CALCULATING THE RESULTS AND REPORTING THE DATA

The analyst must provide expert advice on the significance of a result.

Once the concentration of analyte in the prepared sample solution has been determined, the results are used to calculate the amount of analyte in the original sample. Either an *absolute* or a *relative* amount may be reported. Usually, a relative composition is given, for example, percent or parts per million, along with the mean value for expressing accuracy. Replicate analyses can be performed (three or more), and a precision of the analysis may be reported, for example, standard deviation. A knowledge of the precision is important because it gives the degree of uncertainty in the result (see Chapter 3). The analyst should critically evaluate whether the results are reasonable and relate to the analytical problem as originally stated. Remember that the customer often does not have a scientific background so will take a number as gospel. Only you, as analyst, can put that number in perspective, and it is important that you have good communication and interaction with the "customer" about what the analysis represents.

1.4 Validation of a Method—You Have to Prove It Works!

Great care must be taken that accurate results are obtained in an analysis. Two types of error may occur: *random* and *systematic*. Every measurement has some imprecision associated with it, which results in random distribution of results, for example, a Gaussian distribution. The experiment can be designed to narrow the range of this, but it cannot be eliminated. A systematic error is one that biases a result consistently in one direction. Such errors may occur when the sample matrix suppresses the instrument signal, a weight of an analytical balance may be in error, skewed either high or low, or a sample may not be sufficiently dried.

The best way to validate a method is to analyze a standard reference material of known composition.

Proper calibration of an instrument is only the first step in assuring accuracy. In developing a method, samples should be spiked with known amounts of the analyte (above and beyond what is already in the sample). The amounts determined (recovered) by the analysis procedure (after subtraction of the amount apparently present in the sample as determined by the same procedure) should be close to what was added. This is not a foolproof approach, however, and only assures that the intended analyte is measured. It cannot assure that some interferent present in the sample is not measured. A new method is better validated by comparison of sample results with those obtained with another accepted method. There are various sources of certified standards or reference materials that may be analyzed to assure accuracy by the method in use. For example, environmental quality control standards for pesticides in water or priority pollutants in soil are commercially available. The National Institute of Standards and Technology (NIST) prepares standard reference materials (SRMs) of different matrix compositions (e.g., steel, ground leaves) that have been certified for the content of specific analytes, by careful measurement by at least two independent techniques. Values are assigned with statistical ranges. Different agencies and commercial concerns can provide samples for round-robin or blind tests in which control samples are submitted to participating laboratories for analysis at random; the laboratories are not informed of the control values prior to analysis.

Standards should be run intermittently with samples. A *control sample* should also be run at least daily and the results plotted as a function of time to prepare a *quality control chart*, which is compared with the known standard deviation of the method. The measured quantity is assumed to be constant with time, with a Gaussian distribution, and there is a 1 in 20 chance that values will fall outside two standard deviations from the known value, and a 1 in 100 chance it will be 2.5 standard deviations away. Numbers exceeding these suggest uncompensated errors, such as instrument malfunction, reagent deterioration, or improper calibration.

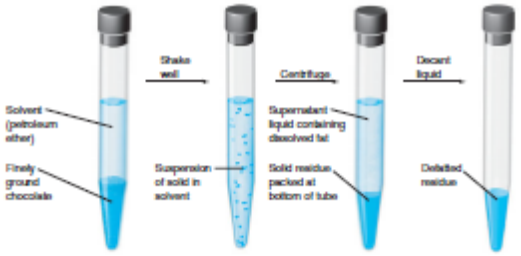

Figure 0-8 shows the next part of the procedure. A 10-mL portion of the solvent, petroleum ether, was added to the tube, and the top was capped with a stopper. The tube was shaken vigorously to dissolve fat from the solid chocolate into the solvent. Caffeine and theobromine are insoluble in this solvent. The mixture of liquid and fine particles was then spun in a centrifuge to pack the chocolate at the bottom of the tube. The clear liquid, containing dissolved fat, could now be **decanted** (poured off) and discarded. Extraction with fresh portions of solvent was repeated twice more to ensure complete removal of fat from the chocolate. Residual solvent in the chocolate was finally removed by heating the centrifuge tube in a beaker of boiling water. The mass of chocolate residue could be calculated by weighing the centrifuge tube plus its content of defatted chocolate residue and subtracting the known mass of the empty tube.

Substances being measured—caffeine and theobromine in this case—are called **analytes.** The next step in the sample preparation procedure was to make a **quantitative transfer** (a complete transfer) of the fat-free chocolate residue to an Erlenmeyer flask and to dissolve the analytes in water for the chemical analysis. If any residue were not transferred from the tube to the flask, then the final analysis would be in error because not all of the analyte would be present. To perform the quantitative transfer, Denby and Scott added a few milliliters of pure water to the centrifuge tube and used stirring and heating to dissolve or suspend as much of the chocolate as possible. Then they poured the **slurry** (a suspension of solid in a liquid) into a 50-mL flask. They repeated the procedure several times with fresh portions of water to ensure that every bit of chocolate was transferred from the centrifuge tube to the flask.

To complete the dissolution of analytes, Denby and Scott added water to bring the volume up to about 30 mL. They heated the flask in a boiling water bath to extract all the caffeine and theobromine from the chocolate into the water. To compute the quantity of analyte later, the total mass of solvent (water) must be accurately known. Denby and Scott knew the mass of chocolate residue in the centrifuge tube and they knew the mass of the empty Erlenmeyer flask. So they put the flask on a balance and added water drop by drop until there were exactly 33.3 g of water in the flask. Later, they would compare known solutions of pure analyte in water with the unknown solution containing 33.3 g of water.

Before Denby and Scott could inject the unknown solution into a chromatograph for the chemical analysis, they had to clean up the unknown even further (Figure 0-9). The slurry of chocolate residue in water contained tiny solid particles that would surely clog their expensive chromatography column and ruin it. So they transferred a portion of the slurry to a centrifuge tube and centrifuged the mixture to pack as much of the solid as possible at the bottom of the tube. The cloudy, tan, **supernatant liquid** (liquid above the packed solid) was then filtered in a further attempt to remove tiny particles of solid from the liquid.

It is critical to avoid injecting solids into a chromatography column, but the tan liquid still looked cloudy. So Denby and Scott took turns between classes to repeat the centrifugation and filtration five times. After each cycle in which the supernatant liquid was filtered and centrifuged, it became a little cleaner. But the liquid was never completely clear. Given enough time, more solid always seemed to precipitate from the filtered solution.

The tedious procedure described so far is called **sample preparation**—transforming a sample into a state that is suitable for analysis. In this case, fat had to be removed from the chocolate, analytes had to be extracted into water, and residual solid had to be separated from the water.

Table 1.1
Comparison of Different Analytical Methods

Method	Approx. Range (mol/L)	Approx. Precision (%)	Selectivity	Speed	Cost	Principal Uses
Gravimetry	10^{-1}–10^{-2}	0.1	Poor–moderate	Slow	Low	Inorg.
Titrimetry	10^{-1}–10^{-4}	0.1–1	Poor–moderate	Moderate	Low	Inorg., org.
Potentiometry	10^{-1}–10^{-6}	2	Good	Fast	Low	Inorg.
Electrogravimetry, coulometry	10^{-1}–10^{-4}	0.01–2	Moderate	Slow–moderate	Moderate	Inorg., org.
Voltammetry	10^{-3}–10^{-10}	2–5	Good	Moderate	Moderate	Inorg., org.
Spectrophotometry	10^{-3}–10^{-6}	2	Good–moderate	Fast–moderate	Low–moderate	Inorg., org.
Fluorometry	10^{-6}–10^{-9}	2–5	Moderate	Moderate	Moderate	Org.
Atomic spectroscopy	10^{-3}–10^{-9}	2–10	Good	Fast	Moderate–high	Inorg., multielement
Chromatography—Mass Spectrometry	10^{-4}–10^{-9}	2–5	Good	Fast–moderate	Moderate–high	Org., multi-component
Kinetics methods	10^{-2}–10^{-10}	2–10	Good–moderate	Fast–moderate	Moderate	Inorg., org., enzymes

bar can be determined gravimetrically by dissolving a small sample in nitric acid and precipitating AgCl with chloride and weighing the purified precipitate.

Most methods require calibration with a standard.

The various methods of determining an analyte can be classified as either **absolute** or **relative**. Absolute methods rely upon accurately known fundamental constants for calculating the amount of analyte, for example, atomic weights. In gravimetric analysis, for example, an insoluble derivative of the analyte of known chemical composition is prepared and weighed, as in the formation of AgCl for chloride determination. The precipitate contains a known fraction of the analyte, in this case, fraction of Cl = at wt Cl/f wt AgCl = 35.453/143.32 = 0.24737.[2] Hence, it is a simple matter to obtain the amount of Cl contained in the weighed precipitate. Gravimetry, titrimetry and coulometry are examples of absolute methods. Most other methods, however, are relative in that they require comparison against some solution of known concentration (also called calibration or standardization, see below).

INSTRUMENT STANDARDIZATION

A calibration curve is the instrument response as a function of concentration.

Most instrumental methods of analysis are relative. Instruments register a signal due to some physical property of the solution. Spectrophotometers, for example, measure the fraction of electromagnetic radiation from a light source that is absorbed by the sample. This fraction must be related to the analyte concentration by comparison against the fraction absorbed by a known concentration of the analyte. In other words, the instrumentation must be **standardized**.

Instrument response may be linearly or nonlinearly related to the analyte concentration. Calibration is accomplished by preparing a series of standard solutions of the analyte at known concentrations and measuring the instrument response to each of these (usually after treating them in the same manner as the samples) to prepare an **analytical calibration curve** of response versus concentration. Figure 1.2 shows examples of calibration curves obtained in a mass spectrometry experiment. The concentration of an unknown can then be determined from

[2] at wt = atomic weight; f wt = formula weight.

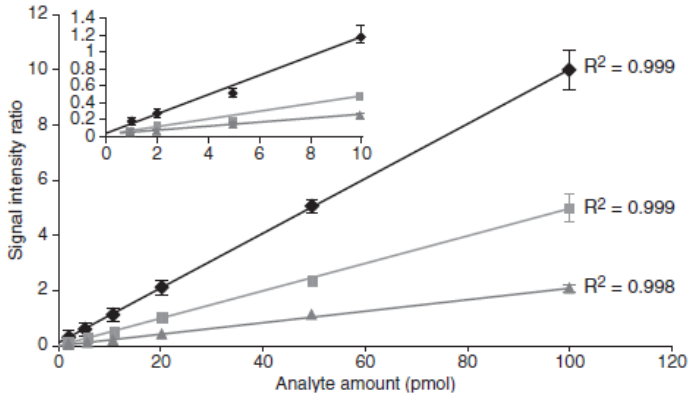

Fig. 1.2. Calibration curves for the measurement of proteins using matrix-assisted laser desorption ionization (MALDI)—mass spectrometry and an ionic liquid matrix (Courtesy of Prof. Michael Gross, Washington University in St. Louis. Reprinted with permission).

the response, using the calibration curve. With modern computer-controlled instruments, this is done electronically or digitally, and direct readout of concentration is obtained.

METHOD OF STANDARD ADDITIONS

Standard additions calibration is used to overcome sample matrix effects.

The sample matrix may affect the instrument response to the analyte. In such cases, calibration may be accomplished by the **method of standard additions**. A portion of the sample is spiked with a known amount of standard, and the increase in signal is due to the standard. In this manner, the standard is subjected to the same environment as the analyte. These calibration techniques are discussed in more detail when describing the use of specific instruments.

See Section 17.5 and the website supplement for that section for a detailed description of the standard additions method and calculations using it. Section 20.5 illustrates its use in gas chromatography, and Example 14.8 illustrates how it is used in potentiometry. Experiments 33 (atomic spectrometry) and 35 (solid-phase extraction) on the text website employ the method of standard additions.

INTERNAL STANDARD CALIBRATION

An instrumental response is often subject to variations from one measurement to the next due to changing instrument conditions, resulting in imprecision. For example, in gas chromatography, the volume of injected sample or standard from a Hamilton microliter syringe (see Chapter 2) may vary. In atomic absorption spectrometry, fluctuations in gas flows and aspiration rates for sample introduction may occur. In order to compensate for these types of fluctuations, internal standard calibration may be used. Here, a fixed concentration of a different analyte, that is usually chemically similar to the sample analyte, is added to all solutions to be measured. Signals for both substances are recorded, and the ratio of the sample to internal standard signals is plotted versus sample analyte concentration. So, if say the volume of injected sample is 10% lower than assumed, each signal is reduced 10%, and the ratio at a given sample analyte concentration remains constant.

See Sections 17.5 (atomic spectrometry) and 20.5 (gas chromatography) for illustrations of internal standard use.

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