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Proper Laboratory Protocols for Analytical Instrumentation

The proper operation of analytical instruments requires hands-on experience, even though studying the various components of instrument and understanding the theory is important. Experiential learning is a must for most students, especially when dealing with analytical instruments. This chapter is divided into two sections: Section 1.1 contains preliminary information that is not included in most textbooks but is necessary to truly understand the difficulty in measuring analytes in the parts-per-billion (ppb) and parts-per-trillion (ppt) concentrations; Section 1.2 deals with trace analysis and concerns the proper setup and testing of instruments to ensure that accurate and reproducible numbers are obtained and a brief discussion on quality assurance/quality control is provided.

1.1 Laboratory Preliminaries

The analysis of metals in the ppb and ppt concentrations is a difficult and tedious process that requires optimum laboratory conditions and analytical skills. Many laboratories even have their sample handling and preparation sections of the lab physically separated from their instrument rooms in order to avoid contaminating instruments and cross contamination of samples. Error associated with laboratory work can be divided into three general categories: sampling, sample preparation, and analysis. Entire books and statistics courses have been dedicated to proper sampling design and implementation in order to avoid errors (referred to as bias) in the collection of samples, such as in the proper sampling of lake water. Laboratory error has been significantly reduced by standardizing sample extraction procedures for a particular sample, element, compound, industry, and even a specific technician.

This beginning section of this chapter is dedicated to a few of the more important factors associated with the instrumental analysis of samples. Before actual experimental procedures and results are presented, it is necessary to comment on sample preparation, special chemicals, and the calibration of an instrument. These topics are not normally covered in textbooks but are important for first-time instrument users. The types of samples that are commonly analyzed by analytical instruments will be discussed, including proper procedures that are critical to avoid common laboratory errors.

1.1.1 Types of Samples and Sample Preparation

There are various sample types that need to be analyzed for their analyte concentration. For example, a geochemist may be interested in determining the concentration of a particular

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metal, such as lead (Pb), in a terrestrial or oceanic rock, or a fisheries biologist may be interested in determining the concentration of mercury in a particular fish species. In addition, a wide range of instrumentation is available for the analysis of organic compounds such as hydrocarbons and pesticides. However, the various instruments that could perform this task at a trace level (ppm or lower) are unable to accommodate the introduction of a rock, fish, or soil matrix. As a result, sample preparation, specifically sample digestion, is needed before an instrument can be utilized. The chemical process to transform the solid sample into an aqueous sample must be undertaken carefully with the proper reagents and glassware. After the concentration of the digested sample is determined, simple calculations can be performed to accurately determine the concentrations of Pb in a rock, mercury in a fish, or pesticide in a soil or sediment sample. If sample preparation is not performed properly, no degree or expense of instrumental components or laboratory technique can correct for a sampling or digestion error. Despite the obviousness of some of these techniques, this component of analytical chemistry is still a considerable portion of error associated with the final analyte concentration.

1.1.1.1 Samples

Before a procedure of sample preparation can be created (commonly referred to as a standard operating procedure, or SOP), the analyte(s) of interest must be identified as well as the matrix. The most common type of sample matrix for metals and organic compounds is aqueous. The first step when analyzing water samples is to determine whether the total or dissolved metal concentration is of interest. All natural water samples contain particles; some are extremely small, while others are visible to the naked eye. Frequently, particle size is used to differentiate between dissolved and suspended solids. For example, in environmental chemistry and other disciplines, most scientists accept that constituents of a sample are *dissolved* if they pass through a 0.45-μm glass-fiber filter; anything not passing through the filter is considered *particulate*. This will be the distinction between the dissolved and particulate phases used in this text. If the total analyte concentration needs to be determined, the water sample may require strong acidification and heating for metals or solvent extraction for organic compounds prior to analysis depending on the concentration in the solid phase. If the dissolved solid concentration is desired, the water is first filtered and then acidified or extracted, usually with high-purity nitric acid (HNO_3) (1–3% acid) in a plastic container or with high-purity (chromatography-grade) organic solvents. The purpose of the extraction is to permanently dissolve any analyte that is adsorbed to colloidal (very small) particles or the container walls. Some procedures require that samples be stored at 4 °C for less than one month prior to analysis. Each industry, governmental agency, or company will have an SOP that must be rigidly followed.

Other sample matrices, such as gaseous, solid, and biological tissues, are usually chemically converted to aqueous samples through digestions or extractions. Metals and organic analytes contained in the atmosphere from geological processes, dust from wind, and high-temperature industrial processes are frequently measured to model the fate and transport of pollutants. Analytes may be in the atomic gaseous state, such as mercury vapor, or associated with suspended particles, such as cadmium (Cd) or Pb ions adsorbed to clay particles. One common sampling method is to utilize a vacuum to pull large volumes of air (tens to hundreds of cubic meters) through a filter. The filter is then removed and the metals or organics are extracted into an aqueous or organic solution that is then analyzed. This type of sampling and analysis is commonly performed in urban areas to monitor atmospheric metal emissions and organic pollution. Standardized methods for these types of measurements have been developed by the US Environmental Protection Agency (EPA) and other governmental agencies.

The metal concentration in a solid sample or geological material is also frequently analyzed by flame atomic absorption spectrometry (FAAS) and inductively coupled plasma-mass spectrometry (ICP-MS). Organic analytes are commonly measured by gas chromatography (GC) with flame ionization detection (FID) or MS detection, or high-pressure liquid chromatography-mass spectrometry (HPLC-MS). Sometimes analytes are analyzed directly by special attachments to FAAS and ICP units (as described in Chapters 4 and 5) but are more commonly digested or extracted with acids followed by analysis. Similarly, organic compounds in soil, sediment, and biological tissue are extracted with organic solvents and analyzed by GC or HPLC. The resulting solution is then analyzed as described in Section 1.1.3. However, the specific type of digestion/extraction depends on the ultimate goal of the analysis. If adsorbed metal concentrations are of interest, soil/sediment samples are simply placed in acid (usually 1–5% HNO_3), heated for a specified time, diluted, filtered, and then the filtrate is analyzed as if it were a liquid sample. If the total metal concentration is desired, as in many geological applications, the sample is completely digested with a combination of hydrofluoric (HF), HNO_3, and $HClO_4$; again, the samples are then diluted, filtered, and analyzed as an aqueous sample. The extraction of organic analytes can be far more complicated and may involve liquid–liquid extraction for water samples, digestion of biological tissues followed by extraction, or extensive Soxhlet extraction for soil and sediment samples. Procedures are available from the US EPA and the US Geological Survey (USGS).

Biological tissues present considerable problems as do soil and sediment samples. In order to create an aqueous sample, the tissue sample is first digested in acid to oxidize all biological matter and "free" up any present metal or organic analyte. This can be accomplished with a combination of sulfuric acid (H_2SO_4) and HNO_3 (1–5% each) and 30% hydrogen peroxide (H_2O_2), after digesting for 24 hours, and when necessary, heat. After the tissue has been dissolved, the sample is diluted, filtered, and analyzed as an aqueous sample. Each of the digestion procedures described above dilutes the original metal concentration in the sample. Thus, a careful accounting of all dilutions of a sample is required. After a concentration is obtained from an instrument, it must be adjusted for the dilution(s) to determine the analyte concentration in the original sample. Calculations concerning these dilutions will be discussed in the final paragraphs of this section.

1.1.1.2 Chemical Reagents

A variety of chemicals are used in the trace analysis. Mostly, these include reagents used in the digestion of solid samples or tissues to release metals into an acidic aqueous solution. The majority of these chemicals fall into two categories: oxidants and acids. Common digestion reagents include oxidants such as H_2O_2, potassium permanganate ($KMnO_4$), and potassium perchlorate ($KClO_4$) for oxidizing organic matter or tissue. Common acids include hydrochloric acid (HCl), HF, H_2SO_4, HNO_3, and perchloric acids ($HClO_4$). Organic solvents used in extraction of organic analytes must be extremely pure. For example of 20-L barrel of standard-grade acetone may cost \$10–20 (US), but an analytical grade 4-L bottle of acetone could be as high as \$50–100 (US). As manufacturers continue to produce commercially available instruments with lower and lower (better) detection limits, the purity of the reagents that are utilized to prepare samples must also improve in order to take advantage of these instrumental advances. Many of the reagents commonly used to prepare samples are commercially available in ultrapure grades. However, purchasing these ultrapure reagents is often expensive because of the relatively large volume of the acids or the oxidants needed to oxidize and remove the matrix prior to analysis. In addition, some ultrapure acids can still contain some metals at the ppt-to-ppb concentrations, especially for analytes such as Pb and mercury. Some laboratories that analyze large sample volumes choose to prepare their own acids by redistilling even the ultrapure acids. While this may seem like a major inconvenience,

the cost savings are obvious when a 2-L bottle of ultratrace metal HNO_3 (double distilled and packaged under clean room conditions) currently lists for several hundreds of dollars while a 2.5-L bottle of ACS-grade HNO_3 costs approximately only tens of dollars. A Teflon® sublimation distillation apparatus used to produce purer versions of these acids in-house only costs approximately $5000–10 000 (US).

While the purity of the chemical reagents is important, the purity of dilution water is also important. Due to the presence of trace metals and salts, the use of tap water is out of the question. House resin-deionized (DI) water may be sufficiently pure for some flame-based methods but may be inadequate for emission and mass spectrometry techniques. For these techniques, house DI is usually passed through a commercially available secondary resin filter system (such as the Nanopure® water filtration system from Millipore and Bronstead manufacturers) that removes metal concentrations down to the parts-per-trillion concentration or less, and organic concentrations down to the ppt levels. If concentrations in samples are to be measured below this level, cleaner water must be obtained. Commonly available resin-based systems cost approximately $5000–10 000 (US).

Despite the extensive preparations and costs associated with acquiring pure reagents, there is usually some detectable metal concentration in the reagents. In order to correct for the presence of trace metals, their concentration in a reagent blank must be determined. A reagent blank is a solution that contains every reagent in the digestion and analysis procedure but lacks the actual sample. This blank allows for any metal concentrations present in the reagents (including dilution water) to be accounted for and subtracted from the metal concentrations found in the sample. This process ensures that the final concentration is representative of the analyte's true presence in the original sample and not from contamination in the added reagents.

1.1.1.3 Glassware

FAAS, flame atomic emission spectroscopy (FAES), and ICP instruments yield concentration data with 3–4 significant figures, while chromatography instruments yield fewer significant figures. Thus, quantitative glassware with a similar number of significant figures must be used in order for the instrumentally significant figures to accurately represent the concentration of the sample. The most accurate dilutions are achieved with Class A pipettes and volumetric flasks. These pipettes and flasks are almost always made out of glass which can present a problem for trace metal analysis in techniques such as ICP-MS. Trace levels of some metals in unacidified water commonly "stick" (adsorb) to glass and some glass containers actually contain metals in their matrix and can release measurable concentrations when acidic solutions are added to them. In order to avoid this source of contamination, plastic or Teflon materials may be required for delivery, digestion, and storage of these samples. The use of manual pipetting systems with plastic tips, however, results in slightly less precise data (approximately three significant figures), but these devices are rapidly being adopted in the laboratory. Similar Class A glassware used in the analysis of organic compounds can be adequately cleaned with high-purity acetone or methanol.

It should be noted that for metal analysis, contact with metal surfaces, such as Al weigh boats should be avoided. Plastic and glass are preferred. For most organic analyses, all contact with plastics should be avoided.

1.1.2 Calibration Techniques

Once a sample has been prepared properly, it is necessary to calibrate the instrument to determine the concentration in the sample. Uncalibrated scientific instruments are random number generators. These number generators are transformed into valuable analytical tools with the use of

calibration techniques. There are various ways to determine the concentration of a sample through the use of external standards, standard addition, or semiquantitative methods.

1.1.2.1 External Standards

The most common technique to determine the concentration of a sample is through the use of external standards. For example, before a sample of water is analyzed for magnesium (Mg), a series of known Mg concentrations in acidified water ranging from 0.010 to 50.0 ppm would be analyzed. The respective absorbance or emission values for each standard are tabulated and a plot of concentration versus detector signal is made. Next, a plot of detector response (in this case, absorbance or counts per second) is made against the respective metal concentration on the *y*- and *x*-axes, respectively. Such a plot is shown in Figure 1.1 where a FAAS was used to measure the concentration of Mg in various external standards.

Note the linearity of the data plot; most calibration "curves" are lines. This linear detector response is preferred since it allows for easier statistical calculations (discussed in Chapter 2). Once an instrument is calibrated with an external standard, it can be used to estimate the concentration of an analyte in an unknown sample. This is accomplished by performing a linear least-squares (LLS) regression on the dataset (see Chapter 2) and obtaining an equation for the calibration line. For Figure 1.1, this equation is $y = 0.012x + 0.000$ where 0.012 is the slope of the line and 0.000 is the *y*-intercept. Next, the equation is rearranged to solve for x (the reader should do this now). When the actual sample is analyzed on the instrument, the readout will give the absorbance of the sample that corresponds to the value of y in the linear regression. For example, if an unknown sample yields an absorbance reading of 0.062, the Mg concentration would be estimated at 5.03 ppm with the rearranged linear regression equation (the reader should perform this calculation now). Another important statistical calculation is determined by r^2 (Figure 1.1) and is a measure of the "goodness of fit" between the linear model and the experimental data. The closer to 1.000 the r^2 value, the more the linear model for the data approaches a perfect fit. Most modern instruments have the built-in ability to perform a LLS and r^2 calculation. But an even more important statistical parameter, s_c, the standard deviation of replicate samples will be discussed in Chapter 2.

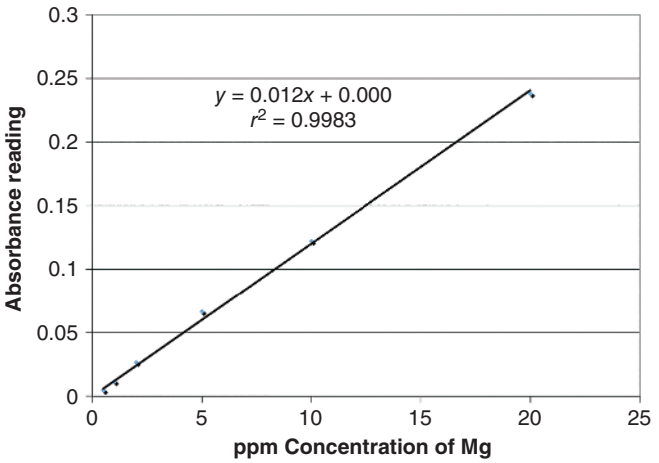

Figure 1.1 Instrument response (absorbance) versus Mg concentration (ppm) by FAAS. (Source: Dunnivant and Ginsbach).

Some calibration lines do not automatically intersect at the origin ($x = 0$, $y = 0$) because of the presence of small concentrations of analyte in the blank sample or because of "unbalanced" electronics (the detector not reading zero absorbance for the blank due to noise). This is especially true as the analyst approaches the detection limit of the instrument. In the past, the blank absorbance reading was simply manually subtracted from other readings prior to plotting the data. Today, modern instruments that are connected to computers perform many of these tasks automatically. Frequently, calibration lines, subtraction of blanks, and estimating unknown sample concentrations and even dilutions can all be calculated or accounted for automatically by the computer. This eliminates the need to re-enter data into another computer and decreases typographical and transposing errors. However, these automatic procedures should be monitored to ensure that accurate data are being generated.

1.1.2.2 Standard Addition

Another form of calibration, standard addition, can be used to account for matrix effects (such as surface tension or viscosity) or other problems (such as chemical interferences). For example, in FAAS and FAES analysis, the sample is drawn into the nebulizer by a constant vacuum source; and if the viscosity of the solutions being analyzed is not all the same, then different flow rates of sample or standard solution will reach the flame and therefore different masses of metal will be measured for each. (This is not a problem in ICP since a peristaltic pump is used to place sample in the nebulizer.) or in chromatography instruments. A sample containing significant amounts of sugar, commonly found in food products, will have a higher viscosity and will move more slowly through the inlet tube in FAAS and FAES systems than standards that are made up of relatively pure water; hence, the resultant signal obtained from this external standard-based analysis will be inaccurately low for the metal of interest. If the standard addition method described below is used, more accurate metal concentrations for the samples will be obtained.

In standard addition analysis, several equal volume aliquots of a sample are added to a volumetric flask (i.e. 15 mL of sample to each 25 mL flask). Increasing reference concentrations of the analyte of interest are added to each flask beginning at zero and increasing to the end of the linear range of the instrument (i.e. one flask will have 0.00 ppm, the next will have 1.00 ppm, the next will have 5.00 ppm, etc.). Acid is added to each flask in the analysis of metals to reach a specific and consistent percent (usually 1.00%). Finally, each flask is filled to the 25-mL volumetric line with high-purity water. Thus, each flask has equal volumes of sample, and a linear increase of known analyte is added starting at 0.00 ppm in the "blank" flask and ending at the highest analyte concentration. After the samples are analyzed, the concentration is plotted as a function of analyte added. An LLS regression ($y = mx + b$) is performed and the linear equation can be rearranged to solve for x, the sample concentration, as a function of y, the detector response. The concentration of the blank sample (the diluted sample containing no reference standard) is determined by computing the x intercept of the line (where $y = 0.00$).

An example of a standard addition analysis of a complex matrix (Ca in beer) is shown in Figure 1.2.

1.1.3 Figures of Merit

The individual calibration lines that instruments generate are dependent upon numerous variables such as laboratory technique, instrument components, and operating parameters. In order to compare different instruments to one another, three *figures of merit* have been developed to quantitatively compare different analytical techniques. These are sensitivity, detection limit, and signal to noise ratio (S/N).

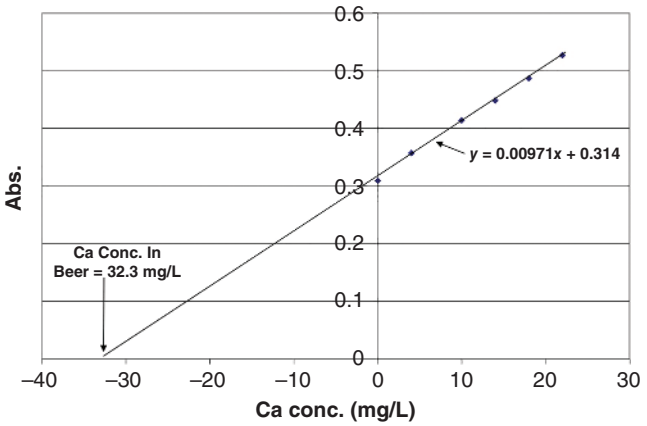

Figure 1.2 Standard addition analysis of Ca in beer. (Source: Dunnivant and Ginsbach).

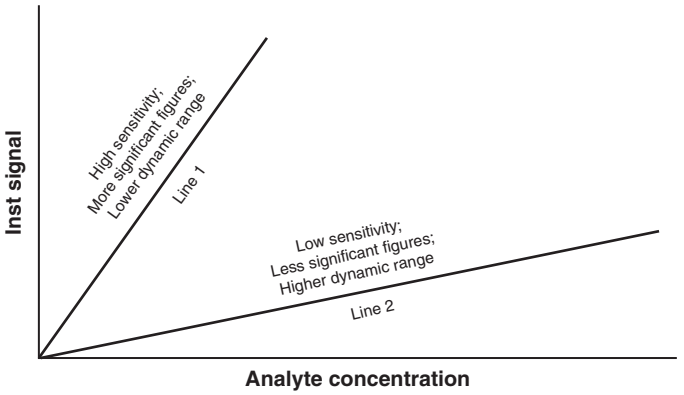

Figure 1.3 Calibration lines with differing sensitivity. (Source: Dunnivant and Ginsbach).

Sensitivity refers to the slope of the calibration line. Figure 1.3 shows two calibration lines, one with a steep slope and another with a shallower slope. The determination of which level of sensitivity is best depends on the situation. Calibration "line 1" is referred to as being more sensitive since it will allow the analyst to distinguish between smaller differences in concentration (i.e. allows the determination of 30.1 from 30.2 as opposed to 30 versus 40). This type of calibration line would be of interest when high degrees of accuracy are needed. If the screening of samples for gross differences in concentration is required, "line 1" would be of little use since it has only a limited dynamic range (small range in analyte concentrations) and its use could require the dilution of samples outside the calibration range. In this case, calibration "line 2" would be useful since it covers a larger dynamic range, but again, yields less accuracy (fewer significant figures).

Another related parameter, calibration sensitivity, is mathematically defined as

$$m = \frac{\Delta\text{Signal}}{\Delta\text{Concentration}} \tag{1.1}$$

where m is the slope of the calibration line from the linear regression. Another form of sensitivity is analytical sensitivity, defined as

$$\frac{m}{\text{std dev}} \tag{1.2}$$

where std dev is the standard deviation of the slope estimate. A range of sensitivities for the identical analyte are usually possible on the same instrument. High sensitivity is accomplished by "tuning" an instrument to its maximum sensitivity before testing the external standards. A lower sensitivity can be accomplished by "detuning" the instrument to obtain a lower slope and a broader range of useful analyte concentrations. The term sensitivity is commonly, but incorrectly, interchanged with detection limit.

Another figure of merit is the detection limit; this is one of the most important ways to compare two analytical techniques and brands of instruments, and is a major competitive selling/advertising metric used by instrument manufacturers. The detection limit determines the limitations of the instrument or technique and is commonly and incorrectly referred to as "zero" concentration of the analyte. For example, it is often stated that "no" pollutant was found in a sample. What does "no" mean? Absolute zero? Probably not. Zero concentration does not typically mean that no analyte exists since there are almost always a few atoms or molecules of any substance in any sample. Today, with modern instrumentation, there is no "zero concentration" for more environmental analytes. For example, the EPA-recommended limit (maximum contaminant level or MCL) for Pb in drinking water is 15 ppb, but the detection limit by ICP-MS is 0.1 ppb. No Pb concentration is healthy for consumption, but if we set the MCL to "zero," virtually no water would be safe to drink. Additionally, most people think the air they breathe is "free" of hazardous chemicals; few know it, but the air that they just took into their lungs contains measurable concentrations of PCBs and Hg. However, these concentrations are only measurable with rigorous analytical procedures. Instead, it would be more accurate to report the detection limit of the technique and indicate that the sample was below that limit (for example, the sample has less than 26.1 ppt of pollutant). So, how does an analyst quantitatively determine the detection limit? To illustrate this problem, Figure 1.4 shows three signal responses (these can be measures of absorbance or counts per second). Which of these signals can we confidently say is attributed to the analyte of interest instead of random background noise?

Most of everyone would agree that the right-hand sample signal would be attributed to the analyte. Some would also agree with the middle figure, but fewer analysts, if any, would call the left-hand spike the analyte. A quantitative way of distinguishing noise from a sample signal has been developed using the results from the linear regression process described earlier. In order to complete this process, a range of calibration standards is analyzed on an instrument with blank samples being run before, during, and after the standards. The responses for 5–10 blanks are averaged and a standard deviation is calculated. Next, a linear regression is completed for the results of the external calibration data. This data is then utilized to determine the detection limit by using the following equations.

There are two ways of determining the detection limit. First, from a purely statistical viewpoint, the reported detection limit should be the lowest concentration of the reference standard. But some analytical chemists find this definition too restrictive.

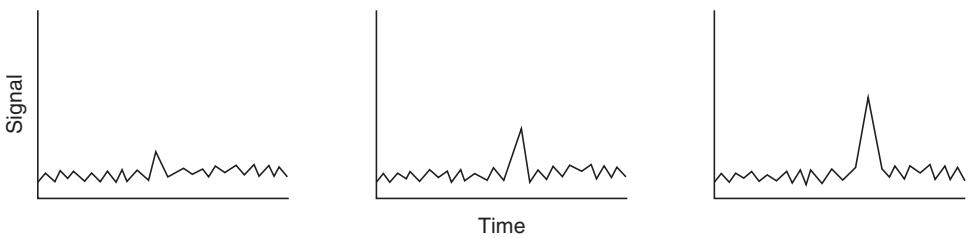

Figure 1.4 Illustration of signal to noise and instrument responses for a sample. (Source: Dunnivant and Ginbach).

From a more analytical standpoint, the minimum signal that can be distinguished from the background is determined by defining a constant k, usually equal to three (3.00…, a defined number), in the equation

$$S_m = S_{blank} + k(\text{s. d.}_{blank}) \tag{1.3}$$

where S_m is the minimum signal discernable from the noise, S_{blank} is the average blank signal, and s. $d._{blank}$ is the standard deviation of the blank measurements. The k value of 3 is common across most disciplines and basically means that a signal can be attributed to the analyte if it has a value that is larger than the noise (blank concentration) plus three times the standard deviation.

Next, recall the calibration line equation

$$S = mC + b \tag{1.4}$$

Redefining S to S_m and b to S_{blank} (to be consistent with most statistics textbooks) yields

$$S_m = S_{blank} + k(\text{s. d.}_{blank}) = mC + C_{blank}$$

And upon rearranging yields $C = \frac{k(\text{s. d.}_{blank})}{m}$.

Recalling that $S_m = S_{blank} + k(\text{s. d.}_{blank})$ and upon rearranging yields: $S_m - S_{blank} = k(\text{s. d.}_{blank})$.

Substitution into the above equation yields

$$C = \frac{S_m - S_{blank}}{m}$$

where C is the minimum detection limit.

This method gives a quantitative and consistent way of determining the detection limit once a value of k is determined (again, usually 3.00).

The final figure of merit quantifies the noise in a system by calculating the S/N ratio. Sources of noise are commonly divided into environmental, chemical, and instrumental sources that were discussed in the detector section of the FAAS chapter (Chapter 4) and are relevant to almost all detector systems.

The S/N is determined by:

$$\frac{S}{N} = \sqrt{n}\frac{S_x}{N_x} \tag{1.5}$$

where S_x and N_x are the signal and noise readings for a specific setting and n is the number of replicate measurements. One of the most common noise reduction techniques is to take as many readings as are reasonably possible. The more replicate readings a procedure utilizes, the greater the decrease of S/N by the square root of the number of measurements. By taking two measurements, one can increase the S/N by a factor of 1.4; or by taking four measurements, the analyst can cut the noise in half.

For the topics covered in this book, FAAS and ICP-AES instruments generally allow for multiple measurements or for an average measurement, for example, over 5–10 seconds, to be taken. For ICP-MS, again, multiple measurements are usually taken, usually 3–5 per mass/charge value in a few milliseconds. And, of course, the analyst can analyze a sample multiple times given the common presence of automatic samplers in the modern laboratory. For chromatography techniques, multiple sample runs require considerable time and are rarely conducted.

These statistical calculations, since they are dependent upon the linear calibration curve, can only be accurately applied over the range of measured external standards. This is because of the fact that detectors do not always give linear responses at relatively low or relatively high concentrations. As a result, all sample signals must be within the range of signals contained in the calibration line. This is referred to as "bracketing." If sample signals (and therefore concentrations) are too

high, the samples are diluted and reanalyzed. If the samples are too low, they are concentrated by a variety of techniques or they are reported to be below the minimum detection limit. In some cases, the limit of quantification (LOQ) is at the lowest reference standard. The limit of linearity (LOL) is where the calibration line becomes nonlinear, at the upper and lower ends of the line. In a case where the sample concentration is below the lowest standard but above the calculated detection limit, it is acceptable to record that sample as containing "trace concentrations" for the analyte but usually no actual number is quoted.

Technically, a fourth figure of merit, selectivity, exists but this is more obvious. Selectivity refers to the ability of a technique or instrument to distinguish between two different analytes (i.e. calcium (Ca) versus Mg or ^{206}Pb versus ^{207}Pb or 2-2′-dichlorobiphenyl versus 2,4-dichlorobiphenyl). For example, AAS and inductively coupled plasma-optical emission spectrometry (ICP-AES) can distinguish (are selective) between Ca and Mg but not different isotopes of the same element. ICP-MS can be selective for isotopes and elements depending on the mass resolution of the instrument.

1.1.4 Calculating Analyte Concentrations in the Original Sample

Dilutions and digestions are routine practices in the analysis of metal compounds. Once the concentration of the aqueous sample is prepared, an interesting problem arises, "How does the analysis relate the concentration in a diluted sample to the concentration in the original sample?" In general, this is accomplished by carefully performing and recording all steps of the sample preparation. A more detailed explanation of the calculation is offered in the following example problem:

Problem Statement: An analysis takes 0.500 g of wet sediment and prepares it for Cd analysis by FAAS. The purpose of the analysis is to determine the concentration of Cd adsorbed to the sediment on a dry weight basis. The water content in the wet sample was gravimetrically determined to be 24.8%. The wet sample is then measured out and digested in 25 mL of DI water and 3.00 mL of ultrapure HNO_3 on a hot plate at 80 °C for one hour. The sample is then cooled and filtered through a 0.45-μm filter into a 100-mL volumetric flask that is then filled to the mark with DI. Previous analysis indicated that the sample needed to be diluted by a factor of 10 to be within the linear concentration range of the instrument. The diluted sample was analyzed on an instrument and a concentration of 1.58 mg/L was measured for the aqueous sample. What is the concentration of Cd in the original, dry-weight sample?

First, the data are tabulated.

Next, the analyte concentration measured by the instrument is back-calculated to the concentration in the original sample. Note the accounting of each of the measurements in this calculation.

$$\text{Conc. in sample in} \frac{\text{mg}}{\text{kg}} = \frac{\left(\frac{1.58\ \text{mg}}{\text{L}}\right)\left(\frac{1\ \text{L}}{1000\ \text{mL}}\right)\left(\frac{100.0\ \text{mL}}{10.00\ \text{mL}}\right)(100\ \text{mL})}{(0.500\ \text{g wet sediment})\left(\frac{1\ \text{kg}}{1000\ \text{g}}\right)\left(\frac{100-28\ \text{g dry}}{100\ \text{g wet}}\right)}$$

$$\text{Conc. in sample in} \frac{\text{mg}}{\text{kg}} = 4390$$

1.2 Standard Practices

1.2.1 Ensuring Adequate Rinsing Between Samples and Standards

The cleanliness of all instruments at the beginning of a run or between every sample and reference standard is insured by running a blank solvent sample through the system. For FAAS and ICP-MS, this is relatively easy and does not require much time. For chromatography systems, this can be very time-intensive. With the advent of computer-controlled instruments, this is automatically performed by most modern AAS systems by the automatic sampler. Additional rinsing after a series of samples will usually prevent any buildup of analytes in the inlet of the instruments.

The part-per-trillion detection limits of ICP-MS and in the GC-ECD (electron capture detector) systems create the need for more thorough rinsing between samples and standards. Usually, the rinsing part of the sample sequence is the most time-consuming.

1.2.2 Quality Assurance/Quality Control

Most laboratories have a quality assurance/quality control (QA/QC) program, and usually, one person is in control of all QA/QC matters. QA/QC starts with a laboratory technician being certified on a particular instrument and for a particular analysis. Certification is completed with extensive training, a required analysis time on the instrument, and by passing a test examining known concentrations of analyte in reference check samples (described later). Laboratories and personnel have to be certified to conduct most governmental, consulting, and industry analyses.

QA/QC begins with a sampling plan, which is a document detailing how, when, and where samples will be taken, handling of the samples, preservation of the samples, and storage of the samples. Next, an SOP will be created outlining exactly how any sample will be analyzed. The SOP can contain, at a minimum, what quality of chemicals will be used, detailed procedures of sample digestion, detailed procedures for analyte extraction for undigested and digested samples, instruments to be used in the measurement of analytes, data analysis, statistical analysis, and reporting and long-term storage of the data. Many of these exact details of the SOP are government- and industry-specific.

A rigorous SOP will include field blanks, reagent blanks, a daily calibration curve, duplicate (or more) sample analysis, and analysis of blanks between samples, spiked samples, and reference check samples. Field blanks are samples containing any preservative present in the actual samples that are taken to the field to serve as a blank for the sample container and any conditions that the samples may be exposed to. Reagent blanks are samples that are analyzed on the instrument that contain any chemicals used in preservation, digestion, and extraction of the samples. Calibration of the instruments was discussed earlier in this chapter, but is usually run every day on the instrument of interest. Many sampling plans require more than one sample to be taken at a specific location or time. It may also be required to analyze a particular sample multiple times to determine the reproducibility of a technique. It is always a good analytical practice to run reagent blanks between each sample and standard to ensure that no carryover of analyte occurs in the introduction of sample to an instrument. Autosamplers have made this large increase in the number of samples possible in a timely and economical manner. It is also common practice to take a replicate sample and spike it with a known quantity of analyte. This allows an analyte recovery check to be conducted on the digestion and extraction procedures. Finally, to ensure that your SOP, instrument, and overall

analysis are accurate, reference check samples of many sample matrices (i.e. water, sediment, fish, etc.), containing known concentrations of analytes, can be purchased and analyzed.

A typical instrument run will include:

(1) several blank analyses at the beginning of the run sequence to ensure that all parts of the instrument are clean;
(2) a set of calibration standards ranging from the lowest possible detection limit to the end of the dynamic range of the instrument, from low to high concentrations, and with reagent blanks between each standard to ensure that no carryover of analyte occurs;
(3) the samples to be analyzed, including replicates, blanks, field blanks, reagent blanks, and spiked samples; and
(4) in some cases, the calibration standards are analyzed again at the end to ensure that instrument signal drift has not occurred.

Finally, the results from the instrument must be analyzed in a way to relate the instrument response to sample concentration, as discussed earlier in this chapter. Calibration data and all sample results can be stored on the instrument computer or a printed-out hard copy placed in laboratory notebooks. Most results must be archived for at least three years.

The true test of an adequate QA/QC program is documentation in the form of a legally defensible lab notebook (with procedures given in the laboratory experiments chapter at the end of this book). The term "legally defensible" is used since almost all data today in government, industry, and academia can be introduced as evidence in court and there are agency- and discipline-specific rules on documentation. Students start their education in lab notebook documentation in college, but this will continue for the rest of their professional lives as the system, and legal requirements continue to evolve. In academia, we mostly still use hardcopy, paper notebooks. Many industry groups have shifted to electronic notebooks that start with a barcode being attached to each sample entering the laboratory that is used to track the sample through the complete experimental analysis, data analysis, data reporting, and billing. This approach deters data fraud and aids in long-term storage of data, a requirement in some sectors of chemical analysis.

1.2.3 Computer-Controlled Instruments

There have been many notable advances in the history of instrumentation and these will be discussed in the following chapters, instrument by instrument. But over the entire history of instrumentation, the addition of a computer to each instrument has been the most important. Prior to the 1990s, an instrumental methods lab would consist of bored students manually injecting standards into the instrument, and maybe one or two samples before the lab period ended. Today, the student focuses more on sample preparation and programming of the computer and instrument that runs for hours or days collecting the results of their experiment. The computer-controlled system consists of an autosampler, the instrument, and a computer to manage the instrument and collect the data. The computer control is divided into two programs, the method and the sequence.

The method controls the physical parts of the instrument. In FAAS, FAES, and ICP, it controls the gas flows, turns on the flame or plasma, selects the intensity and wavelength(s) to measure, and monitors the instrument for safety. In GC systems, the computer controls the incoming gas pressures, the various gas flows via mass flow regulators, and the injector, oven, and detector temperatures. In LC, the computer controls the high-pressure pumps, the proportioning valve, the UV–vis

Table 1.1 Selected costs of analysis by chemical parameter.

Metal analysis (water and wastewater) (US dollars)	
ICP/FAAS digestion	$10.00
ICP-OES (per metal)	$11.00
ICP-MS (per metal)	$18.00
Pb and Cu	$35.00
Hg	$25.00
Heavy metal suite	$150.00
Cr^{+6}	$70.00
Organic analysis (water and wastewater (US dollars)	
Aromatics (GC-FID)	$100.00
Chlorinated HCs (GC-ECD)	$185.00
Volatiles (GC purge and trap)	$225.00
Gasoline components	$65.00

(Source: Basis 2022 data from various laboratories)

wavelengths of interest, and parameters of the mass spectrometer. In capillary electrophoresis (CE), the computer controls the potential across the column and temperature zones.

The sequence program is a data management tool. It controls and correlates the position of the sample in the autosampler and the method file to the data storage file. By combining these two computer assets, an instrument user can program the system in a few minutes, press the start key, and the instrument can operate for hours or days depending on the duration of each sample run.

The net result of a computer-controlled instrument is multifold. First, a student can spend time learning the programming of the instrument instead of waiting to inject the next sample. The cost and time savings of a computer-controlled system are enormous. Today, a technician can essentially program several days' worth of work in an hour or so. To illustrate this, we have compared the cost of a single chemical parameter analysis in the 1970s to today, and the costs are essentially the same. Examples are given in Table 1.1 for specific parameters and the costs have not significantly increased in these decades due to computer-controlled systems and mass throughput (the ability to process large numbers of samples in a shorter time period, while the cost of a chemical technician has doubled or tripled.

1.3 Questions

1. List three types of sample matrices.
2. Why does analytical chemistry not use standard reagent-grade chemicals such as acids and solvents?
3. Which types of weigh boats are best for use with (i) metals and (ii) organic chemicals?
4. Explain how external calibration works.

5 Explain how internal calibration works.

6 What is the most important "figure of merit" in the sale of an instrument?

7 What is meant by the statement "There is no zero concentration anymore"?

8 What was the most important advancement in instrumentation?

9 Describe the difference between sensitivity and selectivity.

10 Describe the results of a linear least-squares analysis.

1.4 Problems

1 Benzene is commonly analyzed using GC or HPLC. The following dataset was obtained by using the external standard calibration technique and an unknown was analyzed five times. The dataset follows:

Standard conc. ppm benzene	Peak area resulting from analysis
100	653
250	1312
600	2848
800	3726
1200	5482
Unknown	1507, 1525, 1501, 1516, 1490

Analyze the data using the linear least-squares technique on your calculator. Prepare a summary (one-page maximum) of your results and discuss (at a minimum): the equation governing the calibration curve, the errors associated with the *y*-intercept and slope, some measure of how linear the line is, the minimum detection limit that you would report (and why you selected the number that you did), and the estimated value for your unknown.

2 Most quantitative measurements require several steps in a given procedure, including weighing, dilution, and various quantification approaches. Each of these processes has an error associated with it. Suppose that you are analyzing a liver sample for a given toxin *X*. You weigh out 1.05 g of liver, dry it, extract it, dilute it, and analyze your dilution. These following steps, and the error associated with each step, are summarized in the following data summary:

Operation	Value of operation	Error associated with each operation (as ± s)
Weight (of wet liver)	1.05 g	0.01 g
Determination of dry weight (g dry liver/g wet liver)	0.40	0.05
Total volume that toxin is extracted into	100 mL	0.05 (mL)

Operation	Value of operation	Error associated with each operation (as ± s)
Extraction efficiency	0.90	0.05
Dilution 1 (1–10)	0.10	0.001
Dilution 2 (1–100)	0.01	0.0001
Volume of solvents (in most dilute solutions) analyzed	1.00 µL	0.05 µL
Error from least squares	5.62 pg	0.08 pg
Analysis and calibration curve (the amount detected in 1.00 µL of injected solvent)		

Calculate the concentration of toxin *X* in your original sample (on a dry liver basis) and the total error associated with the measurement (propagation of error; as from your quantitative analysis course). Report concentrations in µg of toxin per gram of dry liver. Show all calculations for credit.