Introduction to Instrumental Analysis

Classification of Analytical Techniques

Introduction

In quantitative chemical analysis, a sample is prepared and then analyzed to determine the concentration of one (or more) of its components. The following figure gives a general overview of this process.

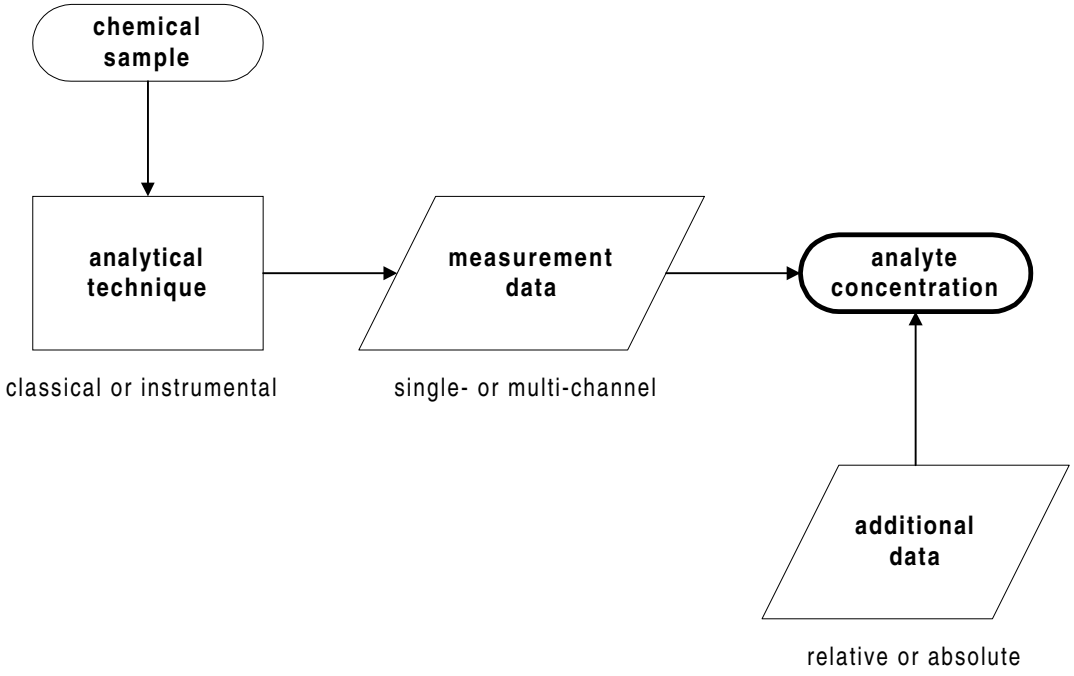

Figure 1: Schematic showing measurement steps involved in quantitative chemical analysis of a sample. There are three ways of classifying the process, based on the technique (classical *vs* instrumental), the measurement data (single-channel *vs* multi-channel), or on whether additional data is needed to estimate the analyte concentration (relative *vs* absolute).

There are a very large number of techniques used in chemical analysis. It can be very useful to classify the measurement process according to a variety of criteria:

- by the type of analytical technique – ***classical*** or ***instrumental*** techniques;
- by the nature of the measurement data generated – ***single-channel*** or ***multi-channel*** techniques; and
- by the quantitation method (by which the analyte concentration is calculated) – ***relative*** or ***absolute*** techniques.

In the next few sections, we will use these classifications to describe the characteristics of a variety of analytical techniques.

Classical *vs* Instrumental Techniques

In ***classical*** analysis, the signal depends on the chemical properties of the sample: a reagent reacts completely with the analyte, and the relationship between the measured signal and the analyte concentration is determined by chemical stoichioimetry. In ***instrumental*** analysis, some physical property of the sample is measured, such as the electrical potential difference between two electrodes immersed in a solution of the sample, or the ability of the sample to absorb light.

Classical methods are most useful for accurate and precise measurements of analyte concentrations at the 0.1% level or higher. On the other hand, some specialized instrumental techniques are capable of detecting individual atoms or molecules in a sample! Analysis at the ppm (μg/mL) and even ppb (ng/mL) level is routine.

The advantages of instrumental methods over classical methods include:

1. The ability to perform *trace analysis*, as we have mentioned.
2. Generally, large numbers of samples may be analyzed very quickly.
3. Many instrumental methods can be automated.
4. Most instrumental methods are multi-channel techniques (we will discuss these shortly).
5. Less skill and training is usually required to perform instrumental analysis than classical analysis.

Because of these advantages, instrumental methods of analysis have revolutionized the field of analytical chemistry, as well as many other scientific fields. However, they have not entirely supplanted classical analytical methods, due to the fact that the latter are generally more accurate and precise, and more suitable for the analysis of the major constituents of a chemical sample. In addition, the cost of many analytical instruments can be quite high.

Instrumental analysis can be further classified according to the principles by which the measurement signal is generated. A few of the methods are listed below. [The underlined methods are to be used in the round-robin experiments.]

1. ***Electrochemical*** methods of analysis, in which the analyte participates in a redox reaction or other process. In potentiometric analysis, the analyte is part of a galvanic cell, which generates a voltage due to a drive to thermodynamic equilibrium. The magnitude of the voltage generated by the galvanic cell depends on the concentration of analyte in the sample solution. In voltammetric analysis, the analyte is part of an electrolytic cell. Current flows when voltage is applied to the cell due to the participation of the analyte in a redox reaction; the conditions of the electrolytic cell are such that the magnitude of the current is directly proportional to the concentration of analyte in the sample solution.

2. ***Spectrochemical*** methods of analysis, in which the analyte interacts with electromagnetic radiation. Most of the methods in this category are based on the measurement of the amount of light absorbed by a sample; such *absorption-based* techniques include atomic absorption, molecular absorption, and nmr methods. The rest of the methods are generally based on the measurement of light emitted or scattered by a sample; these *emission-based* techniques include atomic emission, molecular fluorescence, and Raman scatter methods.

3. The technique of ***mass spectroscopy*** is a powerful method for analysis in which the analyte is ionized and subsequently detected. Although in common usage, the term "spectroscopy" is not really appropriate to describe this method, since electromagnetic radiation is not usually involved in mass spectroscopy. Perhaps the most important use of mass spectrometers in quantitative analysis is as a gas or liquid chromatographic detector. A more recent innovation is the use of an inductively coupled plasma (ICP) as an ion source for a mass spectrometer; this combination (ICP-MS) is a powerful tool for elemental analysis.

Although they do not actually generate a signal in and of themselves, some of the more sophisticated separation techniques are usually considered "instrumental methods." These techniques include ***chromatography*** and ***electrophoresis***. These techniques will separate a chemical sample into its individual components, which are then typically detected by one of the methods listed above.

Finally, we should note that a number of methods that are based on stoichiometry, and so must be considered "classical," still have a significant "instrumental" aspect to their nature. In particular, the techniques of ***electrogravimetry***, and ***potentiostatic*** and ***amperostatic coulometry*** are relatively sophisticated classical methods that have a significant instrumental component. And let us not forget that instrumental methods can be used for endpoint detection in titrimetric analysis. Even though potentiostatic titrimetry uses an instrumental method of endpoint detection, it is still considered a classical method.

Single-Channel *vs* Multi-Channel Techniques

So now we have classified analytical methods according to the method by which they generate the measurement data. Another useful distinction between analytical techniques is based on the information content of the data generated by the analysis:

- ***single-channel*** techniques will generate but a single number for each analysis of the sample. Examples include gravimetric and potentiometric analysis. In the former, the signal is a single mass measurement (e.g., mass of the precipitate) and in the latter method the signal is a single voltage value.

- ***multi-channel*** techniques will generate a series of numbers for a single analysis. Multi-channel techniques are characterized by the ability to obtain measurements while changing some independently controllable parameter. For example, in a molecular absorption method, an absorption *spectrum* may be generated, in which the absorbance of a sample is monitored as a function of the wavelength of the light transmitted through the sample. Measurement of the sample thus produces a series of absorbance values.

Any multi-channel technique can thus produce a plot of some type when analyzing a single sample, where the signal is observed as a function of some other variable: absorbance as a function of wavelength (in molecular absorbance spectroscopy), electrode potential as a function of added titrant volume (potentiometric titrimetry), diffusion current as a function of applied potential (voltammetry), etc. Multi-channel methods provide a lot more data – and information – than single-channel techniques.

Multi-channel methods have two important advantages over their single-channel counterparts:

1. They provide the ability to perform *multicomponent analysis*. In other words, the concentrations of more than one analyte in a single sample may be determined.
2. Multi-channel methods can detect, and sometimes correct for, the presence of a number of types of interferences in the sample. If uncorrected, the presence of the interference will result in biased estimates of analyte concentration.

Multi-channel measurements simply give more information than a single-channel signal. For example, imagine that measurement of one of the calibration standards gives the data pictured in fig 2(a):

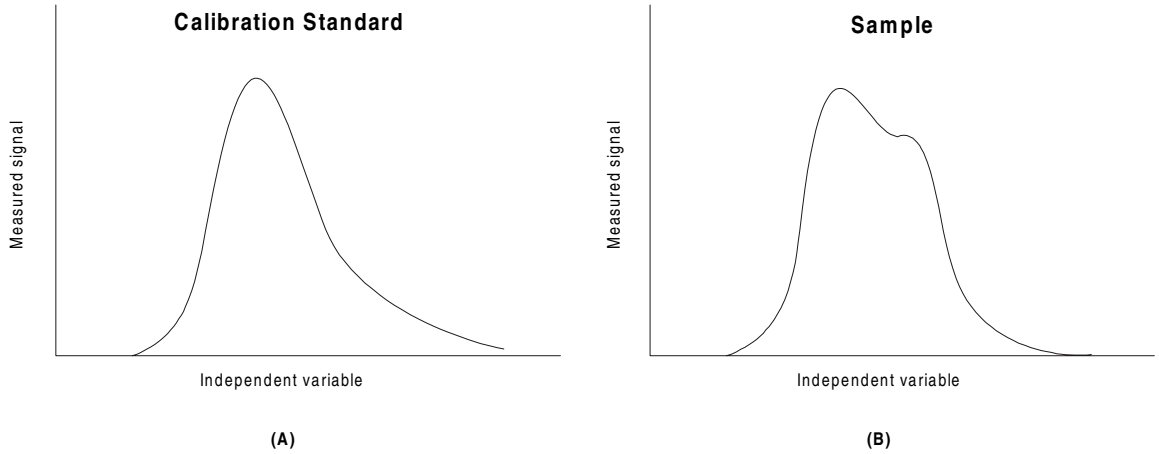

Figure 2: illustration of how multi-channel data allow for the detection of interferences. Comparison of the multi-channel signal of the (a) the calibration standard, and (b) the sample reveals that there is interference in the latter. A likely explanation is that another component of the sample (absent from the calibration standard) also gives a measurable response. The left side of the peak appears relatively unaffected by the presence of the interferent; it may be possible to obtain an unbiased estimate of analyte concentration by using one of these channels for quantitation.

Plots of the measurements of the other calibration standards (assuming they are not contaminated) should give the same general shape, although the magnitude of the signal will of course depend on the analyte concentration.

Now imagine that you obtain multi-channel measurements of a sample, recording the following data shown in fig 2(b). It is immediately obvious that the shape has changed due to some interference. A likely explanation is that some component of the sample matrix is also contributing to the measured signal, so that the result is the sum of the two (or perhaps more than two) sample components. Another possibility is that the sample matrix alters the response of the analyte, giving rise to an altered peak shape.

More than just *identifying* the presence of an interfering substance, multi-channel data often allows the analyst to *correct* for its presence. For example, if it is suspected that the altered peak in fig 2(b) is due to an additional component, then a channel can be chosen for quantitation where the interfering substance does not contribute. The left side of the peak looks unaltered, so perhaps the data in one of these channels can be used to estimate analyte concentration.

An important point: although multi-channel methods are *capable* of collecting measurements on multiple channels (e.g., different wavelengths), it is possible to use them in "single-channel" mode. In other words, to decrease measurement time, the analyst has the option of measuring the response on only a single channel (e.g., the wavelength corresponding to the peak response). If the nature of the sample or standard is well known, this may be perfectly acceptable. However, the analyst must realize that a lot of information is being thrown away – the advantages of multi-channel data described above (multicomponent analysis and detection/correction of interferences) will be lost. As a general guideline, it is always a good idea to collect the multi-channel response of *at least* one of the calibration standards to see what the analyte response looks like, and then to collect the multi-channel response of *at least* one of the samples to ensure that no interferences are present.

One last item: there is another way of classifying analytical techniques according to the measurement data produced. Rather than single- and multi-channel techniques, we may speak of the ***order*** of the analytical technique. The order is equal to the number of independent parameters that are controlled as the data is collected for each sample. Thus, single-channel techniques would be *zeroth order* methods, since only a single data point is collected. If absorbance is measured as a function of wavelength, as in molecular absorption spectroscopy, the technique is labelled *first order*. Examples of second order techniques include the following:

- gas chromatography with mass spectrometric detection (the two independent parameters are retention time and ion mass/charge ratio);
- liquid chromatography with uv/vis spectrophotometric detection (signal is determined as a function of retention time and wavelength); and
- molecular fluorescence (signal measured as a function of both excitation wavelength and emission wavelength).

As discussed, techniques with first-order data are able to identify, and in many cases correct, for the presence of interferes. Due to their ability to provide data with higher information content, second-order techniques are even more powerful than first-order methods; further discussion of the additional capabilities of these methods is beyond the scope of this course.

Relative *vs* Absolute Techniques

Another way of classifying analytical techniques is according to the method by which the analyte concentration is calculated from the data:

- in ***absolute*** analytical techniques, the analyte concentration can be calculated directly from measurement of the sample. No additional measurements are required (other than a measurement of sample mass or volume).

• in ***relative*** analytical techniques, the measurement of the sample must be compared to measurements of additional samples that are prepared with the use of analyte *standards* (e.g., solutions of known analyte concentration).

The following figure illustrates the difference between the two types of methods.

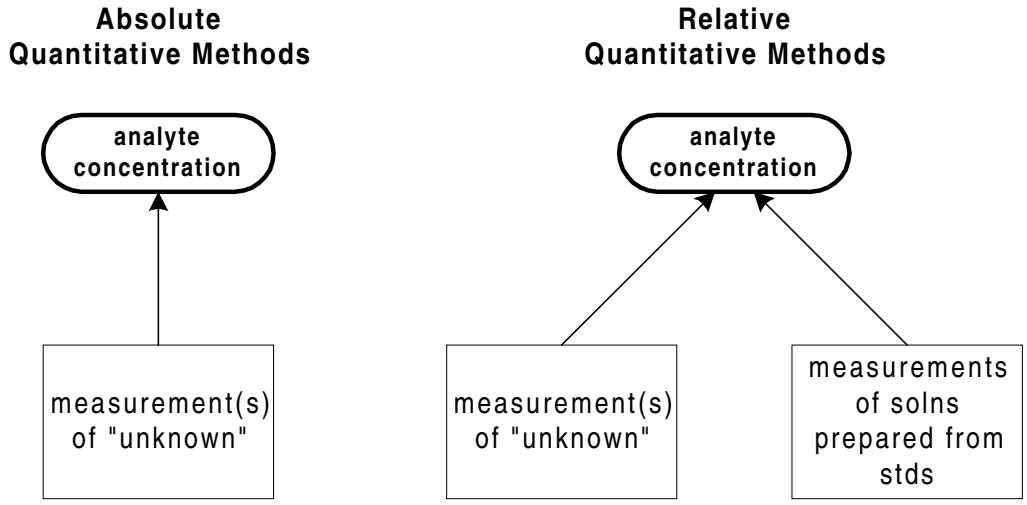

Figure 3: The difference between *absolute* and *relative* techniques is that the latter requires additional measurements in order to obtain an estimate of the analyte concentration.

Classical methods of analysis are considered absolute techniques, because there is a direct and simple relationship between the signal (mass in gravimetry; endpoint volume in titrimetry) and the analyte concentration in the sample. The vast majority of instrumental methods of analysis are relative methods: the measurement of the sample solution must be compared to the measurement of one or more solutions that have been prepared using standard solutions. The most common methods of quantitation for instrumental analysis will be described shortly.

Summary: Characterization of Analytical Techniques

There are a large number of techniques used for quantitative chemical analysis. As we have discussed, any analytical technique may be classified according to a variety of criteria, revealing something of the its characteristics. Table 1 on the following page summarizes the techniques discussed in this course.

Table 1: Characterization of analytical techniques discussed in this course. For each technique, the table states the property being measured (second column); for multi-channel techniques, the independent parameter is also given. So, for example, we can see that in potentiostatic coulometry (6th row), current is measured as a function of time. The last column states how the measured quantity is determined by the analyte concentration in the sample.

Technique	Quantity Measured	Single- or multi-channel? (*independent parameter*)	Theoretical Principle
Classical Techniques – all absolute methods[a]			
gravimetry	mass	single-channel	complete/selective rxn of analyte; composition of weighing form is known
electrogravimetry	mass	single-channel	
titrimetry (chemical indicator)	endpoint volume/mass	single-channel	complete/selective rxn of analyte; known stoichiometry of titration reaction
titrimetry (instrum endpt detection)	instrument signal	multi-channel (*volume/mass of titrant solution*)	
amperostatic coulometry	time	single-channel	complete/selective rxn of analyte; Faraday's Law, and the known stoichiometry of titration reaction
potentiostatic coulometry	current	multi-channel (*time*)	
Instrumental Techniques – all relative methods[b]			
potentiometry	potential	single-channel	thermodynamic drive to equilibrium (Nernst)
voltammetry	current	multi-channel (*working electrode potential*)	analyte diffusion controls signal (Fick's Law)
atomic absorption	attenuation of light intensity	single-channel[c]	Beer's Law
molecular absorption	attenuation of light intensity	multi-channel (*wavelength*)	
atomic emission	emitted light intensity	multi-channel (*wavelength*)	signal is proportional to excited-state concentration
molecular fluorescence	fluorescence light intensity	multi-channel[d] (*excitation wavelength* and *emission wavelength*)	

[a]strictly speaking, titrimetry is only an absolute method if the titrant solution is prepared from a primary standard

[b]molecular absorption can be an absolute method if the analyte absorptivity is known and the instrument is properly calibrated

[c]although usually single-channel, atomic absorption may be multi-channel in some cases

[d]molecular fluorescence is the only second-order technique listed in this table: the signal can be measured as a function of two independently adjustable parameters

Methods of Quantitation for Instrumental Analysis

Instrumental techniques are almost all *relative* in nature: the signal obtained from the analysis of the sample must be compared to other measurements in order to determine the analyte concentration in the sample. Since these other measurements naturally contain measurement error, relative quantitation increases the overall error in the estimate of analyte concentration – we shall refer to this source of error as ***calibration error***. Calibration error can contain both random and systematic components. One of the advantages of classical methods over instrumental methods is the absence of calibration error, since classical methods are *absolute* in nature.

Classical methods are absolute because of the direct relationship between the quantity measured and the analyte concentration. Why isn't the same thing true of instrumental methods? There are a wide variety of instruments used for analysis, and they can generally be broken down into four components:

1. A *signal generator*, in which the analyte in the sample results in the production of some form of energy (such as light or heat);
2. A *transducer*, or "detector," that transforms the energy produced in the signal generator into an electrical signal (usually a voltage or a current);
3. Various *electronic components*, such as amplifiers and filters, that "clean up" the electrical signal; and
4. A *read-out device*, such as a chart recorder, an analog meter, an oscilloscope or a computer, that converts the electrical signal into a form that is usable by the analyst.

For example, let's consider the process involved in the method of *flame atomic emission*, in which the analyte solution is "sprayed" into a hot combustion flame.

- the analyte atoms will absorb thermal energy from the flame, and will release some of this energy in the form of light. The light is collected by and separated into its component wavelengths. This part of the instrument can be considered the "signal generator," because the amount of light at a certain wavelength will be proportional to the concentration of analyte atoms in the flame.
- light of the proper color (i.e., light emitted by the analyte atoms) is directed to strike a photon detector, which produces a current.
- this photon-induced current is amplified and converted into a voltage
- the voltage is then used to drive the pen of a strip-chart recorder.

This description illustrates that there is no simple relationship between the data produced by the read-out device of an analytical instrument and the concentration of the analyte in the chemical sample. This is in contrast to the case in classical analysis, where the relationship between measured values such as mass or volume and the analyte concentration is fairly direct, and can be calculated from the stoichiometry of the chemical reaction.

To see why the relationship between signal and analyte concentration in instrumental analysis is so complicated, let's go back to our example of flame atomic emission, where:

- the distance moved by the pen in the chart recorder is proportional to the voltage output of the electronics in the instrument;
- the voltage output is proportional to the current produced by the light detector;
- the current produced by the light detector is proportional to the light intensity striking the detector;
- the light intensity striking the detector is proportional to the intensity of light emitted by the analyte atoms in the flame;
- the emission intensity from the flame is proportional to the number of analyte atoms in the flame; and finally
- the number of analyte atoms in the flame is proportional to the concentration of analyte in the sample solution.

Whew! Although the end result of all this hand waving is that the data produced by the instrument is proportional to the analyte concentration in the sample, it is not a relationship that is readily amenable to theoretical treatment. Instead, we must estimate the analyte concentration in the sample by using solutions of known analyte concentration.

The two most common methods of calibration in instrumental analysis are (i) the use of ***calibration curves***, and (ii) the ***method of standard additions***. In addition, ***internal standards*** may be used in combination with either of these methods. We will now describe how these methods may be used in quantitative chemical analysis.

Calibration Curve Method

For any instrumental method used for quantitative chemical analysis, there is some functional relationship between the instrument signal, r, and the analyte concentration, C_A:

$$r = f(C_A)$$

The calibration curve approach to quantitation is an attempt to estimate the nature of this functional relationship. A series of ***calibration standards*** are analyzed, and a "best-fit" line or curve is used to describe the relationship between the analyte concentration in the calibration standards and the measured signal. The following figure demonstrates the concept.

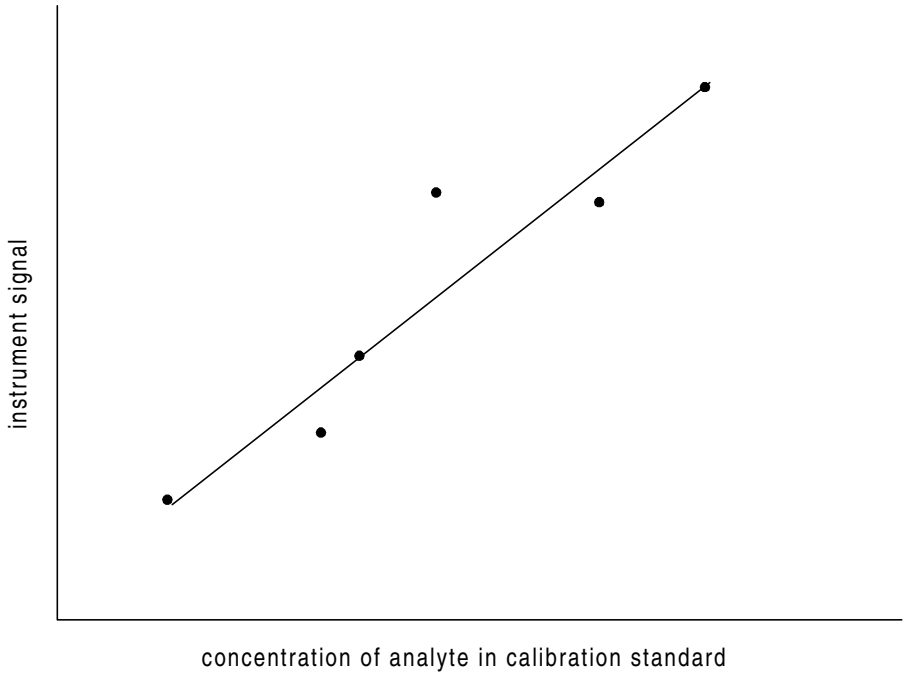

Figure 4. Typical calibration curve. The instrument response is measured for a series of calibration standards, which contain a known concentration of analyte. The curve is a function that describes the functional relationship between signal and concentration. Note that the calibration curve should never be extrapolated (i.e., never extended beyond the range of the calibration measurements).

The least-squares regression technique is the usual method of obtaining the best-fit line. Although the functional form of the fitted function may be dictated from theory, its main purpose is to allow the analyst to predict analyte concentration in measured samples. Thus, the main criterion in choosing the regression model is to choose one that enables accurate (and precise) predictions of analyte concentration in samples of unknown composition.

The following points should be made about this method of quantitation:

1. The central philosophy of the calibration curve method is this: *the function that describes the relationship between signal and concentration for the calibration standards* ***also applies*** *to any other sample* that is analyzed. Any factor that changes this functional relationship will result in a biased estimate of analyte concentration.

2. A linear relationship between signal and concentration is desirable, generally resulting in the best accuracy and precision using the fewest number of calibration standards.

3. Ideally, the analyte concentration should only be calculated by *interpolation*, not by *extrapolation*. In other words, the analyte concentration should be within the range of concentrations spanned by the calibration standards. If the analyte concentration in the sample is too great, then the sample may be diluted. If the analyte concentration is too small, then additional calibration standards can be prepared. For best precision, the concentration is close to the mean concentration of the calibration standards.

If we can assume a linear relationship between signal and concentration, then simple linear regression may be used. A point estimate of the analyte concentration in the "unknown" is calculated from the following equation:

$$\hat{x}_u = \frac{y_u - b_0}{b_1} \qquad \textbf{[1]}$$

where $\hat{x}_u$ is the point estimate, y_u is the signal measured for the "unknown," and b_1 & b_0 are the least-squares estimates of the slope and intercept of the best-fit line.

The point estimate is a random variable because it is calculated from three other random variables. There are two sources of error in the point estimate: measurement error in y_u and error in the least-squares estimates b_1 and b_0. The latter error arises due to error in the measurement of the calibration standards (i.e., due to calibration error).

The random error of $\hat{x}_u$ due to both sources (i.e., random error in "unknown" measurement and in the calibration measurements) can be estimated using the following expression:

$$s(\hat{x}_u) \approx \frac{s_{res}}{b_1}\sqrt{1 + \frac{1}{n} + \frac{(\hat{x}_u - \bar{x})^2}{S_{xx}}} \qquad \textbf{[2]}$$

where s_{res} is the standard deviation of the residuals, n is the number of calibration standards, $\bar{x}$ is the average analyte concentration in the calibration standards, and $S_{xx} = (n-1)s_x^2$, where s_x is th standard deviation of the concentrations of the calibration standards. The second two terms in the square root term accounts for the effects of random error in the calibration measurements.

Exercise 1. The following data was obtained in the analysis of copper using flame atomic absorption spectroscopy

conc, ppm	% transmittance
5.1	78.1
17.0	43.2
25.5	31.4
34.0	18.8
42.5	14.5
51.0	8.7

Following calibration, a sample of unknown copper concentration was analyzed. The measured transmittance was 35.6 %. Report the concentration of analyte in the form of a 95% confidence interval.

Answer: 21.8 ± 3.9 ppm (95% CI). This confidence interval accounts for the uncertainty introduced in measurements of both the "unknown" and of the calibration standards.

Equations 1 and 2 are intended for linear calibration curves. Equation 2 depends on the usual assumptions made in linear regression (e.g., measurement errors of standards and samples are homogeneous). A variety of other regression methods are available to estimate calibration curves, including polynomial regression, nonlinear regression, and weighted regression methods.

Standard Addition Method

Introduction

Inherent in the calibration curve method is the key assumption that the analyte "behaves" (i.e., generates signal) in the sample exactly as it does in the calibration standards. In other words, the calibration curve function is assumed to apply equally to any sample as to the calibration standards. However, the sample may be much more complex than the calibration standards, and the interaction of the analyte with the other components of the sample may alter its signal. Thus, the calibration curve may *not* describe the relationship between analyte concentration and signal that actually exists in the sample.

For example, one may with to determine the concentration of lead in seawater by using flame atomic absorption spectroscopy. The calibration standards might be made by dissolving a lead salt into deionized water. The other components of the seawater may well change the efficiency of lead atomization in the flame, altering its signal. This effect is absent in the calibration standards, and a biased estimate of lead concentration in seawater would be obtained.

The phenomenon just described is an example of a ***matrix effect***. The *sample matrix* is the portion of the sample that does not include the analyte: in other words, the entire sample consists of analyte plus its matrix. A matrix effect occurs whenever some component of the sample matrix changes the analyte's signal – for whatever reason (chemical reaction, changing the ionic strength, etc).

The method of standard additions is meant to be used in this case. Whenever there is reason to suspect that the calibration curve approach will not work due to the presence of a matrix effect, the method of standard additions may give more accurate results.

Standard Additions: Dilution to Constant Volume

One way of using standard additions is as follows. Imagine that we have a sample solution, and we divide it up into four solutions as shown in table 2:

Table 2. One way to perform the standard additions method of analyte quantitation. The sample solution is divided into four equal 10 mL portions. Each portion is eventually diluted to 50 mL; the solutions have different volumes of added standard, and hence different concentrations of analyte.

	#1	#2	#3	#4
volume sample added, mL:	10	10	10	10
volume standard added, mL:	0	0.1	0.2	0.3
volume solvent added, mL:	40	39.9	39.8	39.7
total volume, mL:	50	50	50	50

In other words, from our sample solution we obtain four new 50 mL solutions, each of which contains 10 mL of the original sample solution. In addition, to each new solution a certain volume of standard analyte solution is added (the concentration of this solution is known). The volume of added standard is kept small so that it has little effect on the matrix; presumably, the final solutions have identical sample matrices, and so *the analyte should be affected by the matrix equally in all the solutions*. This is the key assumption in the standard addition method.

Note that we can easily write an expression for the concentration of analyte in the new solutions:

$$C_{new} = \frac{C_{std}V_{std} + C_A V_A}{V_{tot}} \qquad \textbf{[3]}$$

where C_{std} and C_{A} are the concentration of analyte in the standards and the original sample solution, respectively, and V_{std} and V_{A} are the volumes of added standard and sample, respectively. The volume V_{tot} is the total volume of the new "sample" solutions.

Why should we divide up the sample like this? Looking at the four new solutions, it should be obvious that *each of them is exposed to the same matrix.* If we obtain measurements on each of the sample, then these measurements will all be equally affected by chemical interferences. In this manner, we can match matrices for all the measured solutions. Thus, the only task left is to calculate C_{A}, the concentration of analyte in the original sample, from measurements that are obtained from our new samples. This objective can be achieved by using a *standard additions plot*. If the signal is linearly proportional to the analyte concentration, then the plot will look like the following

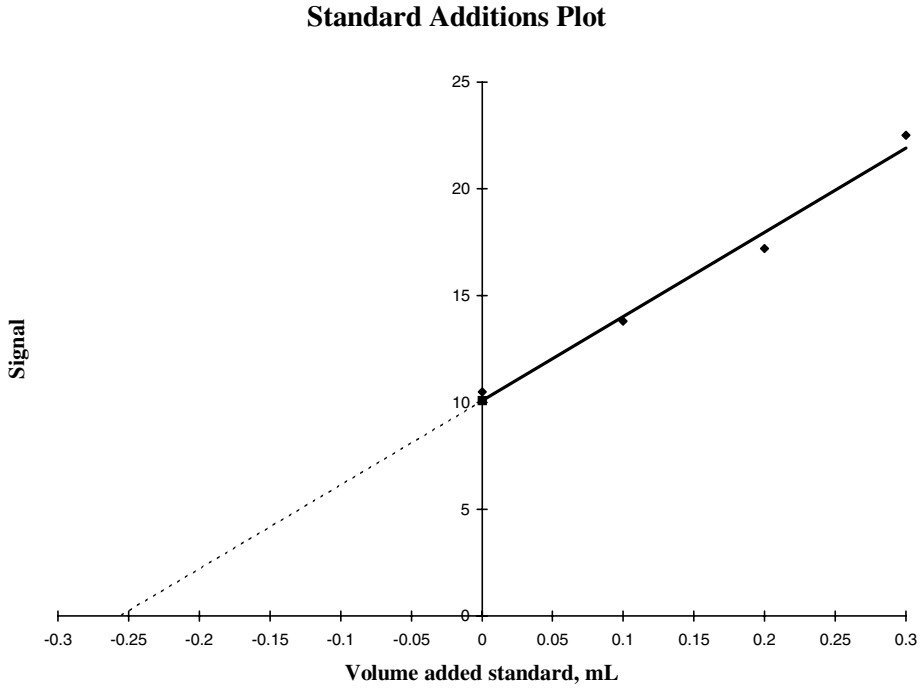

Figure 5: A standard additions plot. The signal is plotted as a function of the volume of added standard. The slope and intercept of the best-fit line can be used to estimate the analyte concentration in the original sample (see text for equations).

In a standard additions plot, the measured signal is plotted as a function of V_{std}, the volume of added standard. How does this plot help us to calculate the concentration of analyte in the original sample? The value of the intercept of the fitted line with the x-axis is $-V'$, which is calculated as

$$V' = \frac{b_0}{b_1}$$

where b_0 and b_1 are the intercept and slope, respectively, of the fitted line. It turns out that the volume, V', is the *volume of the standard solution that contains the same amount of analyte as the original sample*. The following figure shows this concept graphically:

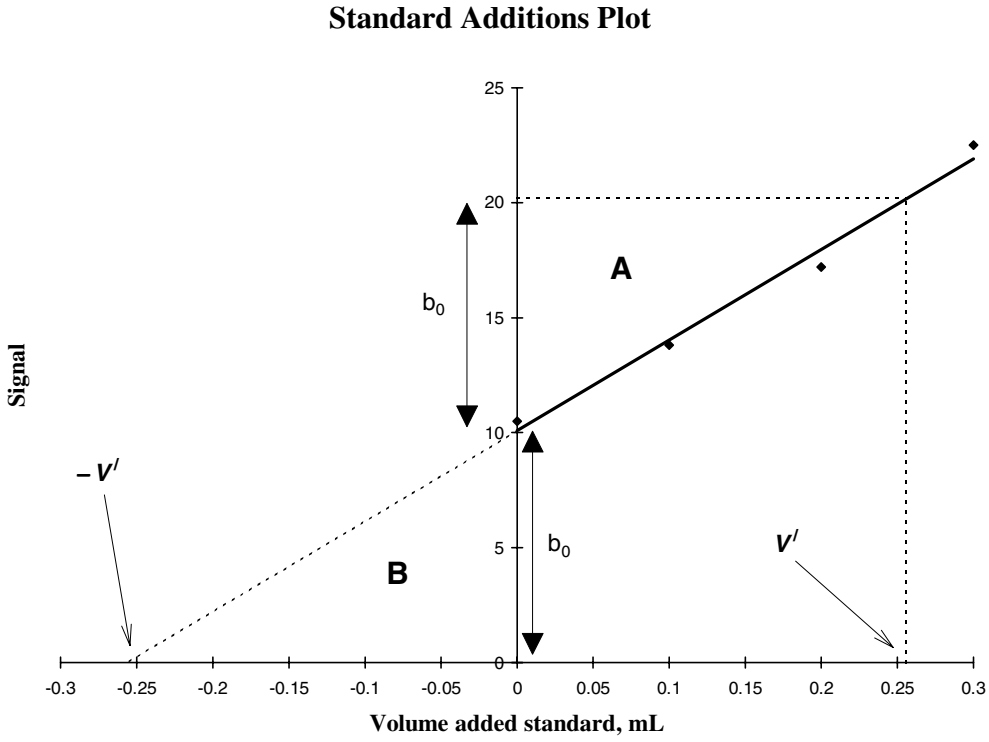

Figure 6. The "geometry" of the standard additions plot, explaining how V' is the volume of standard that would double the measured signal. Since a linear relationship between signal and concentration is assumed, V' is also the volume of standard that contains the same amount of analyte that was present in the original solution.

The goal of the standard additions plot is to obtain the value of V', the volume of standard that contains the same quantity of analyte as in the original sample solution. We can obtain this value in one of two ways: by extrapolating the plot to the x-axis, or by solving for $y = 2b_0$, where b_0 is the y-intercept of the standard additions plot. Both of these are shown in the figure. Since the triangle **A** and **B** are equivalent (i.e., same lengths and angles), then both of these methods of obtaining V' are equally valid. We will use the second approach, solving for $y = 2b_0$, to obtain expressions that allow us to determine the analyte concentration in the sample from the standard addition plot.

The *y*-intercept, b_0, corresponds to the signal from the sample that contains with no added standard; a signal of b_0 is due solely to the contribution of the analyte contained in the original sample. A signal of double this value, $y = 2b_0$, must correspond to *twice* the concentration that was in the original sample; thus, the volume of standard that is required to reach this point ($y = 2b_0$) must contain the same quantity of analyte as was in the original sample. This volume is indicated on the figure as V'.

Mathematically, we can express this idea as follows. We assume that the signal is directly proportional to the analyte concentration,

$$r = kC_A$$

where r is the signal ("response") and k, the constant of proportionality, is the sensitivity of the analytical procedure.

The analyte concentration in the new standard addition solutions is given by eqn. 3; thus, we can say that the signal for these new solutions will be

$$r = k\left(\frac{C_{std}V_{std} + C_A V_A}{V_{tot}}\right) = b_1 V_{std} + b_0 \qquad \textbf{[4]}$$

Thus, a plot of signal r against the volume of added standard, V_{std} will be a straight line with a slope of b_1 and an intercept of b_0, where

$$b_1 = \frac{kC_{std}}{V_{tot}} \qquad b_0 = \frac{kC_A V_A}{V_{tot}} \qquad \textbf{[5]}$$

We want to solve for $r = 2b_0$, where $V' = V_{std}$

$$2b_0 = b_1 V' + b_0$$

$$\boxed{V' = \frac{b_0}{b_1}} \qquad \textbf{[6]}$$

Substituting for b_0 and b_1, we see that

$$V' = \frac{C_A V_A}{C_{std}}$$

Rearranging this expression gives $C_{std}V' = C_A V_A$ **[7]**

Thus, we have just proven that V' is the volume of standard that contains the same quantity of analyte as in the original volume V_A of the sample solution. We can now see how to estimate the analyte concentration in the original sample solution from the standard addition plot:

$$\boxed{C_A = C_{std}\frac{V'}{V_A}} \qquad \textbf{[8]}$$

Thus, eqns 6 and 8 can be used to calculate the concentration of analyte in the original sample.

Standard Additions Method 2: Direct Addition of Standard

In the previous standard additions method, a number of sample solutions were each diluted to a constant volume. Although this approach achieved our goal of perfect matrix matching for each

of the new solutions, there is an important disadvantage with the method: *the analyte is diluted* in the new solutions, thus decreasing the sensitivity of the analysis.

There is another method of standard additions that does not suffer from this disadvantage. Instead of preparing separate solutions, diluted to constant volume, we can use the following procedure:

1. Measure the signal for a known volume of sample solution;
2. Add a *very small* volume of concentrated standard solution directly to the sample solution;
3. Measure the signal for the new solution, after the addition;
4. Repeat steps 2 & 3 as many times as desired.

As before, we want to keep the volume of added standard much smaller than the volume of the original sample so that the sample matrix is not changed by the additional volume. Although the new method does not dilute the analyte, there is one problem: the total volume of the solution will change for each measurement following a standard addition. Equations 3 and 4 show that if we thus plot the signal, r, against the volume of added standard, V_{std}, the slope of the plot will not be constant, since V_{tot} is changing. Thus, the standard addition plot will be slightly curved.

Fortunately, we can correct for the effects of increasing V_{tot}. To see this, notice that we can rearrange eqn. 4 as follows:

$$r\left(\frac{V_{tot}}{V_A}\right) = \frac{kC_{std}}{V_A}V_{std} + kC_A = b_1 V_{std} + b_0$$

If we plot the *dilution corrected response*, $r_{corr} = r\frac{V_{tot}}{V_A}$, against V_{std}, we will obtain a straight line with slope b_1 and intercept b_0, where

$$b_1 = \frac{kC_{std}}{V_A} \qquad b_0 = kC_A \qquad \textbf{[9]}$$

If we again define V' as the volume of added standard that gives a dilution corrected response of twice the y-intercept, $2b_o$, we would again find that

$$V' = \frac{b_0}{b_1} = \frac{C_A V_A}{C_{std}}$$

just as before. Thus, *we can still use the same equations* to calculate the concentration of the analyte in the original sample solution; we just have to use a slightly different standard addition plot.

Calculating Confidence Intervals from a Standard Addition Plot

The expression for V' in eqn. 6 has two random variables, b_0 and b_1, which are calculated from a linear regression of the standard addition plot. Thus, the value of V' will also include random error, which will propagate to the final results, the calculated analyte concentration. It turns out that the standard error of V' is given by the following approximation (which usually works pretty well):

$$\boxed{s(V') \approx \frac{s_{res}}{b_1}\sqrt{1 + \frac{1}{n} + \frac{(V' + \bar{x})^2}{S_{xx}}}} \qquad \textbf{[10]}$$

where s_{res} is the standard deviation of the residuals of the standard addition plot, b_1 is the slope of the standard addition plot, n is the number of points in the standard addition plot, $\bar{x}$ is the average of the x-data (i.e., the standard addition volumes), and $S_{xx} = (n-1)s_x^2$. This equation is very similar to one that describes the standard error in concentrations calculated using a calibration curve; you should note, however, that the third root term involves a *sum* in the numerator $(V' + \bar{x})$ and not a difference, as with the calibration curves.

Once the standard error in V' has been calculated, we can use propagation of error to show that

$$s(C_A) = s(V')\frac{C_{std}}{V_A} \qquad \textbf{[11]}$$

Let's do an example to show how to calculate a confidence interval for the analyte concentration using the method of standard additions.

Exercise 2. Anodic stripping voltammetry can be used to measure the leachable lead content of pottery and crystalware. A sample of pottery being considered for import is leached with 4% acetic acid for 24 hr. A 50.00 mL aliquot is transferred to an electrolysis cell, and after controlled-potential electrolysis at -1.0V (vs SCE) for 2 minutes, an anodic scan is recorded with the differential pulse method. The procedure is repeated with new 50.00 mL aliquots to which 25, 50, and 75 µL spikes of a standard containing 1000 ppm lead have been added.

Volume added Standard, µL	Limiting Current, µA
0	2.5
25	3.7
50	4.9
75	6.0

Answer: 1.07 ± 0.16 ppm (95% CI).

Characteristics of the Standard Additions Method

- advantage over calibration curve method: can be more accurate for complicated sample matrices (corrects for changes in the slope of the calibration line due to the sample matrix)
- caveats: (i) must be linear; (ii) an extrapolation method; (iii) must correct for background; (iv) less precise than calibration curve method; (v) more time consuming when many samples must be analyzed

Internal Standard Method

An ***internal standard*** is a substance that is added to every sample that is analyzed; the concentration of the internal standard is known. In most cases, the concentration of the internal standard is constant in each sample that is analyzed. In this case, the analyte measurement is simply divided by the internal standard measurement, and quantitation proceeds as before. Thus, a "corrected" signal signal may be defined as

$$r_{corr} = \frac{r_A}{r_{is}}$$

where r_A is the signal from the analyte, and r_{is} is the signal from the internal standard. This method may be used in combination with either the calibration curve or standard additions method: the corrected signal, r_{corr}, is used instead of r_A when determining the best fit calibration curve (or in the standard addition plot, as appropriate).

The purpose of the internal standard is to improve the precision of the estimate of analyte concentration. Why is the precision improved? The following example illustrates the general concept.

Exercise 3. In the analysis of sodium metals by flame atomic emission spectroscopy, lithium may be used as an internal standard. Using the data below, calculate the concentration of sodium in the sample. Compare the precision of the result from the internal standard method with that achieved with the calibration curve method (i.e., if the lithium emission signals were ignored).

solution	Na emission	Li emission
0.2 ppm Na, 500 ppm Li	0.22	48
0.5 ppm Na, 500 ppm Li	0.53	47
2.0 ppm Na, 500 ppm Li	2.30	51
5.0 ppm Na, 500 ppm Li	5.00	46
"unknown" sample, 500 ppm Li	0.88	48

Answer: When the internal standard measurements are used, the following confidence interval is obtained:

with internal standards 0.82 ± 0.20 ppm (95% CI)

When the internal standard measurements are ignored, and the usual calibration curve calculations are obtained, the following confidence interval is the result:

without internal standards 0.79 ± 0.87 ppm (95% CI)

That's quite a difference! Why does the presence of the internal standard matter so much?

The basic concept is that the internal standard is supposed to behave just like the analyte, and yet the measurement technique can distinguish between the two (*a multi-channel method is required*). In this way, many sources of error will affect **both** internal standard and analyte. In the above example, fluctuations in sample uptake and in flame temperature will affect the signal of both sodium and lithium, and to approximately the same degree. Since the lithium is present at the same concentration in all of the samples, ideally its emission signal would be constant. Presumably, an increase in analyte signal (due to a change in flame temperature, for example) would be reflected in an increase in the internal standard signal. The correlation between the signals of the internal standard and the analyte is the key to this method.

Interferences in Quantitative Analysis

Types of Interferences

Introduction

Ideally, the only property of a sample that affects the data collected from an analytical procedure is the concentration of the analyte in the analyte. Inevitably, there will be other properties that will affect the measurements obtained in chemical analysis; common examples of such properties include temperature, pH, ionic strength, or solution turbidity. Changes in these properties can thus also cause changes in the measurements that are unrelated to analyte concentration, leading to errors; such properties are thus called ***interferences***, since they "interfere" with the proper determination of analyte concentration.

Interferences may be broadly classified into two types: (a) *chemical* interferences, which are due to the presence of specific chemicals in the sample, and (b) *physical* interferences. The effects of changing ionic strength or pH are examples of chemical effects, while that of temperature or turbidity are physical phenomenon. In this section, we will mostly be concerned with chemical interferences that might be present in the sample, and methods that are used to overcome their presence.

Generally speaking, any chemical sample consists of two parts:

1. the analyte(s) to be quantitatively determined, and
2. the rest of the sample, called the ***sample matrix***.

The sample matrix is simply all the components of the sample for which quantification is not necessary. However, the signal due to any individual analyte can be influenced by any other component of the sample, including other analytes and components of the matrix. The effect of chemical interferences are often called ***matrix effects***; the two terms can be used interchangeably.

Additive and Multiplicative Matrix Effects

The presence of a chemical interferent can affect the measured signal in one of two ways:

1. The interferent can *directly* affect the signal, usually causing an increase in the signal. For example, the electrode used for pH measurements will respond to sodium cations as well as hydronium cations, so that the presence of a high concentration of Na^+ will cause an increase in the measured voltage. Likewise, in the gravimetric analysis of Cl^- using Ag^+ as a precipitating agent, the presence of the interferent Br^- will cause an increase in the mass of the precipitant. These types of interferences are sometimes called ***blank interferences***. Even if the analyte is not present in the sample, the presence of a blank interferent will cause a measurable signal.

2. The interferent can *indirectly* affect the signal, most commonly causing a decrease in the signal. For example, consider the gravimetric determination of Al using NH_3 as a precipitating agent (which causes precipitation of hydrous aluminum oxide). The presence of fluoride in the solution will cause the formation of aluminum fluoride complex, which will not precipitate. Another example: the presence of dissolved oxygen in solution will reduce (*quench*) the fluorescence of many organic molecules. These type of interferences are sometimes referred to as an ***analyte interferences***, since they usually alter the response of the analyte to the analytical method. If the analyte is not present in the sample, then no signal will be measured, since the analyte interferents do not themselves directly cause a measurable signal.

The effects of chemical interferences can also be described in another way. Consider that most analytical procedures are described by a linear response of the type

$$y = \beta_1 x + \beta_0$$

where y is the measured signal, x is the analyte concentration. The value of β_1 is the slope of the calibration line, also called the *sensitivity*, while β_0 is the intercept of the line, sometimes called the *blank response*. Since the term "interference" is defined as a change in signal that is not due to the change in analyte concentration, *the presence of chemical interferents must change either the sensitivity*, β_1, *or the blank response*, β_0:

- chemical interferents that *indirectly* affect the signal (the so-called "analyte interference" effect) change the sensitivity of the analyte. This type of a matrix effect can be called a ***multiplicative effect***.
- chemical interferents that *directly* change the signal (the "blank interference" effect) change the value of β_0, the blank response. This type of a matrix effect can be termed an ***additive effect***.

The following figures show the effect of the presence of an additive or multiplicative interferent on the response function of an analytical technique.

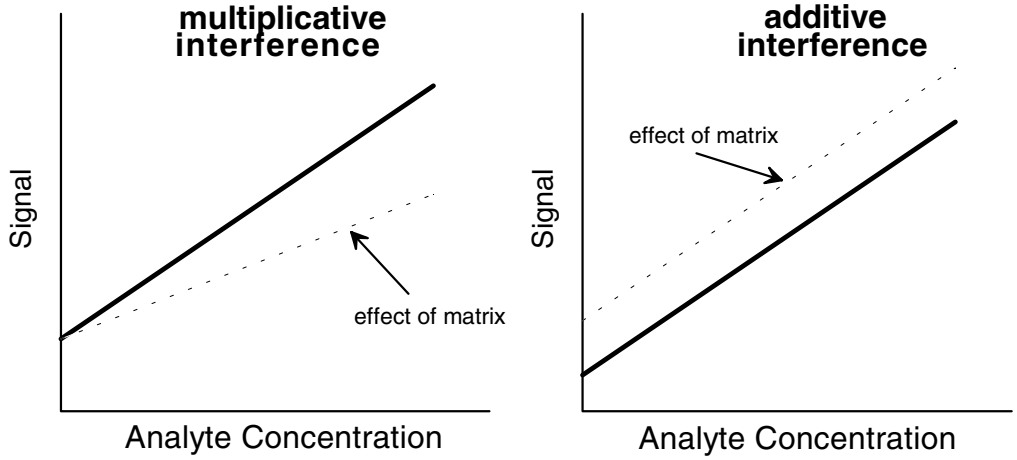

Figure 7. Illustration of the effect of the matrix on the relationship between analyte concentration and measured signal. An additive effect introduces an additional offset to the observed signal, while a multiplicative effect changes the analyte's sensitivity.

Both types of interferences, ***if uncorrected***, will result in systematic error in the determination of analyte concentration. We will discuss methods used to correct for (mostly chemical) interferences in quantitative analysis.

Correction for Interferences: General Methods

We will now discuss a few general approaches that can be used to correct for the effect of interferences (both chemical and physical) in quantitative analysis.

Elimination

The most straightforward method of dealing with interferences is to eliminate the source, if possible. For example, chromatographic methods are commonly used for the analysis of compounds in complicated sample matrices, since the analytes are separated from the other components of the matrix. There are numerous other methods used to separate the analyte(s) from interferences in the sample matrix, such as solvent extraction, solid phase extraction, centrifugation, immunoassays, electrophoresis, and many others.

Control

In some cases, elimination of interferences is either not possible or too difficult. For example, the analyte signal is often affected by temperature of pH, and these effects cannot be removed. In such cases, it is possible to eliminate errors caused by the interferences by simply controlling the effect of the interference. If the interference is present to the same degree in all the calibration standards and the samples that are analyzed, then it will not cause any systematic error. For example, if pH affects the signal, we would buffer all standards and samples to the same pH value. Likewise, we can keep the temperature constant for all standards and samples.

Correction

There are situations where interference can neither be completely eliminated nor controlled. For example, in environmental analysis it is often desirable to perform rapid "on-site" analysis, rather than transporting samples back to a laboratory. You may want to measure the pH of the water in a lake, a measurement that is affected by temperature. Obviously, you cannot control the temperature of the lake water, or eliminate the interference. What you might do instead is to measure the temperature of the water at the same time as you measure the pH (some pH meters actually do this automatically). Then you can adjust for the effect of temperature on your pH measurement.

Correcting for the effects of interference by this method means that you must make at least two measurements: one that relates to the analyte concentration (and includes the effect of the interference) and one to assess the magnitude of the interference. There are two general methods of then adjusting for the interference:

- use some *theoretical* expression to correct for the interference; or
- use an *empirical* adjustment factor, based on the results of previous experiments.

The empirical approach is probably more common. For example, in the measurement of the pH of water that contains sodium cations, the measured voltage is known to exhibit the following behavior:

$$E_{meas} = B + m\log([H^+] + k_s[Na^+])$$

where the values of B, m and k_s must be determined empirically (i.e., during the calibration of the instrument). Once the values of these parameters are known, then two measurements are necessary to determine the pH of water containing substantial amounts of sodium cation: one measurement with the pH meter, and another measurement to determine the concentration of sodium cations. This second measurement must be made by some other analytical techniques (perhaps using a sodium ion-selective electrode).

Correction for Matrix Effects (Chemical Interferences)

In the next few sections we will concerned with a few methods that correct for a variety of matrix effects (i.e., additive and multiplicative chemical interferences). The most direct method to correct for matrix effects is to separate the analyte from the matrix by a separation method such as extraction or chromatography. Essentially, the matrix effect is eliminated by getting rid of the matrix! This is the "brute force" approach

In this section, we will be discussing methods that can be used instead of (or perhaps in addition to) this approach. These methods do not necessarily *eliminate* the source of the chemical interference, but instead try to correct for the bias introduced by the interference. The methods we will discuss are basically specific applications of the general principles of *control* and *correction* discussed in the last section.

Before plunging into this topic, it is probably worthwhile to quickly describe some of the most common ways in which the matrix can cause systematic errors.

- the matrix of the sample is different than that of the calibration standards, causing either an additive or multiplicative effect on the signal. Essentially, the calibration curve does not really "apply" to the sample.
- you are analyzing a number of samples, and the matrix of these samples is somewhat variable. Likewise the additive and/or multiplicative effect of the matrix of the samples will be variable. In this instance, it is difficult[1] to prepare a single set of calibration standards that will apply to each of the samples.

Of course, there are many other ways in which matrix effects can cause error(s) in quantitative analysis. Nevertheless, the methods described in this section are remarkably general in their ability to correct for matrix effects.

Blank Measurements

Recall that a sample is composed of (a) analyte and (b) everything else (i.e., the sample matrix). A ***blank*** is a sample that contains no analyte, only the sample matrix. If a blank is available for a

[1] but not impossible! Some multivariate calibration methods – which are a little too advanced to be discussed in this class – can handle this sort of situation.

particular sample, it can be used to correct for additive interferences by simply subtracting the signal measured for the blank from that observed for the sample:

$$\text{net signal} = \text{signal from sample} - \text{signal from blank}$$

Since the sample matrix may change from sample to sample, this would mean that, theoretically, every sample needs a different blank. Most commonly, however, it is assumed that the sample matrix does not change for a group of related samples (e.g., seawater samples), and so a single blank is prepared for this group. If the amount of additive interference changes for one of the samples in the group, then subtraction of the blank will not fully correct for the interference. Obviously, the ability to correct for additive interference will depend on the ability to obtain a true blank.

The use of multi-channel analytical methods can sometimes help correct for additive interferences without the use of a blank. For example, consider the situation in the following figure, which depicts the multi-channel response that might be obtained for a sample.

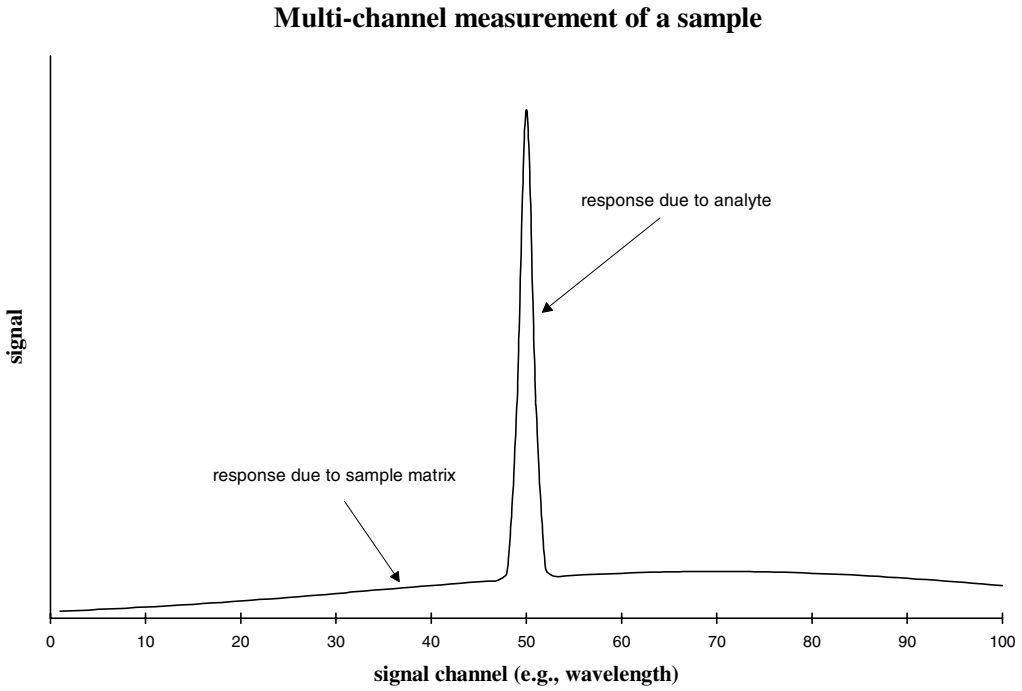

Figure 8. Detecting additive matrix interference using multi-channel data. When the sample matrix gives a very broad background, as shown here, it is particularly easy to correct for the interference (no blank is necessary); see fig 9 for a very simple correction method.

This type of response could easily be seen in an atomic spectroscopy technique, such as atomic emission, where the analyte response generally consists of narrow spectral "lines," and interferents (such as small molecules) might result relatively broad spectral "bands" appearing in the data. It is possible in such situations to correct for the presence of the additive interference without the use of a blank. In this example, the maximum analyte response occurs at signal channel #50 (which would correspond to a particular wavelength in the case of a spectroscopic method); the signal on this channel will contain contributions from both the analyte and the

interferent. However, there are many signal channels where only the interferent will give a response; we can use one of these to correct for the contribution of the interferent. In this case, for example, the following figure graphically depicts a very simple, but effective, method of correcting for the interference.

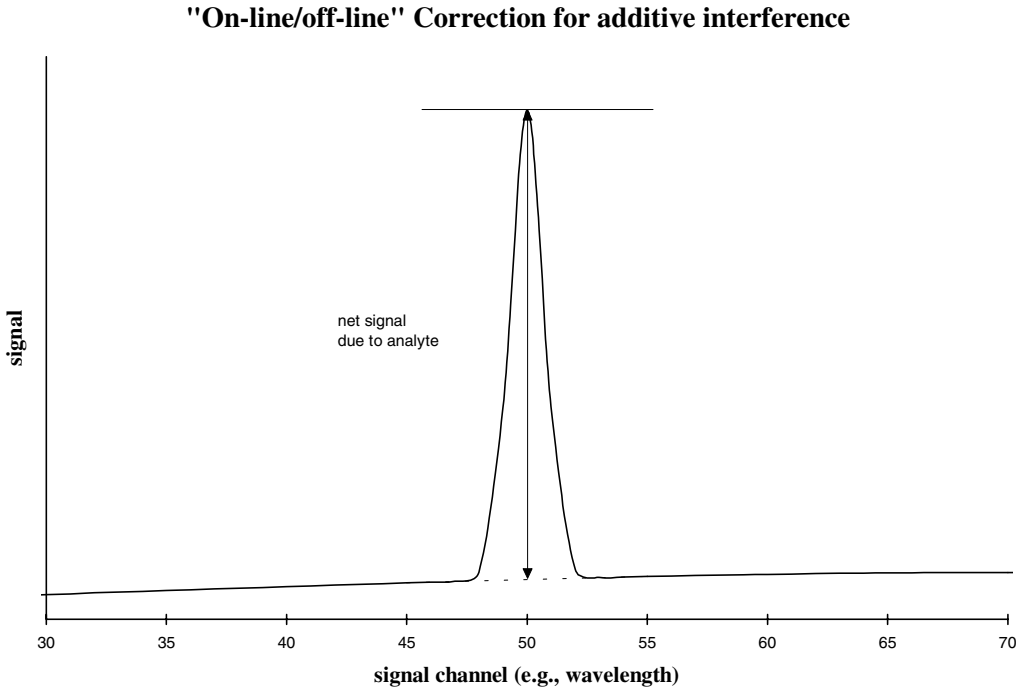

Figure 9. Simple method to correct for additive interference with a multi-channel method. The advantage of multi-channel correction is that a blank is not necessary.

We see that we need to measure the instrument response on at least two channels to correct for the additive interference by this method: one channel at the maximum analyte response (the "on-line" channel) and one channel where only the interferent responds (the "off-line" channel). In atomic spectroscopy, if the off-line channel is near enough to the on-line channel, then we can often assume that the net signal due to the analyte is equal to the difference between the on-line and off-line signals:

on-line/off-line approach net signal = on-line signal – off-line signal

In a way, this multi-channel approach is like taking a blank measurement without actually having to prepare a blank.

The ability of an analytical technique to collect data on multiple signal channels allows some very useful methods to be used to correct for the presence of interferences; some of these methods are far more sophisticated than this simple "on-line/off-line" approach.

Matrix Matching

The blank-measurement approaches described in the previous section will correct for additive but not multiplicative interferences. A "brute force" method of correcting for *all* matrix effects is ***matrix matching***. In this approach, all calibration standards and samples will (ideally) have the

same sample matrix, so that any matrix effects in the sample will be reproduced in the standards. The calibration curve obtained in this case will thus correct for all additive and multiplicative effects.

For example, if you collect seawater samples for analysis, then you might want to create a series of calibration standardsin“seawater.”This could be achieved by either (a) obtaining some seawater that is known to be free of the analyte, and then preparing the standards using this seawater; or (b) creating some artificial “seawater” that will (hopefully) act like the real thing with respect to our analytical method.

It is difficult to artificially duplicate complicated sample matrices, such as that of seawater. However, it might not be necessary to match the sample matrix in it’s entirety in order to correct for matrix effects. If it is known, for example, that it is the ionic strength of seawater that cases interference, then perhaps it is only necessary to adjust the ionic strength (using any inert electrolyte) of the calibration standards to match that of the seawater.

In another form of matrix matching, the nature of the sample matrix is actually changed to conform to that of the calibration standards. To give a simple example, if pH is known to affect the analyte response, then both the samples and the standards will be buffered to the same pH value.

Dilution Method

In this method, the samples are simply diluted with a solvent. The idea is that some interferents do not affect the signal if they are present at low concentrations. Of course, the analyte is diluted too, and the measurement technique must be sensitive enough to quantify the analyte at the diluted level.

The problem with this method is that the effective detection limits are degraded; frequently the measurement precision is worse at lower concentrations as well. However, this technique can still be quite effective in situations where detection limits are not a concern: for example, in the analysis of metal cations in blood using a very sensitive technique, such as graphite furnace atomic absorption spectroscopy, or anodic stripping voltammetry. Depending on the expected concentrations of the analyte in the blood, the sample can be diluted tenfold or even hundredfold.

Saturation Method

The philosophy of the saturation method is almost the exact opposite of the dilution method. In the dilution method, we attempt to reduce the concentration of interferents to low (and hopefully insignificant) levels. In the saturation method, high concentrations of interferent is added to each sample and every calibration standard. This method is particularly effective when the sample matrix may vary from sample to sample. By increasing the concentration of interferent to a high level, the sample-to-sample variation will be “swamped out” by the large added conc.

As an example of this method, consider the fact that the signal in potentiometry is usually sensitive to the ionic strength of the sample solution. One way to correct for systematic error introduced by this dependence is to use the saturation method: add a fairly high concentration (e.g., 4 M) of an inert electrolyte solution (e.g., potassium nitrate) to all samples and calibration standards. This means that every solution analyzed will have almost the same ionic strength, so

that the matrix effect on each measurement will be the same. The electrolyte solution used in this fashion is called an *ionic strength buffer*.

Another example of this method is the use of *ionization suppressors* in flame spectroscopy. An ionization suppressor is an alkali salt; the alkalis are easily ionized in the flame. The presence of the ionization suppressor acts, as the name implies, to reduce the ionization of the analyte in the flame atomizer. Without the presence of the suppressor, the degree of analyte ionization may vary according to the sample matrix; this variation would cause a corresponding change in signal.

The main disadvantage of the saturation method is that it can sometimes degrade LOD's, sensitivity and measurement precision.

Matrix Modifiers

A *matrix modifier* is a chemical that reacts with either the interferent or the analyte. The purpose of a matrix modifier is to correct matrix effects that are usually due to chemical reactions involving the interferent. For example, *masking agents* are a type of matrix modifier that is commonly used in gravimetry and titrimetry. A problem in these techniques is that an interferent may react with the precipitating agent (in gravimetry) or titrant (in titrimetry); the masking agent reacts with the interferent to prevent this from occurring.

Matrix modifiers are also commonly used in atomic absorption spectroscopy to increase the volatility of the analyte. They achieve this by either reacting with interferents that tend to form involatile salts of the analyte (these matrix modifiers are called *releasing agents*) or by reacting with the analyte to form volatile salts (in this case the modifier is a *protecting agent*).

Acid-base pH buffers can (sort of) be thought of in this context. Whenever the analyte signal depends on the pH, we can think of H_3O^+ or OH^- as an "interferent." In aqueous solutions, of course, it is not possible to get rid of these ions; the buffer reacts with them to keep the pH at a constant level in all samples and standards.

The Method of Standard Additions

- brief review of the method
- describe how it corrects for multiplicative matrix effects

Evaluation of Analytical Techniques

Overview: Figures of Merit

• importance

Sensitivity

• definition (briefly)

Detection Limits and Related FOMs

• general idea: give an indication of the lower limit of an analytical method. Very popular FOMs

• LOD (also called IDL). Show how to calculate (with example)

• characteristic concentration (for atomic and molecular absorption). Advantage: much more convenient to measure

• MDL

• detection *vs* quantitation. LOQ (other names?)

• alternate formula for LOQ

(Linear) Dynamic Range

• definition and calculation

Measurement Precision

• RSD and S/N

Selectivity

• general idea: immunity from interferences (both additive and multiplicative)

• definition for ISEs

• general definition (?) for multi-channel techniques

• different types of selectivity: e.g., chemical *vs* spectral