

commodate approximately 98% of the samples encountered in a clinical laboratory. Samples outside this range can usually be handled by suitable dilutions.

Extensive performance testing of these devices generally reveals a good correlation between the data they

produce and the results by standard procedures. Precision of 1 to 10% relative is reported depending upon the type of test, which again is comparable with the data from automated standard methods.

## 28E QUESTIONS AND PROBLEMS

- 28-1 List sequentially a set of laboratory unit operations that might be used to  
 (a) ascertain the presence or absence of lead in flakes of dry paint.  
 (b) determine the iron content of multiple vitamin/mineral tablets.
- 28-2 Sketch a flow-injection system that could be used for the determination of  $K^+$  and  $Na^+$  in blood based upon flame photometric measurements.
- 28-3 Sketch a flow-injection system that might be employed for determining lead in the aqueous effluent from an industrial plant based upon the extraction of lead ions with a carbon tetrachloride solution of dithizone, which reacts with lead ion to form an intensely colored product.
- 28-4 Sketch a flow-injection apparatus for the determination of sodium sulfite in aqueous samples.

# Appendix 1

## Evaluation of Analytical Data

This appendix describes the types of errors that are encountered in analytical chemistry and how their magnitudes are estimated and reported. Estimation of the probable accuracy of results is a vital part of any analysis because data of unknown reliability are essentially worthless.

### a1A PRECISION AND ACCURACY

Two terms are widely used in discussions of the reliability of data: *precision* and *accuracy*.

#### a1A-1 Precision

Precision describes the reproducibility of results—that is, the agreement between numerical values for two or more replicate measurements, or measurements that have been made *in exactly the same way*. Generally, the precision of an analytical method is readily obtained by simply repeating the measurement.

Three terms are widely used to describe the precision of a set of replicate data: *standard deviation*, *variance*, and *coefficient of variation*. These terms have statistical significance and are defined in Section a1B-1.

#### a1A-2 Accuracy

Accuracy describes the correctness of an experimental result. Strictly speaking, the only type of measurement that can be completely accurate is one that involves counting objects. All other measurements contain errors and give only an approximation of the truth.

Accuracy is a relative term in the sense that what is an accurate or inaccurate method very much depends upon the needs of the scientist and the difficulty of the analytical problem. For example, an analytical method that yields results that are within  $\pm 10\%$ , or one part per billion, of the correct amount of mercury in a sample of fish tissue that contains 10 parts per billion of the metal would usually be considered to be reasonably accurate. In contrast, a procedure that yields results that are within  $\pm 10\%$  of the correct amount of mercury in an ore that contains 20% of the metal would usually be deemed unacceptably inaccurate.

Accuracy is expressed in terms of either absolute error or relative error. The *absolute error*  $E_a$  of the mean

(or average)  $\bar{x}$  of a small set of replicate analyses is given by the relationship

$$E_a = \bar{x} - x_t \quad (a1-1)$$

where  $x_t$  is an accepted value of the quantity being measured. Often, it is useful to express the accuracy in terms of *relative error*, where

$$\text{relative error} = \frac{\bar{x} - x_t}{x_t} \times 100\% \quad (a1-2)$$

TABLE a1-1  
Replicate Absorbance Measurements\*

Trial	Absorbance, A	Trial	Absorbance, A	Trial	Absorbance, A
1	0.488	18	0.475	35	0.476
2	0.480	19	0.480	36	0.490
3	0.486	20	0.494†	37	0.488
4	0.473	21	0.492	38	0.471
5	0.475	22	0.484	39	0.486
6	0.482	23	0.481	40	0.478
7	0.486	24	0.487	41	0.486
8	0.482	25	0.478	42	0.482
9	0.481	26	0.483	43	0.477
10	0.490	27	0.482	44	0.477
11	0.480	28	0.491	45	0.486
12	0.489	29	0.481	46	0.478
13	0.478	30	0.469‡	47	0.483
14	0.471	31	0.485	48	0.480
15	0.482	32	0.477	49	0.483
16	0.483	33	0.476	50	0.479
17	0.488	34	0.483		

Mean absorbance = 0.482  
Standard deviation = 0.0056

\* Data listed in the order obtained  
† Maximum value  
‡ Minimum value

Frequently, the relative error is expressed as a percentage as shown; in other cases the quotient is multiplied by 1000 to give the error in parts per thousand (ppt).

Note that both absolute and relative errors bear a sign, a positive sign indicating that the measured result is greater than its true value and a negative sign the reverse.

We will be concerned with two types of errors: *random*, or *indeterminate, errors* and *systematic*, or

*determinate, errors*.<sup>1</sup> The error in the mean of a set of replicate measurements is then the sum of these two types of errors:

$$E_a = E_r + E_s \quad (a1-3)$$

where  $E_r$  is the random error associated with a measurement and  $E_s$  is the systematic error.

**RANDOM ERRORS**

Whenever analytical measurements are repeated on the same sample, the data obtained are scattered, as is shown in Table a1-1, because of the presence of random, or indeterminate errors—that is, the presence of random errors is reflected in the imprecision of the data. The data in columns 2, 4, and 6 of the table are absorbances (Section 7A-2) obtained with a spectrophotometer on 50 replicate red solutions produced by treating identical aqueous samples containing 10 ppm of Fe(III) with an excess of thiocyanate ion. The measured absorbances are directly proportional to iron concentration.

The distribution of random errors in these data is more easily comprehended if they are organized into equal-size, contiguous data groups, or *cells*, as shown in Table a1-2. The relative frequency of occurrence of data in each cell is then plotted as in Figure a1-1A to give a bar graph called a *histogram*.

It is reasonable to suppose that if the number of analyses were much larger than that shown in Table a1-2 and if the size of the cells were made much smaller, then, ultimately, a smooth curve such as that shown in Figure a1-1B would be obtained. A smooth curve of this type is called a *Gaussian curve*, or a *normal error curve*. It is found empirically that the results of replicate chemical analyses are frequently distributed in an approximately Gaussian, or normal, form.

The frequency distribution exhibited by a Gaussian curve has the following characteristics:

1. The most frequently observed result is the mean  $\mu$  of the set of data.

<sup>1</sup> A third type of error occasionally encountered is *gross error*, which arises in most instances from the carelessness, ineptitude, laziness, or bad luck of the experimenter. Typical sources include transposition of numbers in recording data, spilling of a sample, using the wrong scale on a meter, accidental introduction of contaminants, and reversing the sign on a meter reading. A gross error in a set of replicate measurements appears as an *outlier*—a datum that is noticeably different from the other data in the set. We will not consider gross errors in this discussion.

2. The results cluster symmetrically around this mean value.
3. Small divergences from the central mean value are found more frequently than are large divergences.
4. In the absence of systematic errors, the mean of a large set of data approaches the true value.

Characteristic 4 means that, in principle, it is always possible to reduce the random error of an analysis to something that approaches zero. Unfortunately, it is seldom practical to achieve this goal, because to do so requires performing 20 or more replicate analyses. Ordinarily, a scientist can only afford the time for two or three replicated measurements, and a significant random error is to be expected for the mean of such a small number of replicate measurements.

Statisticians usually use  $\mu$  to represent the mean of an infinite collection of data (see Figure a1-1B), and  $\bar{x}$  for the mean of a small set of replicate data. The random error  $E_r$  for the mean of the small set is then given by

$$E_r = \bar{x} - \mu \quad (a1-4)$$

It is found that the mean for a finite set of data rapidly approaches the true mean when the number of measurements  $N$  is greater than perhaps 20 or 30. Thus, as is shown in the following example, we can sometimes

TABLE a1-2  
Frequency Distribution of Data from Table a1-1

Absorbance Range A	Number in Range y	Relative Frequency, y/N*
0.469 to 0.471	3	0.06
0.472 to 0.474	1	0.02
0.475 to 0.477	7	0.14
0.478 to 0.480	9	0.18
0.481 to 0.483	13	0.26
0.484 to 0.486	7	0.14
0.487 to 0.489	5	0.10
0.490 to 0.492	4	0.08
0.493 to 0.495	1	0.02

\* N = total number of measurements = 50.

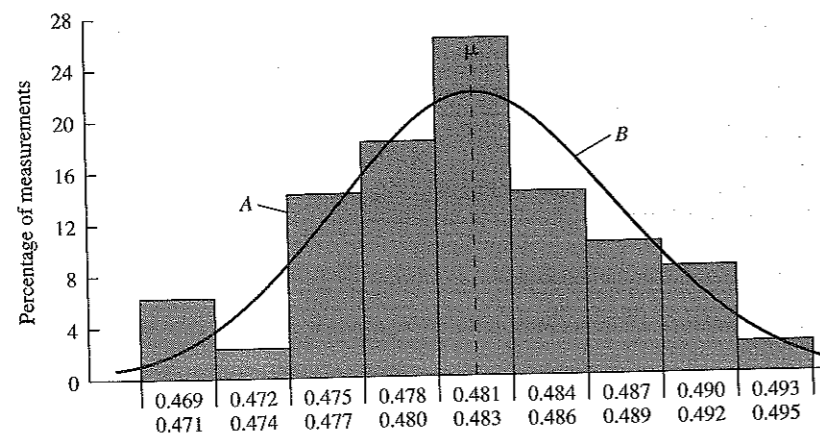


FIGURE a1-1 A, Histogram showing distribution of the 50 results in Table a1-1. B, Gaussian curve for data having the same mean and same standard deviation as the data in A.

determine the random error in an individual datum or in the mean of a small set of data.

#### EXAMPLE a1-1

Calculate the random error for (a) the second datum in Table a1-1 and (b) the mean for the first three entries in the table.

The mean for the entire set of data is 0.482, and because this mean is for 50 measurements, we may assume that the random error in it is approximately zero. Thus, the limiting mean  $\mu$  can be taken as 0.482.

(a) Here, the random error for a single measurement  $x_2$  is

$$E_r = x_2 - \mu = 0.480 - 0.482 = -0.002$$

(b) The mean  $\bar{x}$  for the first three entries in the table is

$$\bar{x} = \frac{0.488 + 0.480 + 0.486}{3} = 0.485$$

Substituting into Equation a1-4 gives

$$E_r = \bar{x} - \mu = 0.485 - 0.482 = +0.003$$

The random nature of indeterminate errors makes it possible to treat these effects by the methods of statistics. Statistical techniques are considered in Section a1B.

#### SYSTEMATIC ERRORS—BIAS

Systematic errors have a definite value, an assignable cause, and are of the same sign and magnitude for replicate measurements made in exactly the same way. Systematic errors lead to *bias* in a measurement technique. Bias is illustrated by the two curves in Figure a1-2, which show the frequency distribution of replicate results in the analysis of identical samples by two methods that have random errors of identical size. Method

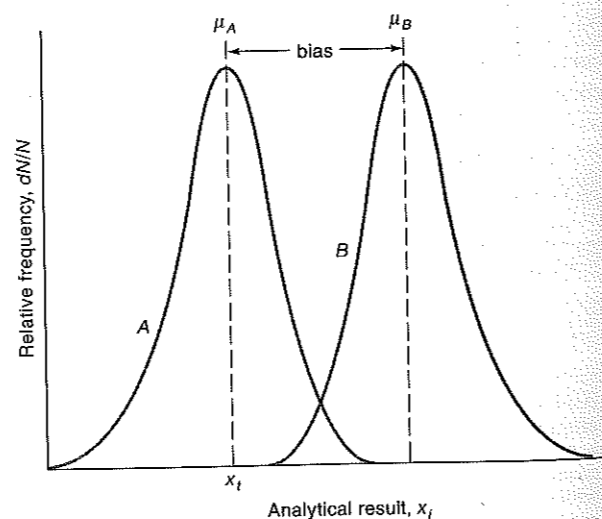


FIGURE a1-2 Illustration of bias: bias =  $\mu_B - \mu_A$ .

A has no bias, so the limiting mean is the true value  $x_r$ . Method B has a bias that is given by

$$\text{bias} = \mu_B - x_r = \mu_B - \mu_A \quad (\text{a1-5})$$

Note that bias affects all of the data in a set and that it bears a sign.

Systematic errors have three sources: *instrumental*, *personal*, and *method*.

**Instrumental Errors.** Typical sources of instrumental errors include drift in electronic circuits, leakage in vacuum systems, temperature effects on detectors, currents induced in circuits from ac power lines, decreases in voltages of batteries with use, and calibration errors in meters, weights, and volumetric equipment.

Systematic instrument errors are commonly detected and corrected by calibration with suitable standards. Periodic calibration of instruments is always desirable because the response of most instruments changes with time as a consequence of wear, corrosion, or mistreatment.

**Personal Errors.** Personal errors are those introduced into a measurement by judgments that the experimentalist must make. Examples include estimating the position of a pointer between two scale divisions, the color of a solution at the end point in a titration, the level of a liquid with respect to a graduation in a pipet, or the relative intensity of two light beams. Judgments of this type are often subject to systematic, unidirectional uncertainties. For example, one person may read a pointer consistently high, another may be slightly slow in activating a timer, and a third may be less sensitive to color. Color blindness or other physical handicaps often exacerbate determinate personal errors.

Number bias is another source of personal systematic error that is widely encountered and varies considerably from person to person. The most common bias encountered in estimating the position of a needle on a scale is a preference for the digits 0 and 5. Also prevalent is a preference for small digits over large and even ones over odd.

A near-universal source of personal error is prejudice. Most of us, no matter how honest, have a natural tendency to estimate scale readings in a direction that improves the precision in a set of results or causes the results to fall closer to a preconceived notion of the true value for the measurement.

Most personal errors can be minimized by care and self-discipline. Thus, most scientists develop the habit of systematically double-checking instrument readings,

notebook entries, and calculations. Robots, automated systems, computerized data collection, and computerized instrument control have the potential of minimizing or eliminating personal systematic errors.

**Method Errors.** Method-based errors are often introduced from nonideal chemical and physical behavior of reagents and reactions upon which an analysis is based. Possible sources include slowness or incompleteness of chemical reactions, losses by volatility, adsorption of the analyte on solids, instability of reagents, contaminants, and chemical interferences.

Systematic method errors are usually more difficult to detect and correct than are instrument and personal errors. The best and surest way involves *validation* of the method by employing it for the analysis of standard materials that resemble the samples to be analyzed both in physical state and in chemical composition. The analyte concentrations of these standards must, of course, be known with a high degree of certainty. For simple materials, standards can sometimes be prepared by blending carefully measured amounts of pure compounds. Unfortunately, more often than not, materials to be analyzed are sufficiently complex to preclude this simple approach.

The National Institute of Standards and Technology<sup>2</sup> offers for sale a variety of *standard reference materials* (SRMs) that have been specifically prepared for the validation of analytical methods.<sup>3</sup> The concentration of one or more constituents in these materials has been determined by (1) a previously validated reference method, (2) two or more independent, reliable measurement methods, or (3) analyses from a network of cooperating laboratories, technically competent and thoroughly familiar with material being tested. Most standard reference materials are substances that are commonly encountered in commerce or in environmental, pollution, clinical, biological, and forensic studies. A few examples include trace elements in coal, fuel oil, urban particulate matter, sediments from estuaries, and

<sup>2</sup> In 1989, the name of the National Bureau of Standards (NBS) was changed to the National Institute of Standards and Technology (NIST). At this time, several of the NIST publications still bear the NBS label.

<sup>3</sup> See U.S. Department of Commerce, *NIST Standard Reference Materials Catalog 1990-91*, NIST Special Publication 260. Washington: Government Printing Office, 1990. For a description of the NIST reference material program, see R. A. Alvarez, S. D. Raspberry, and G. A. Urano, *Anal. Chem.*, **1982**, *54*, 1226A; and G. A. Urano, *ASTM Standardization News*, **1979**, *7*, 8.

water; lead in blood samples; cholesterol in human serum; drugs of abuse in urine; and a wide variety of elements in rocks, minerals, and glasses. In addition several commercial supply houses now offer a variety of analyzed materials for method testing.<sup>4</sup>

### a1B STATISTICAL TREATMENT OF RANDOM ERRORS

Randomly distributed data of the kind described in the section labeled "random errors" are conveniently analyzed by the techniques of statistics, which are considered in the next several sections.<sup>5</sup>

#### a1B-1 Populations and Samples

In the statistical treatment of data, it is assumed that the handful of replicate experimental results obtained in the laboratory is a minute fraction of the infinite number of results that could in principle be obtained given infinite time and an infinite amount of sample. Statisticians call the handful of data a *sample* and view it as a subset of an infinite *population*, or *universe*, of data that in principle exists. The laws of statistics apply strictly to populations only; when applying these laws to a sample of laboratory data, it is necessary to assume that the sample is truly representative of the population. Because there is no assurance this assumption is valid, statements about random errors are necessarily uncertain and must be couched in terms of probabilities.

#### DEFINITION OF SOME TERMS USED IN STATISTICS

**Population Mean ( $\mu$ ).** The *population mean*, or *limiting mean*, of a set of replicate data is defined by the equation

$$\mu = \lim_{N \rightarrow \infty} \frac{\sum_{i=1}^N x_i}{N} \quad (\text{a1-6})$$

<sup>4</sup> See C. Veillon, *Anal. Chem.*, **1986**, *58*, 851A.

<sup>5</sup> For a more detailed treatment of statistics, see R. Calcutt and R. Boddy, *Statistics for Analytical Chemistry*. New York: Chapman and Hall, 1983; J. Mandel, in *Treatise on Analytical Chemistry*, 2nd ed., I. M. Kolthoff and P. J. Elving, Eds., Part I, Vol. 1, Chapter 5. New York: Wiley, 1978; J. K. Taylor, *Quality Assurance of Chemical Measurements*. Chelsea, Michigan: Lewis Publishers, Inc., 1987; and H. Mark and J. Workman, *Statistics in Spectroscopy*. San Diego: Academic Press, 1991.

where  $x_i$  represents the value of the  $i$ th measurement. As indicated by this equation, the mean of a set of measurements approaches the population mean as  $N$ , the number of measurements, approaches infinity. It is important to add that in the absence of bias,  $\mu$  is the true value for the quantity being measured.

**Population Standard Deviation ( $\sigma$ ) and the Population Variance ( $\sigma^2$ ).** The population standard deviation and the population variance provide statistically significant measures of the precision of a population of data. Thus,

$$\sigma = \sqrt{\lim_{N \rightarrow \infty} \frac{\sum_{i=1}^N (x_i - \mu)^2}{N}} \quad (\text{a1-7})$$

where  $x_i$  is again the value for the  $i$ th measurement. Note that the population standard deviation is the root mean square of the individual *deviations from the mean* for the population.

Statisticians prefer to express the precision of data in terms of variance, which is simply the square of the standard deviation ( $\sigma^2$ ), because variances combine additively. That is, if  $n$  independent sources of random error exist in a system, the total variance  $\sigma_t^2$  is given by the relationship

$$\sigma_t^2 = \sigma_1^2 + \sigma_2^2 + \dots + \sigma_n^2 \quad (\text{a1-8})$$

where  $\sigma_1^2, \sigma_2^2, \dots, \sigma_n^2$  are the individual variances.

Chemists generally prefer to describe the precision of measurements in terms of standard deviation rather than variance because the former carries the same units as the measurement itself.

**Sample Mean ( $\bar{x}$ ).** The sample mean is the mean, or average, of a finite set of data. Because  $N$  in this case is a finite number,  $\bar{x}$  often differs somewhat from the population mean  $\mu$ , and thus the true value, of the quantity being measured. The use of a different symbol in this case emphasizes this important distinction.

**Sample Standard Deviation ( $s$ ) and Sample Variance ( $s^2$ ).** The standard deviation ( $s$ ) for sample of data that is of limited size is given by the equation

$$s = \sqrt{\frac{\sum_{i=1}^N (x_i - \bar{x})^2}{N - 1}} \quad (\text{a1-9})$$

Note that the sample standard deviation differs in three ways from the population standard deviation as defined by Equation a1-7. First,  $\sigma$  is replaced by  $s$  in order to emphasize the difference between the two terms. Sec-

ond, the true mean  $\mu$  is replaced by  $\bar{x}$ , the sample mean. Finally,  $N - 1$ , which is defined as the *number of degrees of freedom*, appears in the denominator rather than  $N$ .<sup>6</sup>

**Relative Standard Deviation (RSD) and Coefficient of Variation (CV).** Relative standard deviations are often more informative than are absolute standard deviations. The relative standard deviation of a data sample is given by

$$\text{RSD} = \frac{s}{\bar{x}} \times 10^{\%} \quad (\text{a1-10})$$

When  $z = 2$ , the relative standard deviation is given as a percent; when it is 3, the deviation is reported in parts per thousand. In the former case, the relative standard deviation is also known as the *coefficient of variation* (CV) for the data. That is,

$$\text{CV} = \frac{s}{\bar{x}} \times 100\% \quad (\text{a1-11})$$

In dealing with a population of data,  $\sigma$  and  $\mu$  are used in place of  $s$  and  $\bar{x}$  in Equations a1-10 and a1-11.

**An Alternate Way of Calculating Sample Standard Deviations.** In calculating  $s$  with a handheld calculator that does not have a standard deviation function, the following algebraic identity to Equation a1-9 is somewhat more convenient to use:

$$s = \sqrt{\frac{\sum x_i^2 - (\sum x_i)^2/N}{N - 1}} \quad (\text{a1-12})$$

#### EXAMPLE a1-2

The following replicate data were obtained for the concentration of  $\text{SO}_2$  in the air near a paper mill: 1.96, 1.91, 1.88, and 1.94 parts per million (ppm). Calculate (a) the mean, (b) the absolute standard deviation, and (c) the coefficient of variation for the data.

<sup>6</sup> By definition, the degrees of freedom are the number of data that remain independent when  $s$  is evaluated. The standard deviation of a set of experimental data is calculated based upon  $(N - 1)$  degrees of freedom because the mean  $\bar{x}$  is used in the calculation. By substituting the mean and any subset of  $(N - 1)$  data into the algebraic equation for the mean, the numerical value of the one excluded datum can be calculated. The fact that the numerical value of any datum point can be calculated from the remaining data and the mean illustrates that one degree of freedom is lost anytime a mean is used in the calculation of any subsequent statistic.

	ppm $\text{SO}_2$	
	$x_i$	$x_i^2$
$x_1$	1.96	3.8416
$x_2$	1.91	3.6481
$x_3$	1.88	3.5344
$x_4$	1.94	3.7636
	$\Sigma x_i = 7.69$	$\Sigma x_i^2 = 14.7877$

(a)  $\bar{x} = 7.69/4 = 1.9225 = 1.92 \text{ ppm } \text{SO}_2$

(b) Applying Equation a1-12, we obtain

$$\begin{aligned} s &= \sqrt{\frac{14.7877 - (7.69)^2/4}{4 - 1}} \\ &= \sqrt{\frac{14.7877 - 14.784025}{3}} \\ &= \sqrt{\frac{0.003675}{3}} = 0.035 \text{ ppm } \text{SO}_2 \end{aligned}$$

(c)  $\text{CV} = \frac{0.035 \text{ ppm}}{1.92 \text{ ppm}} \times 100\% = 1.8\%$

Note that the difference between  $\Sigma x_i^2$  and  $(\Sigma x_i)^2/N$  in Example a1-2 is so small that premature rounding would have led to a serious error in the computed value of  $s$ . Because of this source of error, Equation a1-12 should never be used to calculate the standard deviation for numbers containing five or more digits; instead, Equation a1-9 should be used. It is also important to note that handheld calculators and small computers with a standard deviation function usually employ a version of Equation a1-12. Consequently, large errors in  $s$  are to be expected when these devices are applied to data having five or more significant figures.<sup>7</sup>

#### THE NORMAL ERROR LAW

In Gaussian statistics, the results of replicate measurements arising from indeterminate errors are assumed to be distributed according to the *normal error law*, which states that the fraction of a population of observations,  $dN/N$ , whose values lie in the region  $x$  to  $x + dx$  is given by

$$\frac{dN}{N} = \frac{1}{2\pi\sigma} e^{-(x-\mu)^2/2\sigma^2} dx \quad (\text{a1-13})$$

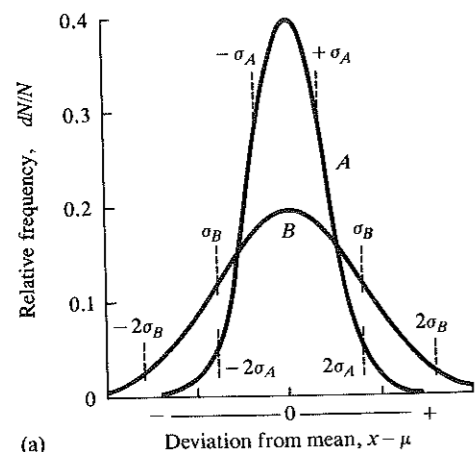
<sup>7</sup> See H. E. Solberg, *Anal. Chem.*, **1983**, *55*, 1661; and P. M. Wanek, et al., *Anal. Chem.*, **1982**, *54*, 1877.

Here,  $\mu$  and  $\sigma$  are the population mean and the standard deviation and  $N$  is the number of observations. The two curves shown in Figure a1-3a are plots of Equation a1-13. The standard deviation for the data in curve B is twice that for the data in curve A.

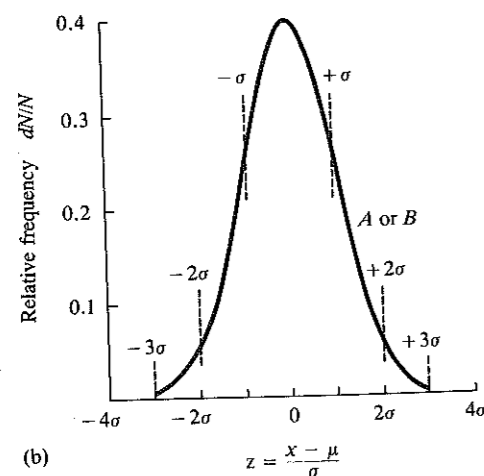
Note that  $(x - \mu)$  in Equation a1-13 is the *absolute deviation of the individual values of  $x$  from the mean* in whatever units are used in the measurement. It is, however, more convenient to express the deviations from the mean in units of standard deviation  $z$  where

$$z = (x - \mu)/\sigma \quad (a1-14)$$

Taking the derivative of this equation with respect to  $x$  gives



(a)



(b)

FIGURE a1-3 Normal error curves. The standard deviation for B is twice that for A, that is,  $\sigma_B = 2\sigma_A$ . (a) The abscissa is the deviation from the mean in the units of measurement. (b) The abscissa is the deviation from the mean in units of  $\sigma$ . Thus, A and B produce identical curves.

$$dz = dx/\sigma \quad (a1-15)$$

Substitution of these two relationships into Equation a1-13 leads to an equation that expresses the distribution in terms of the single variable  $z$ . That is,

$$\frac{dN}{N} = \frac{1}{2\pi} e^{-z^2/2} dz \quad (a1-16)$$

### THE NORMAL ERROR CURVE

Figure a1-3b shows another way of plotting the data for the two curves in Figure a1-3a. The abscissa is now  $z$ , the deviations from the mean of the data in units of standard deviation (Equation a1-14). This function yields a single curve regardless of the magnitude of the mean and standard deviation of the data. The general properties of this curve include (1) zero deviation from the mean occurring with maximum frequency, (2) symmetrical distribution of positive and negative deviations about this maximum, and (3) rapid decrease in frequency as the magnitude of the deviations increases. Thus, small random errors are much more common than large.

**Areas under Regions of the Normal Error Curve.** The area under the curve in Figure a1-3 is the integral of Equation a1-16. The fraction of the population with values of  $z$  between any specified limits is given by the area under the curve between these limits. For example, the area under the curve between  $z = -1\sigma$  and  $z = +1\sigma$  is 0.683, or 68.3% of the total area under the curve. We may therefore conclude that 68.3% of a population of data lies within  $\pm 1\sigma$  of the mean value. Furthermore, 95.5% lies within  $\pm 2\sigma$  and 99.7% within  $\pm 3\sigma$ . Values for  $x - \mu$  corresponding to  $\pm 1\sigma$ ,  $\pm 2\sigma$ , and  $\pm 3\sigma$  are indicated by broken vertical lines in Figure a1-3.

The properties of the normal error curve are useful because they permit statements to be made about the probable magnitude of the net random error in a given measurement or set of measurements *provided the standard deviation is known*. Thus, one can say that the chances are 68.3 out of 100 that the random error associated with any single measurement is smaller than  $\pm 1\sigma$ , that the chances are 95.5 out of 100 that the error is less than  $\pm 2\sigma$ , and so forth. Clearly, the standard deviation is a useful parameter for estimating and reporting the probable net random error for an analytical method.

**Standard Error of a Mean.** The figures on percentage distribution just quoted refer to the probable error of a *single* measurement. If a series of samples, each containing  $N$  data, are taken randomly from a pop-

ulation of data, the mean of each set will show less and less scatter as  $N$  increases. The standard deviation of each mean is known as the *standard error* of the mean and is given the symbol  $s_m$ . It can be shown that the standard error is inversely proportional to the square root of the number of data used to calculate the mean. That is,

$$s_m = s/\sqrt{N} \quad (a1-17)$$

The mean and the standard deviation for a set of data are statistics of primary importance in all types of science and engineering. The mean is important because it usually provides the best estimate of the parameter of interest. The standard deviation of the mean is equally important because it provides information about the precision and thus the random error associated with the mean.

### METHOD FOR OBTAINING A GOOD APPROXIMATION OF $\sigma$

In order to apply a statistical relationship directly to finite samples of data, it is necessary to know that the sample standard deviation  $s$  for the data is a good approximation of the population standard deviation  $\sigma$ . Otherwise, statistical inferences must be modified to take into account the uncertainty in  $s$ . In this section, we consider methods for obtaining reliable values for  $s$  from small samples of experimental data.

**Performing Preliminary Experiments.** Uncertainties in the calculated value for  $s$  decrease as the number of measurements  $N$  in Equation a1-9 increases. Figure a1-4 shows the error in  $s$  as a function of  $N$ . Note that when  $N$  is greater than about 20,  $s$  and  $\sigma$  can

be assumed, for most purposes, to be identical. Thus, when a method of measurement is not excessively time-consuming and when an adequate supply of sample is available, it is sometimes feasible and economic to carry out preliminary experiments whose sole purpose is that of obtaining a reliable standard deviation for the method.

**Pooling Data.** For analyses that are time-consuming, the foregoing procedure is seldom practical. In such cases, however, precision data from a series of similar samples accumulated in the course of time can be pooled to provide an estimate of  $s$  that is superior to the value for any individual subset. Again, one must assume the same sources of random error are present in all the samples. This assumption is usually valid provided the samples have similar compositions and each has been analyzed identically. To obtain a pooled estimate of  $s$ , deviations from the mean for each subset are squared; the squares for all of the subsets are then summed and divided by an appropriate number of degrees of freedom. The pooled  $s$  is obtained by extracting the square root of the quotient. One degree of freedom is lost in obtaining the mean for each subset. Thus, the number of degrees of freedom for the pooled  $s$  is equal to the total number of measurements minus the number of subsets. An example of this calculation follows.

### EXAMPLE a1-3

The mercury in samples of seven fish taken from the Mississippi River was determined by a method based upon the absorption of radiation by gaseous elemental mercury. Calculate a pooled estimate of the standard deviation for the method, based upon the first three columns of data in the table that follows:

Specimen Number	Number of Samples Measured	Hg Content, ppm	Mean, ppm Hg	Sum of Squares of Deviations from Means
1	3	1.80, 1.58, 1.64	1.673	0.0259
2	4	0.96, 0.98, 1.02, 1.10	1.015	0.0115
3	2	3.13, 3.35	3.240	0.0242
4	6	2.06, 1.93, 2.12, 2.16, 1.89, 1.95	2.018	0.0611
5	4	0.57, 0.58, 0.64, 0.49	0.570	0.0114
6	5	2.35, 2.44, 2.70, 2.48, 2.44	2.482	0.0685
7	4	1.11, 1.15, 1.22, 1.04	1.130	0.0170
Number of measurements 28			Sum of sum of squares = 0.2196	

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

$x_i$	$ (x_i - \bar{x}) $	$(x_i - \bar{x})^2$
1.80	0.127	0.0161
1.58	0.093	0.0087
1.64	0.033	0.0011
5.02		Sum of squares = 0.0259
$\bar{x} = \frac{5.02}{3} = 1.673$		

The other data in columns 4 and 5 were obtained similarly. Then

$$s_{\text{pooled}} = \sqrt{\frac{0.2196}{28 - 7}} = 0.102 \text{ ppm Hg}$$

Note that in Example a1-3 one degree of freedom was lost for each of the seven samples. Because the remaining degrees of freedom are greater than 20, however, the computed  $s$  can be considered to be a good approximation of  $\sigma$ , and we may assume that  $s \rightarrow \sigma$ .

### a1B-2 Confidence Limits (CL)

The true or population mean ( $\mu$ ) of a measurement is a constant that must always remain unknown. However, in the absence of systematic errors, limits can be

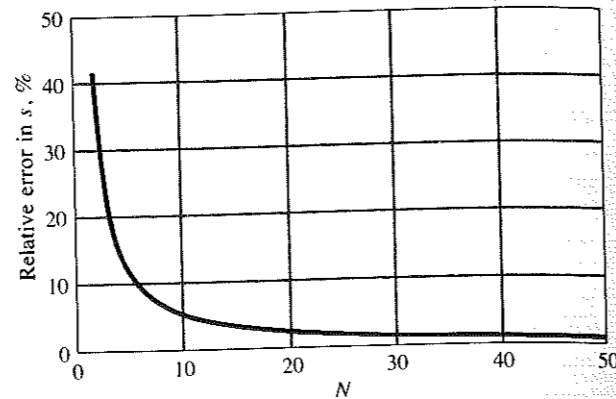


FIGURE a1-4 Relative error in  $s$  as a function of  $N$ .

set within which the population mean can be expected to lie with a given degree of probability. The limits obtained in this manner are called *confidence limits*.

The confidence limit, which is derived from the sample standard deviation, depends upon the certainty with which  $s$  is known. If there is reason to believe that  $s$  is a good approximation of  $\sigma$ , then the confidence limits can be significantly narrower than if the estimate of  $s$  is based upon only two or three measurements.

#### CONFIDENCE LIMIT WHEN $s$ IS A GOOD APPROXIMATION OF $\sigma$

Figure a1-5 is a normal error curve in which the abscissa is the quantity  $z$ , which represents the deviation from the mean in units of the population standard deviation

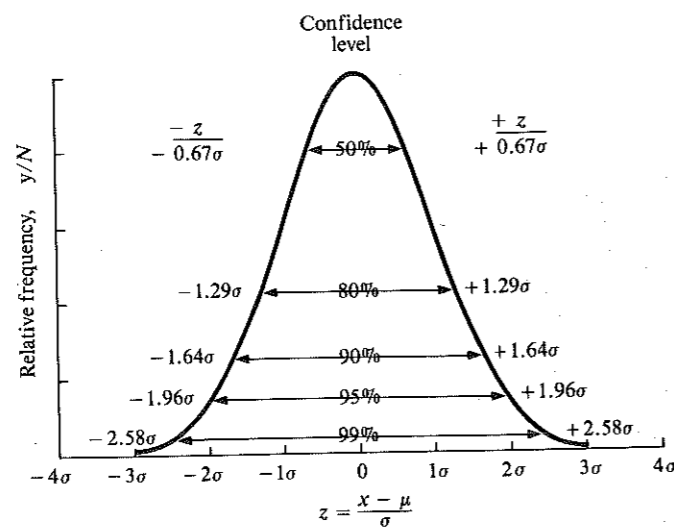


FIGURE a1-5 Confidence levels for various values of  $z$ .

(Equation a1-14). The column of numbers in the center of the figure gives the percent of the total area under the curve that is encompassed by the indicated values of  $-z$  and  $+z$ . For example, 50% of the area under any Gaussian curve lies between  $-0.67\sigma$  and  $+0.67\sigma$ ; 80% of the area lies between  $-1.29\sigma$  and  $+1.29\sigma$ . From the latter limits then, we may assert, with 80 chances out of 100 being correct, that the population mean lies within  $\pm 1.29\sigma$  of any single measurement we make. Here, the *confidence level* is 80% and the *confidence interval* is  $\pm z\sigma = \pm 1.29\sigma$ . A general statement for the confidence limit (CL) of a single measurement is obtained by rearranging Equation a1-14, remembering that  $z$  can take positive or negative values. Thus,

$$\text{CL for } \mu = x \pm z\sigma \quad (\text{a1-18})$$

Equation a1-18 applies to the result of a single measurement. Application of Equation a1-17 shows the confidence interval is decreased by  $\sqrt{N}$  for the average of  $N$  replicate measurements. Thus, a more general form of Equation a1-18 is

$$\text{CL for } \mu = \bar{x} \pm \frac{z\sigma}{\sqrt{N}} \quad (\text{a1-19})$$

Values for  $z$  at various confidence levels are found in Table a1-3.

#### EXAMPLE a1-4

Calculate the 50% and the 95% confidence limits for the mean value (1.67 ppm Hg) for specimen 1 in Example a1-3. Again,  $s \approx \sigma = 0.10$ .

Applying Equation a1-19 to the three measurements yields

$$\begin{aligned} 50\% \text{ CL} &= 1.67 \pm \frac{0.67 \times 0.10}{\sqrt{3}} \\ &= 1.67 \pm 0.04 \\ 95\% \text{ CL} &= 1.67 \pm \frac{1.96 \times 0.10}{\sqrt{3}} \\ &= 1.67 \pm 0.11 \end{aligned}$$

From Example a1-4, we conclude that there is a 50% chance that  $\mu$ , the population mean (and *in the absence of systematic error* the true value), will lie between the limits of 1.63 and 1.71 ppm Hg. Furthermore, there is a 95% chance that  $\mu$  will be found between 1.56 and 1.78 ppm Hg.

#### EXAMPLE a1-5

How many replicate measurements of specimen 1 in Example a1-3 would be needed to decrease the 95% confidence interval to  $\pm 0.07$  ppm Hg?

The pooled value for  $s$  is a good estimate for  $\sigma$ . For a confidence interval of  $\pm 0.07$  ppm Hg, substitution into Equation a1-19 leads to

$$\begin{aligned} 0.07 &= \pm \frac{zs}{\sqrt{N}} = \pm \frac{1.96 \times 0.10}{\sqrt{N}} \\ \sqrt{N} &= \pm \frac{1.96 \times 0.10}{0.07} = 2.80 \\ N &= 7.8 \end{aligned}$$

We conclude, then, that 8 measurements would provide a slightly better than 95% chance of the population mean lying within  $\pm 0.07$  ppm of the experimental mean.

A consideration of Equation a1-19 indicates that the confidence interval for an analysis can be halved by employing the mean of four measurements. Sixteen measurements would be required to narrow the limit by another factor of two. It is apparent that a point of diminishing return is rapidly reached in acquiring additional data. Thus, the chemist ordinarily takes advantage of the relatively large gain afforded by averaging two to four measurements but can seldom afford the time required for further increases in confidence.

In data analysis, it is essential to keep in mind always that confidence limits based on Equation a1-19 apply only *in the absence of systematic errors*.

TABLE a1-3 Confidence Levels for Various Values of  $z$

Confidence Level, %	$z$	Confidence Level, %	$z$
50	0.67	96	2.00
68	1.00	99	2.58
80	1.29	99.7	3.00
90	1.64	99.9	3.29
95	1.96		

**CONFIDENCE LIMITS WHEN  $\sigma$  IS UNKNOWN**

Frequently, a chemist must make use of an unfamiliar method wherein limitations in time or amount of available sample preclude an accurate estimation of  $\sigma$ . Here, a single set of replicate measurements must provide not only a mean but also a precision estimate. As indicated earlier,  $s$  calculated from a small set of data may be subject to considerable uncertainty; thus, confidence limits must be broadened when a good estimate of  $\sigma$  is unavailable.

To account for the potential variability of  $s$ , use is made of the statistical parameter  $t$ , which is defined as

$$t = (x - \mu)/s \quad (a1-20)$$

Note the similarity between Equations a1-20 and a1-14. In contrast to  $z$  in Equation a1-14,  $t$  is dependent not only on the desired confidence level but also upon the number of degrees of freedom available in the calculation of  $s$ . Table a1-4 provides values for  $t$  for a few

TABLE a1-4  
Values of  $t$  for Various Levels of Probability

Degrees of Freedom	Factor for Confidence Interval, %				
	80	90	95	99	99.9
1	3.08	6.31	12.7	63.7	637
2	1.89	2.92	4.30	9.92	31.6
3	1.64	2.35	3.18	5.84	12.9
4	1.53	2.13	2.78	4.60	8.60
5	1.48	2.02	2.57	4.03	6.86
6	1.44	1.94	2.45	3.71	5.96
7	1.42	1.90	2.36	3.50	5.40
8	1.40	1.86	2.31	3.36	5.04
9	1.38	1.83	2.26	3.25	4.78
10	1.37	1.81	2.23	3.17	4.59
11	1.36	1.80	2.20	3.11	4.44
12	1.36	1.78	2.18	3.06	4.32
13	1.35	1.77	2.16	3.01	4.22
14	1.34	1.76	2.14	2.98	4.14
$\infty$	1.29	1.64	1.96	2.58	3.29

degrees of freedom; more extensive tables are found in most mathematical handbooks. Note that the values for  $t$  become equal to those for  $z$  (Table a1-3) as the number of degrees of freedom becomes infinite.

The confidence limit for the mean  $\bar{x}$  of  $N$  replicate measurements can be derived from  $t$  by an equation analogous to Equation a1-19; that is,

$$CL \text{ for } \mu = \bar{x} \pm \frac{ts}{\sqrt{N}} \quad (a1-21)$$

**EXAMPLE a1-6**

A chemist obtained the following data for the alcohol content of a sample of blood; percentage of ethanol = 0.084, 0.089, and 0.079. Calculate the 95% confidence limit for the mean assuming (a) no additional knowledge about the precision of the method and (b) that on the basis of previous experiences it is known that  $s \rightarrow \sigma = 0.006\%$  ethanol.

(a)  $\sum x_i = 0.084 + 0.089 + 0.079 = 0.252$   
 $\sum x_i^2 = 0.0070566 + 0.007921 + 0.006241 = 0.021218$

$$s = \sqrt{\frac{0.021218 - (0.252)^2/3}{3 - 1}} = 0.0050$$

Here,  $\bar{x} = 0.252/3 = 0.084$ . Table a1-4 indicates that  $t = \pm 4.30$  for two degrees of freedom and 95% confidence. Thus;

$$\begin{aligned} 95\% \text{ CL} &= \bar{x} \pm \frac{ts}{\sqrt{N}} \\ &= 0.084 \pm \frac{4.3 \times 0.0050}{\sqrt{3}} \\ &= 0.084 \pm 0.012 \end{aligned}$$

(b) Because a good value of  $\sigma$  is available

$$\begin{aligned} 95\% \text{ CL} &= \bar{x} \pm \frac{z\sigma}{\sqrt{N}} \\ &= 0.084 \pm \frac{1.96 \times 0.006}{\sqrt{3}} \\ &= 0.084 \pm 0.007 \end{aligned}$$

Note from Example a1-6 that a sure knowledge of  $\sigma$  nearly halves the confidence interval.

**a1B-3 Test for Bias**

As noted in Section a1A-2, bias in an analytical method is generally detected by the analysis of one or

more standard reference materials whose composition is known. In all probability, the experimental mean of such an analysis  $\bar{x}$  will differ from the true value  $\mu$  supplied for the standard. In this case, a judgment must be made whether this difference is the consequence of random error in the analysis of the reference material or of bias in the method used.

A common way of treating this problem statistically is to compare the experimental difference  $\bar{x} - \mu$  with the difference that could be expected at a certain probability level if no bias existed. If the experimental  $\bar{x} - \mu$  is larger than the calculated difference, bias is likely. If, on the other hand, the experimental value is equal to or smaller than the computed difference, the presence of bias has not been demonstrated.

This test for bias makes use of the  $t$  statistics discussed earlier. Here we rearrange Equation a1-21 to give

$$\bar{x} - \mu = \pm \frac{ts}{\sqrt{N}} \quad (a1-22)$$

where  $N$  is the number of replicate measurements employed in the test. (If a good estimate of  $\sigma$  is available, the equation can be modified by replacing  $t$  with  $z$  and  $s$  with  $\sigma$ .) If the experimental value of  $\bar{x} - \mu$  is larger than the value of  $\bar{x} - \mu$  calculated from Equation a1-22, the presence of bias in the method is suggested. If, on the other hand, the value calculated using Equation a1-22 is larger, no bias has been demonstrated.

**EXAMPLE a1-7**

A new procedure for the rapid determination of sulfur in kerosenes was tested on a sample known from its method of preparation to contain 0.123% S. The results were % S = 0.112, 0.118, 0.115, and 0.119. Do the data indicate the presence of bias in the method?

$$\sum x_i = 0.112 + 0.118 + 0.115 + 0.119 = 0.464$$

$$\bar{x} = 0.464/4 = 0.116\% \text{ S}$$

$$\bar{x} - \mu = 0.116 - 0.123 = -0.007\% \text{ S}$$

$$\sum x_i^2 = 0.012544 + 0.013924 + 0.013225 + 0.014161 = 0.053854$$

$$\begin{aligned} s &= \sqrt{\frac{0.053854 - (0.464)^2/4}{4 - 1}} \\ &= \sqrt{\frac{0.000030}{3}} = 0.0032 \end{aligned}$$

From Table a1-4, we find that at the 95% confidence level,  $t$  has a value of 3.18 for three degrees of freedom. Thus,

$$\frac{ts}{\sqrt{4}} = \frac{3.18 \times 0.0032}{\sqrt{4}} = \pm 0.0051$$

An experimental mean can be expected to deviate by  $\pm 0.0051$  or greater no more frequently than 5 times in 100. Thus, if we conclude that  $\bar{x} - \mu = -0.007$  is a significant difference and that bias is present, we will, on the average, be wrong fewer than 5 times in 100.

If we make a similar calculation employing the value for  $t$  at the 99% confidence level,  $ts/\sqrt{N}$  assumes a value of 0.0093. Thus, if we insist upon being wrong no more often than 1 time in 100, we must conclude that no bias has been demonstrated. Note that this statement is different from saying that no bias exists.

**EXAMPLE a1-8**

Suppose we know from past experience that the method described in Example a1-7 had a population standard deviation of 0.0032% S. That is,  $s \rightarrow \sigma = 0.0032$ . Is the presence of bias suggested at the 99% confidence level?

Here we write that

$$\begin{aligned} \bar{x} - \mu &= \pm \frac{z\sigma}{\sqrt{N}} \\ &= \pm \frac{2.58 \times 0.0032}{\sqrt{4}} = \pm 0.00413 \end{aligned}$$

The experimental difference of  $-0.007$  is significantly larger than this number. Thus, bias is strongly suggested.

**a1B-4 Propagation of Measurement Uncertainties**

A typical instrumental method of analysis involves several experimental measurements, each of which is subject to an indeterminate uncertainty and each of which contributes to the net indeterminate error of the final result. For the purpose of showing how such indeterminate uncertainties affect the outcome of an analysis, let us assume that a result  $x$  is dependent upon the experimental variables,  $p, q, r, \dots$ , each of which

fluctuates in a random and independent way. That is,  $x$  is a function of  $p, q, r, \dots$ , so we may write

$$x = f(p, q, r, \dots) \quad (a1-23)$$

The uncertainty  $dx_i$  (the deviation from the mean) in the  $i$ th measurement of  $x$  will depend upon the size and sign of the corresponding uncertainties  $dp_i, dq_i, dr_i, \dots$ , and we may write

$$dx_i = f(dp_i, dq_i, dr_i, \dots)$$

The variation in  $dx$  as a function of the uncertainties in  $p, q, r, \dots$  can be derived by taking the total differential of Equation a1-23. That is,

$$dx = \left(\frac{\partial x}{\partial p}\right) dp + \left(\frac{\partial x}{\partial q}\right) dq + \left(\frac{\partial x}{\partial r}\right) dr + \dots \quad (a1-24)$$

In order to develop a relationship between the standard deviation of  $x$  and the standard deviations of  $p, q$ , and  $r$ , it is necessary to square the foregoing equation. In doing so, we will drop the subscripts associated with all partial derivatives. Thus,

$$(dx)^2 = \left[ \left(\frac{\partial x}{\partial p}\right)^2 dp^2 + \left(\frac{\partial x}{\partial q}\right)^2 dq^2 + \left(\frac{\partial x}{\partial r}\right)^2 dr^2 + \dots \right]^2 \quad (a1-25)$$

This equation must then be summed between the limits of  $i = 1$  to  $i = N$ , where  $N$  again is the total number of replicate measurements.

In squaring Equation a1-24, two types of terms from the right-hand side of the equation emerge: (1) square terms and (2) cross terms. Square terms take the form

$$\left(\frac{\partial x}{\partial p}\right)^2 dp^2, \left(\frac{\partial x}{\partial q}\right)^2 dq^2, \left(\frac{\partial x}{\partial r}\right)^2 dr^2, \dots$$

Square terms are always positive and can, therefore, never cancel. In contrast, cross terms may be either positive or negative in sign. Examples are

$$\left(\frac{\partial x}{\partial p}\right)\left(\frac{\partial x}{\partial q}\right) dp dq, \left(\frac{\partial x}{\partial p}\right)\left(\frac{\partial x}{\partial r}\right) dp dr, \dots$$

If  $dp, dq$ , and  $dr$  represent independent and random uncertainties, some of the cross terms will be negative and others positive. Thus, the summation of all such

terms should approach zero, particularly when  $N$  is large.<sup>8</sup>

As a consequence of the canceling tendency of cross terms, the sum of Equation a1-25 from  $i = 1$  to  $i = N$  can be assumed to be made up exclusively of square terms. This sum then takes the form

$$\Sigma(dx_i)^2 = \left(\frac{\partial x}{\partial p}\right)^2 \Sigma(dp_i)^2 + \left(\frac{\partial x}{\partial q}\right)^2 \Sigma(dq_i)^2 + \left(\frac{\partial x}{\partial r}\right)^2 \Sigma(dr_i)^2 + \dots \quad (a1-26)$$

Dividing through by  $N$  gives

$$\frac{\Sigma(dx_i)^2}{N} = \left(\frac{\partial x}{\partial p}\right)^2 \frac{\Sigma(dp_i)^2}{N} + \left(\frac{\partial x}{\partial q}\right)^2 \frac{\Sigma(dq_i)^2}{N} + \left(\frac{\partial x}{\partial r}\right)^2 \frac{\Sigma(dr_i)^2}{N} + \dots \quad (a1-27)$$

From Equation a1-7, however, we see that

$$\frac{\Sigma(dx_i)^2}{N} = \frac{\Sigma(x_i - \mu)^2}{N} = \sigma_x^2$$

where  $\sigma_x^2$  is the variance of  $x$ . Similarly,

$$\frac{\Sigma(dp_i)^2}{N} = \sigma_p^2$$

and so forth. Thus, Equation a1-27 can be written in terms of the variances of the quantities; that is,

$$\sigma_x^2 = \left(\frac{\partial x}{\partial p}\right)^2 \sigma_p^2 + \left(\frac{\partial x}{\partial q}\right)^2 \sigma_q^2 + \left(\frac{\partial x}{\partial r}\right)^2 \sigma_r^2 + \dots \quad (a1-28)$$

The example that follows illustrates how Equation a1-28 is employed to give the variance of a quantity calculated from several experimental data.

#### EXAMPLE a1-9

The number of plates  $N$  in a chromatographic column can be computed with Equation 24-17 (Chapter 24):

$$N = 16 \left(\frac{t_R}{W}\right)^2$$

<sup>8</sup> If the variables are not independent, the cross terms must be kept regardless of the size of  $N$ . See S. L. Meyer, *Data Analysis for Scientists and Engineers*. New York: Wiley, 1975.

where  $t_R$  is the retention time and  $W$  is the width of the chromatographic peak in the same units as  $t_R$ . The significance of these terms is explained in Figure 24-6.

Hexachlorobenzene exhibited a high-performance liquid chromatographic peak at a retention time of 13.36 min. The width of the peak at its base was 2.18 min. The standard deviation  $s$  for the two time measurements was 0.043 and 0.061 min, respectively. Calculate (a) the number of plates in the column and (b) the standard deviation for the computed result.

(a)  $N = 16 \left(\frac{13.36 \text{ min}}{2.18 \text{ min}}\right)^2 = 601 \text{ plates}$

(b) Substituting  $s$  for  $\sigma$  in Equation a1-28 gives

$$s_N^2 = \left(\frac{\partial N}{\partial t_R}\right)_W^2 s_{t_R}^2 + \left(\frac{\partial N}{\partial W}\right)_{t_R}^2 s_W^2$$

Taking partial derivatives of the original equation

$$\left(\frac{\partial N}{\partial t_R}\right)_W = \frac{32t_R}{W^2} \quad \text{and} \quad \left(\frac{\partial N}{\partial W}\right)_{t_R} = \frac{-32t_R^2}{W^3}$$

Substituting these relationships into the previous equation gives

$$\begin{aligned} s_N^2 &= \left(\frac{32t_R}{W^2}\right)^2 s_{t_R}^2 + \left(\frac{-32t_R^2}{W^3}\right)^2 s_W^2 \\ &= \left(\frac{32 \times 13.36 \text{ min}}{(2.18 \text{ min})^2}\right)^2 (0.061 \text{ min})^2 \\ &\quad + \left(\frac{-32(13.36 \text{ min})^2}{(2.18 \text{ min})^3}\right)^2 (0.043 \text{ min})^2 \\ &= 592.1 \end{aligned}$$

$$s_N = \sqrt{592.1} = 24.3 = 24 \text{ plates}$$

$$\text{Thus, } N = 6.0 (\pm 0.2) \times 10^2 \text{ plates}$$

#### a1B-5 Rounding Results from Arithmetic Calculations

Equation a1-28 is helpful in deciding how the results of arithmetical calculations should be rounded. For example, consider the case where the result  $x$  is computed by the relationship

$$x = p + q - r$$

where  $p, q$ , and  $r$  are experimental quantities having sample standard deviations of  $s_p, s_q$ , and  $s_r$ , respectively.

Applying Equation a1-18 (using sample rather than population standard deviations) gives

$$s_x^2 = \left(\frac{\partial x}{\partial p}\right)_{q,r}^2 s_p^2 + \left(\frac{\partial x}{\partial q}\right)_{p,r}^2 s_q^2 + \left(\frac{\partial x}{\partial r}\right)_{p,q}^2 s_r^2$$

But,

$$\left(\frac{\partial x}{\partial p}\right)_{q,r} = \left(\frac{\partial x}{\partial q}\right)_{p,r} = 1 \quad \text{and} \quad \left(\frac{\partial x}{\partial r}\right)_{p,q} = -1$$

Therefore, the variance of  $x$  is given by

$$s_x^2 = (1)^2 s_p^2 + (1)^2 s_q^2 + (-1)^2 s_r^2$$

or the standard deviation of the result is given by

$$s_x = \sqrt{s_p^2 + s_q^2 + s_r^2}$$

Thus, the absolute standard deviation of a sum or difference is equal to the square root of the sum of the squares of the absolute standard deviation of the numbers making up the sum or difference.

Proceeding in this same way yields the relationships shown in column 3 of Table a1-5 for other types of arithmetic operations. Note that in several calculations, relative variances such as  $(s_x/x)^2$  and  $(s_p/p)^2$  are combined rather than absolute standard deviations.

#### EXAMPLE a1-10

Calculate the standard deviation of the result of

$$\frac{[14.3(\pm 0.2) - 11.6(\pm 0.2)] \times 0.050(\pm 0.001)}{[820(\pm 10) + 1030(\pm 5)] \times 42.3(\pm 0.4)} = 1.725(\pm ?) \times 10^{-6}$$

where the numbers in parentheses are absolute standard deviations. First we must calculate the standard deviation of the sum and the difference. The standard deviation  $s_p$  for the difference in the numerator is given by

$$s_p = \sqrt{(\pm 0.2)^2 + (\pm 0.2)^2} = \pm 0.283$$

For the sum in the denominator, the standard deviation  $s_q$  is

$$s_q = \sqrt{(\pm 10)^2 + (\pm 5)^2} = \pm 11.2$$

We may then rewrite the equation as

$$\frac{2.7(\pm 0.283) \times 0.050(\pm 0.001)}{1850(\pm 11.2) \times 42.3(\pm 0.4)}$$

$$= 1.725(\pm ?) \times 10^{-6}$$



The equation now contains only products and quotients, and Equation (2) of Table a1-5 applies:

$$\frac{s_x}{x} = \sqrt{\left(\frac{\pm 0.283}{2.7}\right)^2 + \left(\frac{\pm 0.001}{0.050}\right)^2 + \left(\frac{\pm 11.2}{1850}\right)^2 + \left(\frac{\pm 0.4}{42.3}\right)^2} = \pm 0.107$$

To obtain the absolute standard deviation, we write  $s_x = \pm 0.107 x = \pm 0.0107(1.725 \times 10^{-6}) = \pm 0.185 \times 10^{-6}$  and the answer is rounded to  $1.7 (\pm 0.2) \times 10^{-6}$ .

**EXAMPLE a1-11**

Calculate the absolute deviations of the results of the following computations. The absolute standard deviation for each quantity is given in parentheses.

- (a)  $x = \log [2.00 (\pm 0.02) \times 10^{-4}] = -3.6990 \pm ?$
- (b)  $x = \text{antilog} [1.200 (\pm 0.003)] = 15.849 \pm ?$
- (c)  $x = \text{antilog} [45.4 (\pm 0.3)] = 2.5119 \times 10^{45} \pm ?$

(a) Referring to Equation (4) in Table a1-5 we see

$$s_x = \pm 0.434 \times \frac{0.02 \times 10^{-4}}{2.00 \times 10^{-4}} = \pm 0.004$$

Thus,

$$\log [2.00 (\pm 0.02) \times 10^{-4}] = -3.699 (\pm 0.004)$$

(b) Employing Equation (5) in Table a1-5, we obtain

$$\frac{s_x}{x} = 2.303 \times (\pm 0.003) = \pm 0.0069$$

$$s_x = \pm 0.0069x = \pm 0.0069 \times 15.849 = 0.109$$

Therefore,

$$\text{antilog} [1.200 (\pm 0.003)] = 15.8 \pm 0.1$$

(c)  $\frac{s_x}{x} = 2.303(\pm 0.3) = \pm 0.691$

$$s_x = \pm 0.691 \times 2.511 \times 10^{45} = \pm 1.7 \times 10^{45}$$

Therefore,

$$\text{antilog} [45.4 (\pm 0.3)] = 2.5 (\pm 1.7) \times 10^{45}$$

Example a1-11c demonstrates that a large absolute error is associated with the antilogarithm of a number with few digits beyond the decimal point. This large

uncertainty arises from the fact that the numbers to the left of the decimal (the characteristic) serve only to locate the decimal point. The large error in the antilogarithm results from the relatively large uncertainty in the mantissa of the number (that is,  $0.4 \pm 0.3$ ).

**a1C METHOD OF LEAST SQUARES**

Most analytical methods are based upon a calibration curve in which a measured quantity  $y$  is plotted as a function of the known concentration  $x$  of a series of standards. Figure a1-6 shows a typical calibration curve, which was derived for the chromatographic determination of isooctane in hydrocarbon samples. The ordinate (the dependent variable) is the area under the chromatographic peak for isooctane, and the abscissa (the independent variable) is the mole percent of isooctane. As is typical (and desirable), the plot approximates a straight line. Note, however, that because of the random errors in the measuring process, not all the data fall exactly on the line. Thus, we must try to fit a "best" straight line through the points. A common way of finding such a line is the *method of least squares*.

In applying the method of least squares, we assume that there is a linear relationship between the area of

the peaks ( $y$ ) and the analyte concentration ( $x$ ) as given by the equation

$$y = mx + b$$

where  $m$  is the slope of the straight line and  $b$  is the intercept. We also assume that any deviation of individual points from the straight line results from error in the area measurement and that there is no error in the values of  $x$ —that is, the concentrations of the standard solutions are known exactly.

As illustrated in Figure a1-5, the vertical deviation of each point from the straight line is called a *residual*. The line generated by the least-squares method is the one that minimizes the sum of the squares of the residuals for all of the points.

For convenience, we define three quantities  $S_{xx}$ ,  $S_{yy}$ , and  $S_{xy}$  as follows:

$$S_{xx} = \sum(x_i - \bar{x})^2 = \sum x_i^2 - \frac{(\sum x_i)^2}{N} \quad (\text{a1-29})$$

$$S_{yy} = \sum(y_i - \bar{y})^2 = \sum y_i^2 - \frac{(\sum y_i)^2}{N} \quad (\text{a1-30})$$

$$S_{xy} = \sum(x_i - \bar{x})(y_i - \bar{y}) = \sum x_i y_i - \frac{\sum x_i \sum y_i}{N} \quad (\text{a1-31})$$

Here  $x_i$  and  $y_i$  are the coordinates of the individual data points,  $N$  is the number of pairs of data used in preparation of the calibration curve, and  $\bar{x}$  and  $\bar{y}$  are the average values for the variables, or

$$\bar{x} = \sum x_i / N \quad \text{and} \quad \bar{y} = \sum y_i / N$$

Note that  $S_{xx}$  and  $S_{yy}$  are the sum of the squares of the deviations from the mean for the individual values of  $x$  and  $y$ . The equivalent expressions shown to the far right in Equations a1-29, a1-30, and a1-31 are more convenient when a handheld calculator is being used.

Six useful quantities can be computed from  $S_{xx}$ ,  $S_{yy}$ , and  $S_{xy}$ .

1. The slope of the line  $m$ :

$$m = S_{xy} / S_{xx} \quad (\text{a1-32})$$

2. The intercept  $b$ :

$$b = \bar{y} - m\bar{x} \quad (\text{a1-33})$$

3. The standard deviation  $s_y$  of the residuals, which is given by:

TABLE a1-5 Error Propagation in Arithmetic Calculations

Type of Calculation	Example*	Standard Deviation of $x$	
Addition or Subtraction	$x = p + q - r$	$s_x = \sqrt{s_p^2 + s_q^2 + s_r^2}$	(1)
Multiplication or Division	$x = p \cdot q/r$	$\frac{s_x}{x} = \sqrt{\left(\frac{s_p}{p}\right)^2 + \left(\frac{s_q}{q}\right)^2 + \left(\frac{s_r}{r}\right)^2}$	(2)
Exponentiation	$x = p^y$	$\frac{s_x}{x} = y \frac{s_p}{p}$	(3)
Logarithm	$x = \log_{10} p$	$s_x = 0.434 \frac{s_p}{p}$	(4)
Antilogarithm	$x = \text{antilog}_{10} p$	$\frac{s_x}{x} = 2.303 s_p$	(5)

\*  $p$ ,  $q$ , and  $r$  are experimental variables whose standard deviations are  $s_p$ ,  $s_q$ , and  $s_r$ , respectively;  $y$  is a constant.

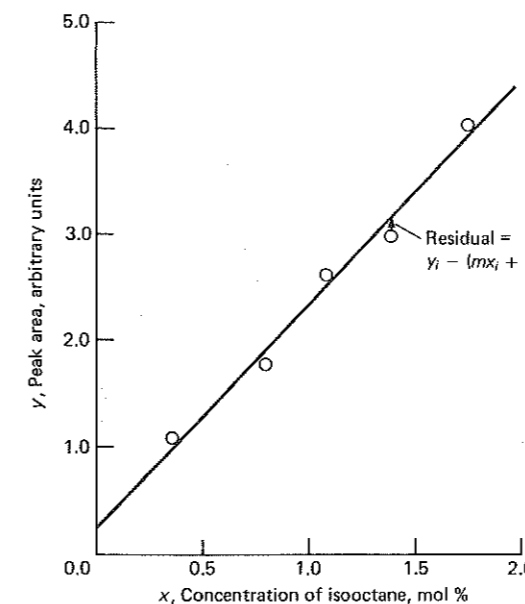


FIGURE a1-6 Calibration curve for determining isooctane in hydrocarbon mixtures.

$$s_y = \sqrt{\frac{S_{yy} - m^2 S_{xx}}{N - 2}} \quad (\text{a1-34})$$

4. The standard deviation of the slope  $s_m$ :

$$s_m = s_y / \sqrt{S_{xx}} \quad (\text{a1-35})$$

5. The standard deviation  $s_b$  of the intercept:

$$s_b = s_y \sqrt{\frac{\sum x_i^2}{N \sum x_i^2 - (\sum x_i)^2}} \\ = s_y \sqrt{\frac{1}{N - (\sum x_i)^2 / \sum x_i^2}} \quad (\text{a1-36})$$

6. The standard deviation  $s_c$  for analytical results obtained with the calibration curve:

$$s_c = \frac{s_y}{m} \sqrt{\frac{1}{L} + \frac{1}{N} + \frac{(\bar{y}_c - \bar{y})^2}{m^2 S_{xx}}} \quad (\text{a1-37})$$

Equation a1-37 permits the calculation of the standard deviation of the mean  $\bar{y}_c$  of a set of  $L$  replicate analyses when a calibration curve that contains  $N$  points is used; recall that  $\bar{y}$  is the mean value of  $y$  for the  $N$  calibration data.

#### EXAMPLE a1-12

Carry out a least-squares analysis of the experimental data provided in the first two columns in Table a1-6 and plotted in Figure a1-6.

Columns 3, 4, and 5 of the table contain computed values for  $x_i^2$ ,  $y_i^2$ , and  $x_i y_i$ ; their sums appear as the last entry of each column. Note that the number of digits carried in the computed values should be the maximum allowed by the calculator or computer, and rounding should not be performed until the calculation is complete.

We now substitute into Equations a1-29, a1-30, and a1-31 to obtain

$$S_{xx} = \sum x_i^2 - (\sum x_i)^2 / N = 6.90201 - (5.365)^2 / 5 \\ = 1.14537$$

$$S_{yy} = \sum y_i^2 - (\sum y_i)^2 / N = 36.3775 - (12.51)^2 / 5 \\ = 5.07748$$

$$S_{xy} = \sum x_i y_i - \sum x_i \sum y_i / N \\ = 15.81992 - 5.365 \times 12.51 / 5 \\ = 2.39669$$

Substituting these quantities into Equations a1-32 and a1-33 yields

$$m = 2.39669 / 1.14537 = 2.0925 = 2.09$$

$$b = \frac{12.51}{5} - 2.0925 \times \frac{5.365}{5} = 0.2567 = 0.26$$

Thus, the equation for the least-squares line is

$$y = 2.09x + 0.26$$

Substitution into Equation a1-34 yields the standard deviation for the residuals:

$$s_y = \sqrt{\frac{S_{yy} - m^2 S_{xx}}{N - 2}} \\ = \sqrt{\frac{5.07748 - (2.0925)^2 \times 1.14537}{5 - 2}} = 0.14$$

and substitution into Equation a1-35 gives the standard deviation of the slope:

$$s_m = s_y / \sqrt{S_{xx}} = 0.14 / \sqrt{1.14537} = 0.13$$

The standard deviation of the intercept is obtained from Equation a1-36. Thus

$$s_b = 0.14 \sqrt{\frac{1}{5 - (5.365)^2 / 6.90201}} = 0.16$$

#### EXAMPLE a1-13

The calibration curve derived in Example a1-12 was used for the chromatographic determination of isooctane in a hydrocarbon mixture. A peak area of 2.65 was obtained. Calculate the mole percent of isooctane and the standard deviation for the result if the area was (a) the result of a single measurement and (b) the mean of four measurements.

In either case,

$$x = \frac{y - 0.26}{2.09} = \frac{2.65 - 0.26}{2.09} = 1.14 \text{ mol } \%$$

(a) Substituting into Equation a1-37, we obtain

$$s_c = \frac{0.14}{2.09} \sqrt{\frac{1}{1} + \frac{1}{5} + \frac{(2.65 - 12.51/5)^2}{(2.09)^2 \times 1.145}} \\ = 0.074 \text{ mol } \%$$

(b) For the mean of four measurements,

$$s_c = \frac{0.14}{2.09} \sqrt{\frac{1}{4} + \frac{1}{5} + \frac{(2.65 - 12.51/5)^2}{(2.09)^2 \times 1.145}} \\ = 0.046 \text{ mol } \%$$

### a1D QUESTIONS AND PROBLEMS

a1-1 Consider the following sets of data:

A	B	C	D
61.45	3.27	12.06	2.7
61.53	3.26	12.14	2.4
61.32	3.24		2.6
	3.24		2.9
	3.28		
	3.23		

Calculate: (a) the mean for each data set, and decide how many degrees of freedom are associated with the calculation of  $\bar{x}$ ; (b) the absolute standard deviation of each set, and decide how many degrees of freedom are associated with the calculation of  $s$ ; (c) the standard error of the mean of each set; (d) the coefficient of variation for the individual data points from the mean.

a1-2 The accepted value for the quantity that provided each of the sets of data in Problem a1-1 is: A 61.71, B 3.28, C 12.23, D 2.75. Calculate: (a) the absolute error for the mean of each set; (b) the percent relative error for each mean.

a1-3 A particular method for the analysis of copper yields results that are low by 0.5 mg. What will be the percent relative error due to this source if the weight of copper in a sample is (a) 25 mg? (b) 100 mg? (c) 250 mg? (d) 500 mg?

a1-4 The method described in Problem a1-3 is to be used to analyze an ore that contains about 4.8% copper. What minimum sample weight should be taken if the relative error due to a 0.5-mg loss is to be smaller than (a) 0.1%? (b) 0.5%? (c) 0.8%? (d) 1.2%?

a1-5 A certain instrumental technique has a standard deviation of 1.0%. How many replicate measurements are necessary if the standard error of the mean is to be 0.01%?

a1-6 A certain technique is known to have a mean of 0.500 and standard deviation of  $1.84 \times 10^{-3}$ . It is also known that Gaussian statistics apply. How many replicate determinations are necessary if the standard error of the mean is not to exceed 0.100%?

TABLE a1-6  
Calibration Data for a Chromatographic Method for the Determination of Isooctane in a Hydrocarbon Mixture

Mole Percent Isooctane, $x_i$	Peak Area, $y_i$	$x_i^2$	$y_i^2$	$x_i y_i$
0.352	1.09	0.12390	1.1881	0.38368
0.803	1.78	0.64481	3.1684	1.42934
1.08	2.60	1.16640	6.7600	2.80800
1.38	3.03	1.90440	9.1809	4.18140
<u>1.75</u>	<u>4.01</u>	<u>3.06250</u>	<u>16.0801</u>	<u>7.01750</u>
5.365	12.51	6.90201	36.3775	15.81992

- a1-7 A constant solubility loss of approximately 1.8 mg is associated with a particular method for the determination of chromium in geological samples. A sample containing approximately 18% Cr was analyzed by this method. Predict the relative error (in parts per thousand) in the results due to this systematic error, if the sample taken for analysis weighed 0.400 g.
- a1-8 Following are data from a continuing study of calcium ion in the blood plasma of several individuals:

Subject	Mean Calcium Content, mg/100 mL	Number of Observations	Derivation of Individual Results from Mean Values
1	3.16	5	0.14, 0.09, 0.06, 0.00, 0.11
2	4.08	4	0.07, 0.12, 0.10, 0.01
3	3.75	5	0.13, 0.05, 0.08, 0.14, 0.07
4	3.49	3	0.10, 0.13, 0.07
5	3.32	6	0.07, 0.10, 0.11, 0.03, 0.14, 0.05

- (a) Calculate  $s$  for each set of values.  
 (b) Pool the data and calculate  $s$  for the analytical method.
- a1-9 A method for determining the particulate lead content of air samples is based upon drawing a measured quantity of air through a filter and performing the analysis on circles cut from the filter. Calculate the individual values for  $s$  as well as a pooled value for the accompanying data.

Sample	$\mu\text{g Pb/m}^3$ Air
1	1.5, 1.2, 1.3
2	2.0, 2.3, 2.3, 2.2
3	1.8, 1.7, 1.4, 1.6
4	1.6, 1.3, 1.2, 1.5, 1.6

- a1-10 Estimate the absolute standard deviation and the coefficient of variation for the results of the following calculations. Round each result so that it contains only significant digits. The numbers in parentheses are absolute standard deviations.
- (a)  $y = 6.75 (\pm 0.03) + 0.843 (\pm 0.001) - 7.021 (\pm 0.001) = 0.572$   
 (b)  $y = 67.1 (\pm 0.3) \times 1.03 (\pm 0.02) \times 10^{-17} = 6.9113 \times 10^{-16}$   
 (c)  $y = 243 (\pm 1) \times \frac{760 (\pm 2)}{1.006 (\pm 0.006)} = 183578.5$   
 (d)  $y = \frac{143 (\pm 6) - 64 (\pm 3)}{1249 (\pm 1) + 77 (\pm 8)} = 5.9578 \times 10^{-2}$   
 (e)  $y = \frac{1.97 (\pm 0.01)}{243 (\pm 3)} = 8.106996 \times 10^{-3}$
- a1-11 Estimate the absolute standard deviation and the coefficient of variation for the results of the following calculations. Round each result to include only significant figures. The numbers in parentheses are absolute standard deviations.
- (a)  $y = -1.02 (\pm 0.02) \times 10^{-7} - 3.54 (\pm 0.2) \times 10^{-8}$   
 $= -1.374 \times 10^{-7}$   
 (b)  $y = 100.20 (\pm 0.08) - 99.62 (\pm 0.06) + 0.200 (\pm 0.004) = 0.780$   
 (c)  $y = 0.0010 (\pm 0.0005) \times 18.10 (\pm 0.02) \times 200 (\pm 1) = 3.62$

$$(d) y = [33.33 (\pm 0.03)]^3 = 37025.927$$

$$(e) y = \frac{1.73 (\pm 0.03) \times 10^{-14}}{1.63 (\pm 0.04) \times 10^{-16}} = 106.1349693$$

- a1-12 Based on extensive past experience, it is known that the standard deviation for an analytical method for gold in sea water is 0.025 ppb. Calculate the 99% confidence limit for an analysis using this method, based on
- (a) a single measurement.  
 (b) three measurements.  
 (c) five measurements.
- a1-13 An established method of analysis for chlorinated hydrocarbons in air samples has a standard deviation of 0.030 ppm.
- (a) Calculate the 95% confidence limit for the mean of four measurements obtained by this method.  
 (b) How many measurements should be made if the 95% confidence limit is to be  $\pm 0.017$ ?
- a1-14 The standard deviation in a method for the analysis of carbon monoxide in automotive exhaust gases has been found, on the basis of extensive past experience, to be 0.80 ppm.
- (a) Estimate the 90% confidence limit for a triplicate analysis.  
 (b) How many measurements would be needed for the 90% confidence limit for the set to be 0.50 ppm?
- a1-15 The certified percentage of nickel in a particular NIST reference steel sample is 1.12%. A new spectrometric method for the determination of nickel produced the following percentages: 1.10, 1.08, 1.09, 1.12, 1.09. Is there an indication of bias in the method at the 95% level?
- a1-16 A titrimetric method for the determination of calcium in limestone was tested by analysis of an NIST limestone containing 30.15% CaO. The mean result of four analyses was 30.26% CaO, with a standard deviation of 0.085%. By pooling data from several analyses, it was established that  $s \rightarrow \sigma = 0.094\%$  CaO.
- (a) Do the data indicate the presence of a determinate error at the 95% confidence level?  
 (b) Do the data indicate the presence of a determinate error at the 95% confidence level if no pooled value for  $\sigma$  was available?
- a1-17 In order to test the quality of the work of a commercial laboratory, duplicate analyses of a purified benzoic acid (68.8% C, 4.953% H) sample was requested. It is assumed that the relative standard deviation of the method is  $s_r \rightarrow \sigma_r = 4$  ppt for carbon and 6 ppt for hydrogen. The means of the reported results are 68.5% C and 4.882% H. At the 95% confidence level, is there any indication of determinate error in either analysis?
- a1-18 The diameter of a sphere has been found to be 2.15 cm, and the standard deviation associated with the mean is 0.02 cm. What is the best estimate of the volume of the sphere, and what is the standard deviation associated with the volume?
- a1-19 A given pH meter can be read with a standard deviation of  $\pm 0.01$  pH units throughout the range 2 to 12. Calculate the standard deviation of  $[\text{H}_3\text{O}^+]$  at each end of this range.
- a1-20 A solution is prepared by weighing 5.0000 g of compound X into a 100-mL volumetric flask. The balance could be used with a precision of 0.2 mg reported as a standard deviation and the volumetric flask could be filled

with a precision of 0.15 mL also reported as a standard deviation. What is the estimated standard deviation of concentration in g/mL?

- a1-21 Estimate the absolute standard deviation in the result derived from the following operations (the numbers in parentheses are absolute standard deviations for the numbers they follow). Report the result to the appropriate number of significant figures.

(a)  $x = \log 878(\pm 4) = 2.94349$   
 (b)  $x = \log 0.4957(\pm 0.0004) = -0.30478$   
 (c)  $p = \text{antilogarithm } 3.64(\pm 0.01) = 4365.16$   
 (d)  $p = \text{antilogarithm } -7.191(\pm 0.002) = 6.44169 \times 10^{-8}$

- a1-22 The sulfate ion concentration in natural water can be determined by measuring the turbidity that results when an excess of  $\text{BaCl}_2$  is added to a measured quantity of the sample. A turbidimeter, the instrument used for this analysis, was calibrated with a series of standard  $\text{Na}_2\text{SO}_4$  solutions. The following data were obtained in the calibration:

mg $\text{SO}_4^{2-}/\text{L}$ , $c_x$	Turbidimeter Reading, $R$
0.00	0.06
5.00	1.48
10.00	2.28
15.0	3.98
20.0	4.61

Assume that a linear relationship exists between the instrument reading and concentration.

- (a) Plot the data and draw a straight line through the points by eye.  
 (b) Derive a least-squares equation for the relationship between the variables.  
 (c) Compare the straight line from the relationship derived in (b) with that in (a).  
 (d) Calculate the standard deviation for the slope and the intercept for the least-squares line.  
 (e) Calculate the concentration of sulfate in a sample yielding a turbidimeter reading of 3.67. Calculate the absolute standard deviation of the result and the coefficient of variation.  
 (f) Repeat the calculations in (e) assuming that the 3.67 was a mean of six turbidimeter readings.

- a1-23 The following data were obtained in calibrating a calcium ion electrode for the determination of pCa. A linear relationship between the potential  $E$  and pCa is known to exist.

pCa	$E$ , mV
5.00	-53.8
4.00	-27.7
3.00	+ 2.7
2.00	+31.9
1.00	+65.1

- (a) Plot the data and draw a line through the points by eye.  
 (b) Derive a least-squares expression for the best straight line through the points. Plot this line.

- (c) Calculate the standard deviation for the slope and the intercept of the least-squares line.  
 (d) Calculate the pCa of a serum solution in which the electrode potential was 20.3 mV. Calculate the absolute and relative standard deviations for pCa if the result was from a single voltage measurement.  
 (e) Calculate the absolute and relative standard deviations for pCa if the millivolt reading in (d) was the mean of two replicate measurements. Repeat the calculation based upon the mean of eight measurements.  
 (f) Calculate the molar calcium ion concentration for the sample described in (d).  
 (g) Calculate the absolute and relative standard deviations in the calcium ion concentration if the measurement was performed as described in (e).

- a1-24 The following are relative peak areas for chromatograms of standard solutions of methyl vinyl ketone (MVK).

Concentration MVK, mmol/L	Relative Peak Area
0.500	3.76
1.50	9.16
2.50	15.03
3.50	20.42
4.50	25.33
5.50	31.97

- (a) Derive a least-squares expression assuming the variables bear a linear relationship to one another.  
 (b) Plot the least-squares line as well as the experimental points.  
 (c) Calculate the standard deviation of the slope and intercept of the line.  
 (d) Two samples containing MVK yielded relative peak areas of 6.3 and 27.5. Calculate the concentration of MVK in each solution.  
 (e) Assume that the results in (d) represent a single measurement as well as the mean of four measurements. Calculate the respective absolute and relative standard deviations.