaporated. oxidation proceeds vigorously with foaming, and the digestion is complete in a few seconds. The digestion time is reduced considerably.

Perchloric acid must be used with caution!

A mixture of nitric and perchloric acids is also commonly used. The nitric acid boils off first, and care must be taken to prevent evaporation of the perchloric acid to near dryness, or a violent explosion may result; this procedure *is not recommended* unless you have considerable experience in digestion procedures. **Perchloric acid should never be added directly to organic or biological material.** Always add an excess of nitric acid first. Explosions with perchloric acid are generally associated with formation of peroxides, and the acid turns dark in color (e.g., yellowish brown) prior to explosion. Certain organic compounds such as ethanol, cellulose, and polyhydric alcohols can cause hot concentrated perchloric acid to explode violently; this is presumably due to formation of ethyl perchlorate.

A mixture of nitric, perchloric, and sulfuric acids allows zinc, selenium, arsenic, copper, cobalt, silver, cadmium, antimony, chromium, molybdenum, strontium, and iron to be quantitatively recovered. Lead is often lost if sulfuric acid is used. The mixture of nitric and perchloric acids can be used for lead and all the above elements. Perchloric acid must be present to prevent losses of selenium. It maintains strong oxidizing conditions and prevents charring that would result in formation of volatile compounds of lower oxidation states of selenium. Samples containing mercury cannot be dry ashed. Wet digestion with heat applied must be done using a reflux apparatus because of the volatile nature of mercury and its compounds. Cold or room temperature procedures are often preferred to obtain partial destruction of organic matter. For example, in urine samples, which contain a relatively small amount of organic matter compared with blood, mercury can be reduced to the element with copper(I) and hydroxylamine hydrochloride and the organic matter destroyed by potassium permanganate at room temperature. The mercury can then be dissolved and the analysis continued.

Many nitrogen-containing compounds can be determined by **Kjeldahl digestion** to convert the nitrogen to ammonium sulfate. The digestion mixture consists of sulfuric acid plus potassium sulfate to increase the boiling point of the acid and thus increase its efficiency. A catalyst is also added (such as copper or selenium). After destruction of the organic matter, sodium hydroxide is added to make the solution alkaline, and the ammonia is distilled into an excess of standard hydrochloric acid. The excess acid is back-titrated with standard alkali to determine the amount of ammonia collected. With a knowledge of the percent nitrogen composition in the compound of interest, the amount of the compound can be calculated from the amount of ammonia determined. This is the most accurate method for determining protein. Protein contains a definite percentage of nitrogen, which is converted to ammonium sulfate during the digestion. See Chapter 8 for further details.

In Kjeldahl digestions, nitrogen is converted to ammonium ion, which is then distilled as ammonia and titrated.

The relative merits of the oxidation methods have been studied extensively. However, there is still no agreement as to which is to be preferred. Dry ashing is recommended for its simplicity and relative freedom from positive errors (contamination) since few or no reagents are added. The potential errors of dry oxidation are volatilization of elements and losses by retention on the walls of the vessel. Adsorbed metals on the vessel may in turn contaminate future samples. Wet digestion is considered superior in terms of rapidity (although it does require more operator attention), low level of temperature maintained, and freedom from loss by retention. The chief error attributed to wet digestion is the introduction of impurities from the reagents necessary for the reaction. This problem has been minimized as commercial reagent-grade acids have become available in greater purity and specially prepared high-purity acids can now be obtained commercially. The time required for ashing or digestion will vary with the sample and the technique employed. Two to 4 h are common for dry ashing and $\frac{1}{2}$ to 1 h is common for wet digestion.

Dry and wet ashing each has advantages and limitations.

MICROWAVE PREPARATION OF SAMPLES

Microwave ovens are now widely used for rapid and efficient drying and acid decomposition of samples. Laboratory ovens are specially designed to overcome limitations of household ovens, and these are discussed below. Advantages of microwave digestions include reduction of dissolution times from hours to minutes and low blank levels due to reduced amounts of reagents required.

1. How Do Microwaves Heat? Microwaves occur between infrared radiation and radio waves in the electromagnetic spectrum, in the frequency range of 300 to 300,000 MHz (3×10^8 to 3×10^{11} Hz, or beginning at about 1000 μm wavelength—see Figure 16.2). Microwaves consist of an electric field and a magnetic field perpendicular to the electric field. The electric field is responsible for energy transfer between the microwave source and the irradiated sample. Microwave energy affects molecules in two ways: dipole rotation and ionic conduction. The first is the more important. When the microwave energy passes through the sample, the molecules having dipole moments will try to align with the electric field, and the more polar ones will have the stronger interaction with the field. This molecular motion (rotation) results in heating. The energy transfer, a function of the dipole moment and the dielectric constant, is most efficient when the molecules are able to relax quickly, that is, when the relaxation time matches the microwave frequency. Large molecules such as polymers relax slowly, but once the temperature increases and they relax more rapidly, they can absorb the energy more efficiently. Small molecules such as water, though, relax more quickly than the resonating microwave energy, and they move farther away from the resonance frequency and absorb less energy as they heat up.

The ionic conduction effect arises because ionic species in the presence of an electric field will migrate in one direction or the other. Energy is transferred from the electric field, causing ionic interactions that speed up the heating of a solution. Ionic absorbers become stronger absorbers of microwave energy as they are heated since ionic conductance increases with temperature. Deionized water heats slowly, but if salt is added, it heats rapidly. Acids, of course, are good conductors and heat rapidly.

Microwaves heat by causing molecules to rotate and ions to migrate.

So microwave energy heats by causing movement of molecules due to dipole rotation and movement of ions due to ionic conductance. The microwave energy interacts with different materials in different ways. Reflective materials such as metals are good heat conductors: They do not heat and instead will reflect the microwave energy. Transparent materials are insulators because they transmit the microwave energy and also do not heat. The absorptive materials, the molecules and ions discussed above, are the ones that receive microwaves and are heated. Microwave energy is too low to break chemical bonds (a feature that has generated interest in using microwave energy to speed up chemical reactions in syntheses). The properties of reflective and insulator materials are utilized in designing microwave digestion systems.

Household microwave ovens don't work for small sample heating.

2. Design of Laboratory Microwave Ovens. Home microwave ovens were initially used for laboratory purposes, but it soon became apparent that modifications were needed. Laboratory samples are usually much smaller than food samples that are cooked and do not absorb much of the energy produced by the magnetron of the oven. The energy not absorbed by the sample is bounced back to the magnetron, causing it to overheat and burn out. Also, arcing could occur. So laboratory ovens are designed to protect the magnetron from stray energy. The main components of these ovens (Figure 2.27) include the magnetron, an isolator, a waveguide, the cavity, and a mode stirrer. Microwave energy generated by the magnetron is propagated down the waveguide into the cavity. The stirrer distributes the energy in different directions. The isolator, made of a ferromagnetic material and placed between the magnetron and the waveguide, deflects the microwave energy returning from the cavity into a fan-cooled ceramic load, keeping it away from the magnetron.

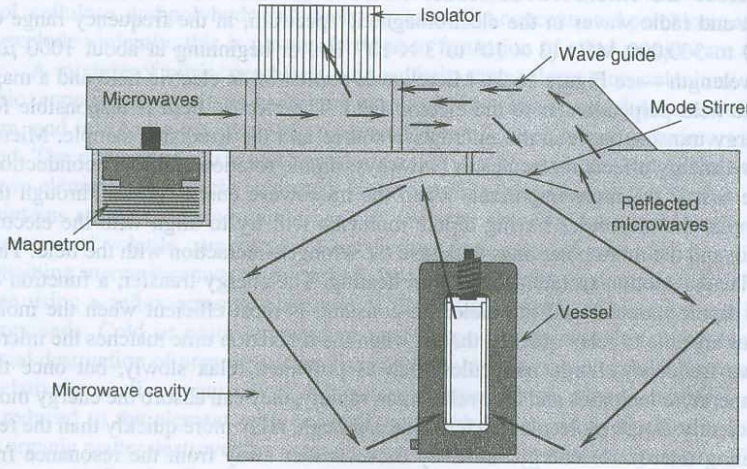


Fig. 2.27. Schematic of a microwave system. [From G. Le Blanc, *LC/GC Suppl.*, **17**(6S) (1999) S30.] (Courtesy of *LC/GC Magazine*.

The frequency used for cooking turns out to be good for chemistry as well, and the standard is 2450 MHz. Powers of 1200 W are typically used.

3. Acid Digestions. Digestions are normally done in closed plastic containers, either Teflon PFA (perfluoroalkoxy ethylene) or polycarbonate (insulators). This is to avoid acid fumes in the oven. It provides additional advantages. Pressure is increased and the boiling point of the acid is raised (the acid is superheated). So digestions occur more rapidly. Also, volatile metals are not lost. Modern ovens provide for control of pressure and temperature (fiber-optic temperature probe, transparent to microwave energy). Temperature control has enabled the use of the oven for microwave-assisted molecular extractions, by maintaining the temperature low enough to avoid molecular decomposition.

PARTIAL DESTRUCTION OR NONDESTRUCTION OF SAMPLE MATRIX

Obviously, when the substance to be determined is organic in nature, nondestructive means of preparing the sample must be employed. For the determination of metallic elements, it is also sometimes unnecessary to destroy the molecular structure of the sample, particularly with biological fluids. For example, several metals in serum or urine can be determined by atomic absorption spectroscopy by direct aspiration of the sample or a diluted sample into a flame. Constituents of solid materials such as soils can sometimes be extracted by an appropriate reagent. Thorough grinding, mixing, and refluxing are necessary to extract the analyte. Many trace metals can be extracted from soils with 1 *M* ammonium chloride or acetic acid solution. Some, such as selenium, can be distilled as the volatile chloride or bromide.

PROTEIN-FREE FILTRATES

See Chapter 24 for the preparation of protein-free filtrates.

Proteins in biological fluids interfere with many analyses and must be removed nondestructively. Several reagents will precipitate (coagulate) proteins. Trichloroacetic acid (TCA), tungstic acid (sodium tungstate plus sulfuric acid), and barium hydroxide plus zinc sulfate (a neutral mixture) are some of the common ones. A measured volume of sample (e.g., serum) is usually treated with a measured volume of reagent. Following precipitation of the protein (approximately 10 min), the sample is filtered through dry filter paper without washing, or else it is centrifuged. An aliquot of the **protein-free filtrate** (PFF) is then taken for analysis. Details for preparing specific types of protein-free filtrates are given in Chapter 22 (under Collection and Preservation of Samples) as well as in experiments requiring them.

LABORATORY TECHNIQUES—FOR DRYING AND DISSOLVING

Take care in drying or dissolving samples.

When a solid sample is to be dried in a weighing bottle, the cap is removed from the bottle and, to avoid spilling, both are placed in a beaker and covered with a ribbed watch glass. Some form of identification should be placed on the beaker.

The weighed sample may be dissolved in a beaker or Erlenmeyer flask. If there is any fizzing action, cover the vessel with a watch glass. After dissolution is complete, wash the walls of the vessel down with distilled water. Also wash the watch glass so the washings fall into the vessel. You may have to evaporate the solution to decrease the volume. This is best done by covering the beaker with a

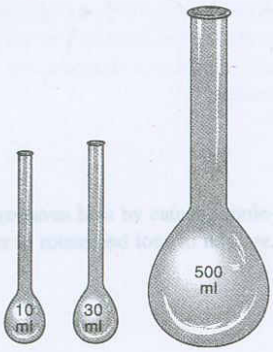


Fig. 2.28. Kjeldahl flasks.

ribbed watch glass to allow space for evaporation. Low heat should be applied to prevent bumping; a steam bath or variable-temperature hot plate is satisfactory.

Use of a **Kjeldahl flask** for dissolution will avoid some of the difficulties of splattering or bumping. Kjeldahl flasks are also useful for performing digestions. They derive their name from their original use in digesting samples for Kjeldahl nitrogen analysis. They are well suited to all types of wet digestions of organic samples and acid dissolution of metals. Kjeldahl flasks come in assorted sizes from 10 to 800 mL. Some of these are shown in Figure 2.28. The sample and appropriate acids are placed in the round bottom of the flask and the flask is tilted while it is heated. In this way the acid can be boiled or refluxed with little danger of loss by "bumping." The flask may be heated with a flame or in special electrically heated Kjeldahl digestion racks, which heat several samples simultaneously.

2.11 Laboratory Safety

You ***must*** familiarize yourself with laboratory safety rules and procedures before conducting experiments! Read Appendix D and the material provided by your instructor. Get a free copy of Ref. 32.

Before beginning any of the experiments, you must familiarize yourself with laboratory safety procedures. Appendix D discusses general safety rules. You should read this material before beginning experiments. Your instructor will provide you with specific guidelines and rules for operation in the laboratory and the disposal of chemicals. For a more complete discussion of safety in the laboratory, you are referred to *Safety in Academic Chemistry Laboratories*, published by The American Chemical Society (Ref. 32). This guide discusses personal protection and laboratory protocol, recommended laboratory techniques, chemical hazards, instructions on reading and understanding material safety data sheets (MSDSs), and safety equipment and emergency procedures. Rules are given for waste disposal, waste classification terminology, Occupational Safety and Health Administration (OSHA) laboratory standards for exposures to hazardous chemicals, and EPA requirements. The handling and treatment of inorganic and organic peroxides are discussed in detail, and an extensive list of incompatible chemicals is given,

Always wear eye protection in the laboratory!

(Courtesy of Merck KGaA. Reproduced by permission.)

and maximum allowable container capacities for flammable and combustible liquids are listed. This resourceful booklet is recommended reading for students and instructors. It is available for free (one copy) from The American Chemical Society, Washington, DC (1-800-227-5558).

The Waste Management Manual for Laboratory Personnel, also published by The American Chemical Society, provides an overview of government regulations (Ref. 33).

Learning Objectives

WHAT ARE SOME OF THE KEY THINGS WE LEARNED FROM THIS CHAPTER?

- Keep a good notebook, p. 20
- Use reagent-grade chemicals, p. 24
- How to use the analytical balance, p. 24
- Volumetric glassware and how to use it, pp. 32, 36
- How to calibrate glassware, p. 39
- How to prepare standard acid and base solutions, pp. 43, 44
- Common laboratory apparatus for handling and treating samples, p. 44
- How to filter and prepare precipitates for gravimetric analysis, p. 48
- How to sample solids, liquids, and gases, p. 52
- How to prepare a solution of the analyte, p. 53

Questions

1. Describe the basic pieces of apparatus used for volumetric measurements. List whether each is designed to contain or to deliver the specified volume.
2. Describe the principle and operation of the analytical balance.
3. Why is a microbalance more sensitive than an analytical balance?
4. What does TD on glassware mean? TC?
5. Explain weighing by difference.
6. Distinguish between the zero point and the rest point of a balance.
7. List the general rules for the use of the balance.
8. Describe the preparation of a standard HCl solution and a standard NaOH solution.
9. Describe the principles of dry ashing and wet digestion of organic and biological materials. List the advantages of each.
10. What are the two principal means of dissolving inorganic materials?
11. What is a PFF? How would you prepare it?
12. What set of conditions must be carefully avoided to use perchloric acid safely for digesting organic materials?
13. What is a gross sample? Sample? Analysis sample? Grab sample?
14. What happens when microwave energy heats samples?

Problems

GLASSWARE CALIBRATION/TEMPERATURE CORRECTIONS

15. You calibrate a 25-mL volumetric flask by filling to the mark with distilled water, equilibrated at 22°C. The dry stoppered flask weighs 27.278 g and the filled flask and stopper is 52.127 g. The balance uses stainless steel weights. What is the volume of the flask? What is it at the standard 20°C. Also insert the weight in air at 22°C into Table 2.4 in your CD, and compare the values obtained.

16. You calibrate a 25-mL pipet at 25°C using steel weights. The weight of the delivered volume of water is 24.971 g. What is the volume of the pipet at 25 and 20°C?

17. You calibrate a 50-mL buret in the winter time at 20°C, with the following corrections:

Buret Reading (mL)	Correction (mL)
10	+0.02
20	+0.03
30	0.00
40	−0.04
50	−0.02

You use the buret on a hot summer day at 30°C. What are the corrections then?

18. You prepare a standard solution at 21°C, and use it at 29°C. If the standardized concentration is 0.05129 *M*, what is it when you use the solution?

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Chapter Three

DATA HANDLING AND SPREADSHEETS IN ANALYTICAL CHEMISTRY

"Facts are stubborn, but statistics are much more pliable."
—Mark Twain

"43.8% of all statistics are worthless."
—Anonymous

Although data handling normally follows the collection of data in an analysis, it is treated early in the text because a knowledge of statistical analysis will be required as you perform experiments in the laboratory. Also, statistics are necessary to understand the significance of the data that are collected and therefore to set limitations on each step of the analysis. The design of experiments (including size of sample required, accuracy of measurements required, and number of analyses needed) is determined from a proper understanding of what the data will represent.

The availability of spreadsheets to process data has made statistical and other calculations very efficient. You will first be presented with the details of various calculations throughout the text, which are necessary for full understanding of the principles. But spreadsheet calculations will also be introduced throughout to illustrate how to take advantage of this software for routine calculations. We will introduce the principles of the use of spreadsheets in this chapter.

3.1 Accuracy and Precision: There Is a Difference

Accuracy is the degree of agreement between the measured value and the true value. An absolute true value is seldom known. A more realistic definition of accuracy, then, would assume it to be the agreement between a measured value and the *accepted* true value.

We can, by good analytical technique, such as making comparisons against a known standard sample of similar composition, arrive at a reasonable assumption about the accuracy of a method, within the limitations of the knowledge of

Accuracy is how close you get to the bullseye. *Precision* is how close the repetitive shots are to one another. It is nearly impossible to have accuracy without good precision.

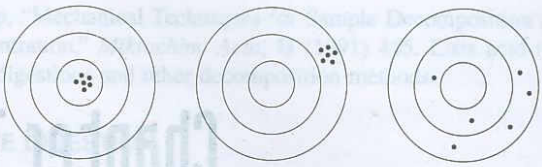


Fig. 3.1. Accuracy versus precision.

the "known" sample (and of the measurements). The accuracy to which we know the value of the standard sample is ultimately dependent on some measurement that will have a given limit of certainty in it.

Good precision does not guarantee accuracy.

Precision is defined as the degree of agreement between replicate measurements of the same quantity. That is, it is the repeatability of a result. The precision may be expressed as the standard deviation, the coefficient of variation, the range of the data, or as a confidence interval (e.g., 95%) about the mean value. Good precision does not assure good accuracy. This would be the case, for example, if there were a systematic error in the analysis. A weight used to measure each of the samples may be in error. This error does not affect the precision, but it does affect the accuracy. On the other hand, the precision can be relatively poor and the accuracy, more or less by chance, may be good. Since all real analyses are unknown, the higher the degree of precision, the greater the chance of obtaining the true value. It is fruitless to hope that a value is accurate if the precision is poor, and the analytical chemist strives for repeatable results to assure the highest possible accuracy.

"To be sure of hitting the target, shoot first, and call whatever you hit the target."—Ashleigh Brilliant

These concepts can be illustrated with a target, as in Figure 3.1. Suppose you are at target practice and you shoot the series of bullets that all land in the bullseye (left target). You are both precise and accurate. In the middle target, you are precise (steady hand and eye), but inaccurate. Perhaps the sight on your gun is out of alignment. In the right target you are imprecise and therefore probably inaccurate. So we see that good precision is needed for good accuracy, but it does not guarantee it.

As we shall see later, the more measurements that are made, the more reliable will be the measure of precision. The number of measurements required will depend on the accuracy required and on the known reproducibility of the method.

3.2 Determinate Errors—They Are Systematic

Determinate or systematic errors are nonrandom and occur when something is wrong with the measurement.

Two main classes of errors can affect the accuracy or precision of a measured quantity. **Determinate errors** are those that, as the name implies, are determinable and that presumably can be either avoided or corrected. They may be constant, as in the case of an uncalibrated weight that is used in all weighings. Or, they may be variable but of such a nature that they can be accounted for and corrected, such as a buret whose volume readings are in error by different amounts at different volumes.

The error can be proportional to sample size or may change in a more complex manner. More often than not, the variation is unidirectional, as in the case of solubility loss of a precipitate (negative error). It can, however, be random in sign. Such an example is the change in solution volume and concentration occurring with changes in temperature. This can be corrected for by measuring the solution temperature. Such measurable determinate errors are classed as **systematic errors**.

Some common determinate errors are:

1. *Instrumental errors.* These include faulty equipment, uncalibrated weights, and uncalibrated glassware.
2. *Operative errors.* These include personal errors and can be reduced by experience and care of the analyst in the physical manipulations involved. Operations in which these errors may occur include transfer of solutions, effervescence and "bumping" during sample dissolution, incomplete drying of samples, and so on. These are difficult to correct for. Other personal errors include mathematical errors in calculations and prejudice in estimating measurements.
3. *Errors of the method.* These are the most serious errors of an analysis. Most of the above errors can be minimized or corrected for, but errors that are inherent in the method cannot be changed unless the conditions of the determination are altered. Some sources of methodic errors include coprecipitation of impurities, slight solubility of a precipitate, side reactions, incomplete reactions, and impurities in reagents. Sometimes correction can be relatively simple, for example, by running a **reagent blank**. A blank determination is an analysis on the added reagents only. It is standard practice to run such blanks and to subtract the results from those for the sample. When errors become intolerable, another approach to the analysis must be made. Sometimes, however, we are forced to accept a given method in the absence of a better one.

Determinate errors may be *additive* or *multiplicative*, depending on the nature of the error or how it enters into the calculation. In order to detect systematic errors in an analysis, it is common practice to add a known amount of standard to a sample and measure its recovery (see Validation of a Method in Chapter 1). The analysis of reference samples also helps guard against method errors or instrumental errors.

3.3 Indeterminate Errors—They Are Random

Indeterminate errors are random and cannot be avoided.

The second class of errors includes the **indeterminate errors**, often called accidental or random errors, which represent the experimental uncertainty that occurs in any measurement. These errors are revealed by small differences in successive measurements made by the same analyst under virtually identical conditions, and they cannot be predicted or estimated. These accidental errors will follow a random distribution; therefore, mathematical laws of probability can be applied to arrive at some conclusion regarding the most probable result of a series of measurements.

It is beyond the scope of this text to go into mathematical probability, but we can say that indeterminate errors should follow a **normal distribution**, or **Gaussian curve**. Such a curve is shown in Figure 3.2. The symbol σ represents the *standard deviation* of an infinite population of measurements, and this measure of precision defines the spread of the normal population distribution as shown in

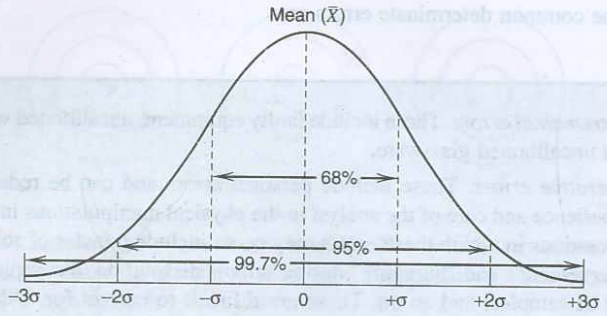


Fig. 3.2. Normal error curve.

Figure 3.2. It is apparent that there should be few very large errors and that there should be an equal number of positive and negative errors.

"Undetectable errors are infinite in variety, in contrast to detectable errors, which by definition are limited."—Tom Gibb

Indeterminate errors really originate in the limited ability of the analyst to control or make corrections for external conditions, or the inability to recognize the appearance of factors that will result in errors. Some random errors stem from the more statistical nature of things, for example, nuclear counting errors. Sometimes, by changing conditions, some unknown error will disappear. Of course, it will be impossible to eliminate all possible random errors in an experiment, and the analyst must be content to minimize them to a tolerable or insignificant level.

3.4 Significant Figures: How Many Numbers Do You Need?

The last digit of a measurement has some uncertainty. You can't include any more digits.

The weak link in the chain of any analysis is the measurement that can be made with the least accuracy. It is useless to extend an effort to make the other measurements of the analysis more accurately than this limiting measurement. The number of significant figures can be defined as **the number of digits necessary to express the results of a measurement consistent with the measured precision**. Since there is uncertainty (imprecision) in any measurement of at least ± 1 in the last significant figure, the number of significant figures includes all of the digits that are known, plus the first uncertain one. Each digit denotes the actual quantity it specifies. For example, in the number 237, we have 2 hundreds, 3 tens, and 7 units.

The digit 0 can be a significant part of a measurement, or it can be used merely to place the decimal point. The number of significant figures in a measurement is independent of the placement of the decimal point. Take the number 92,067. This number has five significant figures, regardless of where the decimal point is placed. For example, 92,067 μm, 9.2067 cm, 0.92067 dm, and 0.092067 m all have the same number of significant figures. They merely represent different ways (units) of expressing one measurement. The zero between the decimal point and the 9 in the last number is used only to place the decimal point. There is no doubt whether any zero that *follows* a decimal point is significant or is used to place the decimal point. In the number 727.0, the zero is not used to locate the decimal point but is a significant part of the figure. Ambiguity can arise if a zero *precedes* a decimal point. If it falls between two other nonzero integers, then it will be significant. Such was the case with 92,067. In the number 936,600, it is impossible to determine whether one or both or neither of the zeros is used merely to place the decimal point or whether they are a part of the measurement. It is best

in cases like this to write only the significant figures you are sure about and then to locate the decimal point by scientific notation. Thus, 9.3660×10^5 has five significant figures, but 936,600 contains six digits, one to place the decimal.

Example 3.1

List the proper number of significant figures in the following numbers and indicate which zeros are significant.

0.216; 90.7; 800.0; 0.0670

Solution

0.216	three significant figures
90.7	three significant figures; zero is significant
800.0	four significant figures; all zeros are significant
0.0670	three significant figures; only the last zero is significant

If a number is written as 500, it could represent 500 ± 100. If it is written as 500., then it is 500 ± 1.

The significance of the last digit of a measurement can be illustrated as follows. Assume that each member of a class measures the length of a rod, using the same meter stick. Assume further that the meter stick is graduated in 1-mm increments. The measurements can be estimated to the nearest 0.1 division (0.1 mm) by interpolation, but the last digit is uncertain since it is only an estimation. A series of class readings, for example, might be

36.4 mm
36.8 mm
36.0 mm
37.1 mm
36.6 mm (average)

MULTIPLICATION AND DIVISION—THINK RELATIVE

The answer of a multiplication or division can be no more accurate than the least accurately known operator.

In many measurements, one estimated digit that is uncertain will be included (e.g., tenth millimeter digit above). This is the last significant figure in the measurement; any digits beyond it are meaningless. In multiplication and division, the uncertainty of this digit is carried through the mathematical operations, thereby limiting the number of certain digits in the answer. There is at least the degree of relative uncertainty (the uncertainty as a function of the answer) in the answer of a multiplication or division as there is in the operator with the least degree of certainty, that is, the one with the least number of significant figures. We shall designate this limiting number as the **key number**. If there is more than one operator with the same lowest number of significant figures, then the one with the smallest absolute magnitude without regard to the decimal point (units) is the key number (since its uncertainty is the greatest). For example, the absolute uncertainty without regard to the decimal point of 0.0344 is 344, and of 5.39 is 539.

Example 3.2

In the following pairs of numbers, pick the one that would represent the key number in a multiplication or division. (a) 42.67 or 0.0967; (b) 100.0 or 0.4570; (c) 0.0067 or 0.10.

Solution

(a) 0.0967 (has three significant figures)

(b) 100.0 (both have four significant figures, but the uncertainty here is 1 part per thousand versus about 1 part in 4600)

(c) 0.10 [both have two significant figures, but the uncertainty here is 10% (1 part in 10) versus about 1 part in 70]

Example 3.3

Give the answer of the following operation to the maximum number of significant figures and indicate the key number.

$$\frac{35.63 \times 0.5481 \times 0.05300}{1.1689} \times 100\% = 88.5470578\%$$

Solution

The key number is 35.63. The answer is therefore 88.55%, and it is meaningless to carry the operation out to more than five figures (the fifth figure is used to round off the fourth). The 100% in this calculation is an absolute number since it is used only to move the decimal point, and it has an infinite number of significant figures. Note that the key number has a relative uncertainty at best of 1 part in 3600, and so the answer has a relative uncertainty at best of 1 part in 3600; thus, the answer has a relative uncertainty at least of this magnitude (i.e., about 2.5 parts in 8900). The objective in a calculation is to express the answer to at least the precision of the least certain number, but to recognize the magnitude of its uncertainty. *The final number is determined by the measurement of significant figures.* (Similarly, in making a series of measurements, one should strive to make each to about the same degree of relative uncertainty.)

A subscript number is used to indicate an added degree of uncertainty. It is used when the number is smaller than the key number.

If the magnitude of the answer without regard to decimal or sign is *smaller* than that of the key number, *one additional figure may be carried in the answer in order to express the minimum degree of uncertainty*, but it is written as a subscript to indicate that it is more doubtful.

Example 3.4

Give the answer of the following operation to the maximum number of significant figures and indicate the key number.

$$\frac{42.68 \times 891}{132.6 \times 0.5247} = 546.57$$

Solution

The key number is 891. Since the absolute magnitude of the answer (its magnitude without respect to units) is less than the key number, it becomes 546_6. The last 6 is written as a subscript to indicate it is more doubtful. Again, the key number has a relative uncertainty of about 1 part in 900, so the answer has an uncertainty of at least 6 parts in 5500 (0.6 parts in 550).

In multiplication and division, the answer from each step of a series of operations can statistically be rounded to the number of significant figures to be retained in the final answer. But for consistency in the final answer, it is preferable to carry one additional figure until the end and then round off.

ADDITION AND SUBTRACTION—THINK ABSOLUTE

The answer of an addition or subtraction is known to the same number of units as the number containing the least significant unit.

Additions and subtractions are handled in a somewhat different manner. We deal with absolute numbers rather than relative numbers. Here, we do not have a key number, and the placing of the decimal point is important in determining how many figures will be significant. Suppose you wish to calculate the formula weight of Ag_2MoO_4 from the individual atomic weights:

Ag	107.87	0
Ag	107.87	0
Mo	95.94	
O	15.99	94
O	15.99	94
O	15.99	94
O	15.99	94
	375.67	76

The atomic weight of molybdenum is known only to the nearest 0.01 atomic unit. Since this unit has an element of uncertainty in it, we cannot justifiably say that we know the formula weight of a compound containing molybdenum to any closer than 0.01 atomic unit. Therefore, the most accurately known value for the atomic weight of Ag_2MoO_4 is 375.68. All numbers being added or subtracted can be rounded to the least significant unit before adding or subtracting. But again, for consistency in the answer, one additional figure should be carried out and then the answer rounded to one less figure.

Summarizing the importance of significant figures, there are two questions to ask. First, how accurately do you have to *know* a particular result? If you only want to learn whether there is 12 or 13% of a substance in the sample, then you need only make all required measurements to two significant figures. If the sample weighs about 2 g, there is no need to weigh it to more than 0.1 g. The second question is, how accurately can you *make* each required measurement? Obviously, if you can read the absorbance of light by a colored solution to only three figures (e.g., $A = 0.447$), it would be useless to weigh the sample to more than three figures (e.g., 6.67 g).

When a number in a measurement is small (without regard to the decimal point) compared with those of the other measurements, there is some justification in making the measurement to one additional figure. This can be visualized as follows. Suppose you wish to weigh two objects of essentially the same mass, and you wish to weigh them with the same precision, for example, to the nearest 0.1 mg, or 1 part per thousand. The first object weighs 99.8 mg, but the second weighs

100.1 mg. You have weighed both objects with equal accuracy, but you have retained an additional significant figure in one of them. This analogy can also be related to the justification for adding an additional significant figure when the answer of a mathematical operation is less than the key number.

When the key number in a series of measurements is known, then, the overall accuracy can be improved if desired either by making the key number larger (e.g., by increasing the sample size) or by making the measurement to an additional figure if possible (e.g., by weighing to one additional figure). This would be desirable when the number is small in magnitude compared to those of the other measurements (without regard to the decimal) in order to bring its uncertainty closer to that of the others.

In carrying out analytical operations, then, you should measure quantities to the same *absolute* uncertainty when adding or subtracting and to the same *relative* uncertainty when multiplying or dividing.

It is good practice to keep an extra figure during stepwise calculations and then drop it in the final number.

"Check the answer you have worked out once more—before you tell it to anybody."—Edmund C. Berkely

If a computation involves both multiplication/division and addition/subtraction, then the individual steps must be treated separately. As good practice, retain one extra figure in the intermediate calculations until the final result (unless it drops out in a subsequent step). When a calculator is used, all digits can be kept in the calculator until the end. Do not assume that a number spit out by a calculator is correct. Always try to estimate the size of the answer you expect. If you expect 2% and the reading is 0.02, you probably forgot to multiply by 100. Or if you expect 20% and the answer is 4.3, you probably made a calculation error or perhaps a measurement error.

Example 3.5

Give the answer of the following computation to the maximum number of significant figures:

$$\frac{\left(\frac{97.7}{32.42} \times 100.0\right) + 36.04}{687}$$

Solution

$$\frac{301_{.36} + 36.04}{687} = \frac{337_{.4}}{687} = .0491_1$$

In the first operation, the key number is 97.7 and the result is $301_{.36}$. We carried an additional fifth figure until the addition step and then rounded to four figures since the division has only three significant figures. In the division step, the key number is 687; but since the absolute magnitude of the answer is less, we carry one more figure. Note that if in the first step we had rounded to $301_{.4}$, the numerator would have become $337_{.5}$ and the final answer would be 0.491_3 (still within the experimental uncertainty).

LOGARITHMS—THINK MANTISSA

In logarithms, it is the mantissa that determines the number of significant figures.

In changing from logarithms to antilogarithms, and vice versa, the number being operated on and the logarithm mantissa have the same number of significant figures. (See Appendix B for a review of the use of logarithms.) All zeros in the

mantissa are significant. Suppose, for example, we wish to calculate the pH of a 2.0×10^{-3} M solution of HCl from $\text{pH} = -\log[\text{H}^+]$. Then,

$$\text{pH} = -\log 2.0 \times 10^{-3} = -(-3 + 0.30) = 2.70$$

The -3 is the characteristic (from 10^{-3}), a pure number determined by the position of the decimal. The 0.30 is the mantissa from the logarithm of 2.0 and therefore has only two digits. So, even though we know the concentration to two figures, the pH (the logarithm) has three figures. If we wish to take the antilogarithm of a mantissa, the corresponding number will likewise have the same number of digits as the mantissa. The antilogarithm of 0.072 (contains three figures in mantissa .072) is 1.18, and the logarithm of 12.1 is 1.083 (1 is the characteristic, and the mantissa has three digits, .083).

3.5 Rounding Off

If the digit following the last significant figure is greater than 5, the number is rounded up to the next higher digit. If it is less than 5, the number is rounded to the present value of the last significant figure:

Always round to the even number, if the last digit is a 5.

$$9.47 = 9.5$$
$$9.43 = 9.4$$

If the last digit is a 5, the number is rounded off to the nearest even digit:

$$8.65 = 8.6$$
$$8.75 = 8.8$$
$$8.55 = 8.6$$

This is based on the statistical prediction that there is an equal chance that the last significant figure before the 5 will be even or odd. That is, in a suitably large sampling, there will be an equal number of even and odd digits preceding a 5. All nonsignificant digits should be rounded off all at once. The even-number rule applies only when the digit dropped is exactly 5 (not . . . 51, e.g.).

3.6 Ways of Expressing Accuracy

There are various ways and units in which the accuracy of a measurement can be expressed, an accepted true value for comparison being assumed.

ABSOLUTE ERRORS

The difference between the true value and the measured value, with regard to the sign, is the **absolute error**, and it is reported in the same units as the measurement. If a 2.62-g sample of material is analyzed to be 2.52 g, the absolute error is -0.10 g. If the measured value is the average of several measurements, the error is called the **mean error**. The mean error can also be calculated by taking the average difference, with regard to sign, of the *individual* test results from the true value.

RELATIVE ERROR

The absolute or mean error expressed as a percentage of the true value is the **relative error**. The above analysis has a relative error of $(-0.10/2.62) \times 100\% = -3.8\%$. The **relative accuracy** is the measured value or mean expressed as a percentage of the true value. The above analysis has a relative accuracy of $(2.52/2.62) \times 100\% = 96.2\%$. We should emphasize that neither number is known to be "true," and the relative error or accuracy is based on the mean of two sets of measurements.

The relative error can be expressed in units other than percentages. In very accurate work, we are usually dealing with relative errors of less than 1%, and it is convenient to use a smaller unit. A 1% error is equivalent to 1 part in 100. It is also equivalent to 10 parts in 1000. This latter unit is commonly used for expressing small uncertainties. That is, the uncertainty is expressed in **parts per thousand**, written as ppt. The number 23 expressed as parts per thousand of the number 6725 would be 23 parts per 6725, or 3.4 ppt. Parts per thousand is often used in expressing precision of measurement.

Example 3.6

The results of an analysis are 36.97 g, compared with the accepted value of 37.06 g. What is the relative error in parts per thousand?

Solution

$$\text{Absolute error} = 36.97\ \text{g} - 37.06\ \text{g} = -0.09\ \text{g}$$

$$\text{Relative error} = \frac{-0.09}{37.06} \times 1000‰ = -2.4\ \text{ppt}$$

‰ indicates parts per thousand, just as % indicates parts per hundred.

3.7 Standard Deviation—The Most Important Statistic

"If reproducibility be a problem, conduct the test only once."
—Anonymous

Each set of analytical results should be accompanied by an indication of the **precision** of the analysis. Various ways of indicating precision are acceptable.

The standard deviation σ of an infinite set of experimental data is theoretically given by

$$\sigma = \sqrt{\frac{\sum(x_i - \mu)^2}{N}} \tag{3.1}$$

where x_i represents the individual measurements and μ the mean of the infinite number of measurements (which should represent the "true" value). This equation holds strictly only as $N \to \infty$, where N is the number of measurements. In practice, we must calculate the individual deviations from the mean of a limited number of measurements, $\bar{x}$, in which it is anticipated that $\bar{x} \to \mu$, although we have no assurance this will be so; $\bar{x}$ is given by $\sum(x_i/N)$.

For a set of N measurements, it is possible to calculate N independently variable deviations from some reference number. But if the reference number chosen is the estimated mean, $\bar{x}$, the sum of the individual deviations (retaining signs) must necessarily add up to zero, and so values of $N - 1$ deviations are adequate to define the Nth value. That is, there are only $N - 1$ independent deviations from the mean; when $N - 1$ values have been selected, the last is predetermined. We have, in effect, used one degree of freedom of the data in calculating the mean, leaving $N - 1$ **degrees of freedom** for calculating the precision.

As a result, the **estimated standard deviation s of a finite set of experimental data** (generally $N < 30$) more nearly approximates σ if the number of degrees of freedom is substituted for N ($N - 1$ adjusts for the difference between $\bar{x}$ and μ).

See Section 3.15 and Equation 3.17 for another way of estimating s for four or less numbers.

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{N - 1}} \tag{3.2}$$

The value of s is only an estimate of σ, then, and will more nearly approach σ as the number of measurements increases. Since we deal with small numbers of measurements in an analysis, the precision is necessarily represented by s.

Example 3.7

Calculate the mean and the standard deviation of the following set of analytical results: 15.67, 15.69, and 16.03 g.

Solution

x_i	$x_i - \bar{x}$	$(x_i - \bar{x})^2$
15.67	0.13	0.0169
15.69	0.11	0.0121
16.03	0.23	0.0529
$\sum$ 47.39	$\sum$ 0.47	$\sum$ 0.0819

$$\bar{x} = \frac{\sum x_i}{N} = \frac{47.39}{3} = 15.80$$

$$s = \sqrt{\frac{0.0819}{3 - 1}} = 0.20 \text{ g}$$

The standard deviation may be calculated also using the following equivalent equation:

$$s = \sqrt{\frac{\sum x_i^2 - (\sum x_i)^2/N}{N - 1}} \tag{3.3}$$

This is useful for computations with a calculator. Many calculators, in fact, have a standard deviation program that automatically calculates the standard deviation from entered individual data.

Example 3.8

Calculate the standard deviation for the data in Example 3.7 using Equation 3.3.

Solution

x_i	x_i^2
15.67	245.55
15.69	246.18
16.03	256.96
$\sum$ 47.39	$\sum$ 748.69

$$s = \sqrt{\frac{748.69 - (47.39)^2/3}{3 - 1}} = 0.21 \text{ g}$$

The difference of 0.01 g from Example 3.7 is not statistically significant since the variation is at least ±0.2 g. In applying this formula, it is important to keep an extra digit or even two in x_i^2 for the calculation.

The precision improves as the square root of the number of measurements.

The standard deviation calculation considered so far is an estimate of the probable error of a single measurement. The arithmetical mean of a series of N measurements taken from an infinite population will show less scatter from the "true value" than will an individual observation. The scatter will decrease as N is increased; as N gets very large the sample average will approach the population average μ, and the scatter approaches zero. The arithmetical mean derived from N measurements can be shown to be $\sqrt{N}$ times more reliable than a single measurement. Hence, the random error in the mean of a series of four observations is one-half that of a single observation. In other words, the **precision of the mean** of a series of N measurements is inversely proportional to the square root of N of the deviation of the individual values. Thus,

$$\text{Standard deviation of the mean} = s_{\text{mean}} = \frac{s}{\sqrt{N}} \tag{3.4}$$

The standard deviation of the mean is sometimes referred to as the *standard error.*

The standard deviation is sometimes expressed as the **relative standard deviation** (rsd), which is just the standard deviation expressed as a fraction of the mean; usually it is given as the *percentage* of the mean (% rsd), which is often called the **coefficient of variation**.

Example 3.9

The following replicate weighings were obtained: 29.8, 30.2, 28.6, and 29.7 mg. Calculate the standard deviation of the individual values and the standard deviation of the mean. Express these as absolute (units of the measurement) and relative (% of the measurement) values.

Solution

x_i	$x_i - \bar{x}$	$(x_i - \bar{x})^2$
29.8	0.2	0.04
30.2	0.6	0.36
28.6	1.0	1.00
29.7	0.1	0.01
Σ 118.3	Σ 1.9	Σ 1.41

$$\bar{x} = \frac{118.3}{4} = 29.6$$

$$s = \sqrt{\frac{1.41}{4-1}} = 0.69 \text{ mg (absolute)}; \quad \frac{0.69}{29.6} \times 100\% = 2.3\% \text{ (coefficient of variation)}$$

$$s_{\text{mean}} = \frac{0.69}{\sqrt{4}} = 0.34 \text{ mg (absolute)}; \quad \frac{0.34}{29.6} \times 100\% = 1.1\% \text{ (relative)}$$

The precision of a measurement can be improved by increasing the number of observations. In other words, the spread $\pm s$ of the normal curve in Figure 3.2 becomes smaller as the number of observations is increased and would approach zero as the number of observations approached infinity. However, as seen above (Equation 3.4), the deviation of the mean does not decrease in direct proportion to the number of observations, but instead it decreases as the square root of the number of observations. A point will be reached where a slight increase in precision will require an unjustifiably large increase in the number of observations. For example, to decrease the standard deviation by a factor of 10 requires 100 times as many observations.

"Randomness is required to make statistical calculations come out right."—Anonymous

The practical limit of useful replication is reached when the standard deviation of the random errors is comparable to the magnitude of the determinate or systematic errors (unless, of course, these can be identified and corrected for). This is because the systematic errors in a determination cannot be removed by replication.

The significance of s in relation to the normal distribution curve is shown in Figure 3.2. The mathematical treatment from which the curve was derived reveals that 68% of the individual deviations fall within one standard deviation (for an infinite population) from the mean, 95% are less than twice the standard deviation, and 99% are less than 2.5 times the standard deviation. So, a good approximation is that 68% of the individual values will fall within the range $\bar{x} \pm s$, 95% will fall within $\bar{x} \pm 2s$, 99% will fall within $\bar{x} \pm 2.5s$, and so on.

The true value will fall within $\bar{x} \pm 2s$ 95% of the time for an infinite number of measurements. See the confidence limit and Example 3.15.

Actually, these percentage ranges were derived assuming an infinite number of measurements. There are then two reasons why the analyst cannot be 95% certain that the true value falls within $\bar{x} \pm 2s$. First, one makes a limited number of measurements, and the fewer the measurements, the less certain one will be. Second, the normal distribution curve assumes no determinate errors, but only random errors. Determinate errors, in effect, shift the normal error curve from the true value. An estimate of the actual certainty a number falls within s can be obtained from a calculation of the *confidence limit* (see below).

It is apparent that there are a variety of ways in which the precision of a number can be reported. Whenever a number is reported as $\bar{x} \pm x$, you should always qualify under what conditions this holds, that is, how you arrived at $\pm x$. It may, for example, represent s, $2s$, s (mean), or the coefficient of variation.

The variance equals s^2.

A term that is sometimes useful in statistics is the **variance**. This is the square of the standard deviation, s^2. We shall use this in determining the propagation of error and in the F test below (Section 3.13).

3.8 Use of Spreadsheets in Analytical Chemistry

A spreadsheet is a powerful software program that can be used for a variety of functions, such as data analysis and plotting. Spreadsheets are useful for organizing data, doing repetitive calculations, and displaying the calculations graphically or in chart form. They have built-in functions, for example, standard deviation and other statistical functions, for carrying out computations on data that are input by the user. Popular spreadsheet programs include Microsoft Excel, Lotus 1-2-3, and Quattro Pro. All operate basically the same but differ somewhat in specific commands and syntax. Because of its widespread availability and popularity, we will use Excel in our illustrations.

You probably have used a spreadsheet program before and are familiar with the basic functions. But we will summarize here the most useful aspects for analytical chemistry applications. You should refer to the spreadsheet manual for more detailed information. Also, the Exel Help on the tool bar provides specific information.

You are referred to the excellent tutorial on using the Excel spreadsheet prepared by faculty at California State University at Stanislaus: http://science.csustan.edu/tutorial/Excel/index.htm. The basic functions in the spreadsheet are described, including entering data and formulas, formatting cells, graphing, and regression analysis. You will find this very helpful and should definitely read it before continuing. The website www.wku.edu/~conteed/CHEM330 at Western Kentucky University gives summary instructions for graphing using either Microsoft Excel or Lotus 1-2-3. Go to the excelhandout.html and lotushandout.html links.

A spreadsheet consists of **cells** arranged in columns (labeled A, B, C, . . .) and **rows** (numbered 1, 2, 3, . . .). An individual cell is identified by its column letter and row number, for example, B3. Figure 3.3 has the identifiers typed into some of the cells to illustrate. When the mouse pointer (the cross) is clicked on an individual cell, it becomes the **active cell** (dark lines around it), and the active cell is indicated at the top left of the formula bar, and the contents of the cell are listed to the right of the equal sign on the bar.

FILLING THE CELL CONTENTS

You may enter *text*, *numbers*, or *formulas* in specific cells. Formulas are the key to the utility of spreadsheets, allowing the same calculation to be applied to many numbers. We will illustrate with calculations of the weights of water delivered by two different 20-mL pipets, from the difference in the weights of a flask plus water and the empty flask. Refer to Figure 3.4 as you go through the steps.

	A	B	C	D	E
1	A1	B1	C1	etc.	
2	A2	B2	C2	etc.	
3	A3	B3	C3	etc.	
4					
5					

Fig. 3.3. Spreadsheet cells.

	A	B	C	D
1	Net weights			
2				
3	Pipet	1	2	
4	Weight of flask + water, g	47.702	49.239	
5	Weight of flask, g	27.687	29.199	
6	Weight of water, g	20.015	20.040	
7				
8	cell B6=B4-B5			
9	cell C6=C4-C5			
10				

Fig. 3.4. Filling cell contents.

Open an Excel spreadsheet by clicking on the Excel icon (or the Microsoft Excel program under Start: Programs). You will enter text, numbers, and formulas. Double click on the specific cell to activate it. Enter as follows (information typed into a cell is entered by depressing the Enter key):

Cell A1: Net weights

Cell A3: Pipet

Cell A4: Weight of flask + water, g

Cell A5: Weight of flask, g

Cell A6: Weight of water, g

You may make corrections by double clicking on a cell; then edit the text. (You can also edit the text in the formula bar.) If you single click, new text replaces the old text. You will have to widen the A cells to accommodate the lengthy text. Do so by placing the mouse pointer on the line between A and B on the row at the top, and dragging it to the right till all the text shows. This moves the other cells to the right.

Cell B3: 1

Cell C3: 2

Cell B4: 47.702

Cell C4: 49.239

Cell B5: 27.687

Cell C5: 29.199

Cell B6: =B4-B5

You can also enter the formula by typing =, then click on B4, then type −, and click on B5. You need to format the cells B4 to C6 to three decimal places. Highlight that block of cells by clicking on one corner and dragging to the opposite corner of the block. In the Menu bar, click on Format:Cells:Number. For Decimal places: type 3, and click OK.

You need to add the formula to cell C6. You can retype it. But there is an easier way, by copying (filling) the formula in cell B6. Place the mouse pointer on the lower right corner of cell B6 and drag it to cell C6. This fills the formula into C6 (or additional cells to the right if there are more pipet columns). You may also fill formulas into highlighted cells by clicking on Edit:Fill:Down(or Right).

Double click on B6. This shows the formula in the cell and outlines the other cells contained in the formula. Do the same for C6. Note that when you activate the cell by either single or double clicking on it, the formula is shown in the formula bar.

SAVING THE SPREADSHEET

Save the spreadsheet you have just created by clicking on File:SaveAs. I like to save documents to the desktop first. Then they can be dragged to whatever file you wish, for example, My Documents. That way they don't get lost. So select Desktop at the top. Give the document a File Name at the bottom, for example, Pipet Calibration. Then click Save. If you wish to place the saved document on a disk, you can drag it from the desktop to the opened disk.

PRINTING THE SPREADSHEET

Click File:Page Setup. Normally, a sheet is printed in the Portrait format, that is, vertically on the 8½- × 11-inch paper. If there are many columns, you may wish to print in Landscape, that is, horizontally. If you want gridlines to print, click on Sheet:Gridlines. Now you are ready to print. Click on Print:OK. Just the working area of the spreadsheet will print, not the column and row identifiers.

RELATIVE VS. ABSOLUTE CELL REFERENCES

In the example above, we used *relative* cell references in copying the formula. The formula in cell B6 said subtract the cell above from the one above it. The copied formula in C6 said the same for the cells above it.

Sometimes we need to include a specific cell in each calculation, containing say, a constant. To do this, we need to identify it in the formula as an *absolute* reference. This is accomplished by placing a $ sign in front of the column and row cell identifiers, for example, $B$2. Placing the sign in front of both assures that whether we move across columns or rows, it will remain an absolute reference.

We can illustrate this by creating a spreadsheet to calculate the means of different series of numbers. Fill in the spreadsheet as follows (refer to Figure 3.5):

A1: Titration means
A3: Titn. No.
B3: Series A, mL
C3: Series B, mL
B4: 39.27
B5: 39.18
B6: 39.30
B7: 39.20
C4: 45.59
C5: 45.55
C6: 45.63
C7: 45.66
A4: 1

We can type in each of the titration numbers (1 through 4), but there are automatic ways of incrementing a string of numbers. Click on Edit:Fill:Series. Check Columns and Linear, and leave Step Value at 1. For Stop Value, enter 4 and click OK. The numbers 2 through 4 are inserted in the spreadsheet. You could also first highlight the cells you want filled (beginning with cell A4). Then you do not have to insert a Stop Value. Another way of incrementing a series is to do it by formula. In cell A5, type =A4+1. Then you can fill down by highlighting from A5 down, and clicking on Edit:Fill:Down. (This is a relative reference.) Or, you can highlight cell A5, click

	A	B	C	D
1	Titration means			
2				
3	Titn. No.	Series A, mL	Series B, mL	
4	1	39.27	45.59	
5	2	39.18	45.55	
6	3	39.30	45.63	
7	4	39.22	45.66	
8	Mean:	**39.24**	45.61	
9	Std.Dev.	**0.053150729**	0.047871355	
10				
11	**Cell B8=**	SUM(B4:B7)/$A$7 Copy right to Cell C8.		
12	**Cell B9=**	STDEV(B4:B7) Copy right to Cell C9.		
13	We have boldfaced the cells with formulas entered.			

Fig. 3.5. Relative and absolute cell references.

on its lower right corner, and drag it to cell A7. This automatically copies the formula in the other cells.

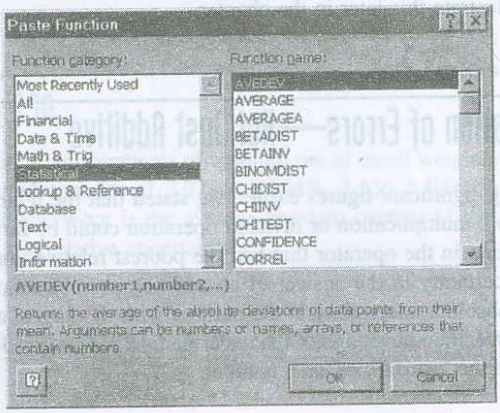
Now we wish to insert a formula in cell B8 to calculate the mean. This will be the sum divided by the number of titrations (cell A7).

B8: =sum(B4:B7)/$A$7

We place the $ signs in the devisor because it will be an *absolute reference* that we wish to copy to the right in cell C8. Placing a $ before both the column and row addresses assures that the cell will be treated as absolute whether it is copied horizontally or vertically. The sum(B4:B7) is a **syntax** in the program for summing a series of numbers, from cell B4 through cell B7. Instead of typing in the cell addresses, you can also type "=(", then click on cell B4 and drag cell B7, and type ")". We have now calculated the mean for series A. We wish to do the same for series B. Highlight cell B8, click on its lower right corner, and drag it to cell C8. *Voilà*, the next mean is calculated! Double click on cell C8, and you will see that the formula has the same divisor (*absolute reference*), but the sum is a *relative reference*. If we had not typed in the $ signs to make the divisor absolute, the formula would have assumed it was relative, and the divisor in cell C8 would be cell B7.

USE OF EXCEL STATISTICAL FUNCTIONS

Excel has a large number of mathematical and statistical functions that can be used for calculations in lieu of writing your own formulas. Let's try the statistical functions to automatically calculate the mean. Highlight an empty cell and click f_x on the tool bar. The Paste Function window appears. Select Statistical in the Function category. The following window appears:



Select AVERAGE for the Function name. Click OK, and for Number, type B4:B7, and click OK. The same average is calculated as you obtained with your own formula. You can also type in the activated cell the syntax =average(B4:B7). Try it.

Let's calculate the standard deviation of the results. Highlight cell B9. Under the Statistical function, select STDEV for the Function name. Alternatively, you can type the syntax into cell B9, =stdev(B4:B7). Now copy the formula to cell C9. Perform the standard deviation calculation using Equation 3.2 and compare with the Exel values. The calculation for series A is ±0.05 mL. The value in the spreadsheet, of course, should be rounded to ±0.05 mL.

USEFUL SYNTAXES

Excel has numerous mathematical and statistical functions or syntaxes that can be used to simplify setting up calculations. Peruse the Function names for the Math & Trig and the Statistical function categories under f_x in the toolbar. Some you will find useful for this text are:

Math and trig functions

LOG10	Calculates the base-10 logarithm of a number
PRODUCT	Calculates the products of a series of numbers
POWER	Calculates the result of a number raised to a power
SQRT	Calculates the square root of a number

Statistical functions

AVERAGE	Calculates the mean of a series of numbers
MEDIAN	Calculates the median of a series of numbers
STDEV	Calculates the standard deviation of a series of numbers
TTEST	Calculates the probability associated with Student's *t* test
VAR	Calculates the variance of a series of numbers

The syntaxes may be typed, followed by the range of cells in parentheses, as we did above.

This tutorial should provide you the basics for other spreadsheet applications. You can write any formula that is in this book into an active cell, and insert appropriate data for calculations. And, obviously, we can perform a variety of data analyses. We can prepare plots and charts of the data, for example, a calibration curve of instrument response versus concentration, along with statistical information. We will illustrate this later in the chapter.

3.9 Propagation of Errors—Not Just Additive

When discussing significant figures earlier, we stated that the relative uncertainty in the answer to a multiplication or division operation could be no better than the relative uncertainty in the operator that had the poorest relative uncertainty. Also, the absolute uncertainty in the answer of an addition or subtraction could be no better than the absolute uncertainty in the number with the largest absolute uncertainty. Without specific knowledge of the uncertainties, we assumed an uncertainty of at least ±1 in the last digit of each number.