CHAPTER 3. INTRODUCTION TO INSTRUMENTAL METHODS

The use of instruments, which have been introduced into chemistry mainly in the last sixty years, for qualitative and quantitative purposes has revolutionized the practice of chemical analysis. There are a wide variety of instrumental methods making use of many different chemical and physical phenomena. Nevertheless, all these diverse methods have many features in common. Rather than discuss these common features in chapters dealing with specific instrumental methods, perhaps leading the reader to think that these features apply only to the method under discussion, they will be presented here in a general discussion.

3.1 INSTRUMENTAL RESPONSE

In any instrumental method, the instrument measures some chemical or physical property of a sample, *e.g.* the amount of light absorbed by a solution containing an unknown substance, often called the *analyte*, and converts the measurement to a numerical value. Usually that value indicates the property measured. For example, if a solution absorbs light, the value reported might be the percent of light transmitted by the sample or the absorbance, which is a function of the amount of light absorbed. If the instrument is sophisticated enough, it may convert the measured property directly to the concentration of analyte and report that result (such instruments will not be considered here). The numerical value might be presented as a meter reading on the instrument, as a line drawn on a piece of chart paper, or as a report on a computer screen. The analyst usually has the task of converting this value to the concentration of the analyte of interest.

In order to convert an instrument response to concentration, the analyst must know the relationship between that response and concentration. Most instruments provide responses that are linear in concentration, but some notable exceptions exist. A few examples of responses that increase as the square of the concentration (quadratic response) are known and pH meters and other ion selective electrochemical methods (see Chapter 5) provide a response that is linear in the *logarithm* of concentration. This chapter will deal strictly with linear response, but much of what is said here can also be applied to other types of instrumental responses.

3.1.1 LINEAR INSTRUMENTAL RESPONSE

If it is known that an instrumental method provides a response linear in concentration, the following equation must apply,

$$R = B + S[\mathrm{A}] \qquad (3\text{-}1)$$

where R is the *instrument response* (the numerical value presented by the instrument), *e.g.* the absorbance of a coloured solution, B is the *background*, S is the *sensitivity*, and [A] is the concentration of analyte.

One normally assumes that if the sample contains no analyte, then the instrument response will be zero. This will be true if there is no background. Background can arise in the instrument itself or in the sample. In the case of a sample which absorbs light, if the sample contains other compounds that absorb light in addition to the analyte, this gives rise to a background absorption which the instrument measures. Some instruments are provided with controls for “zeroing” the background. These add in a signal of opposite polarity to the background and cancel it out. This can be quite convenient, but can only be used if the background is known. In the case where the background is zero or can be properly cancelled, Eqn. (3-1) simplifies to

$$R = S[\mathrm{A}] \qquad (3\text{-}2)$$

The sensitivity, S, is the slope of the response curve. As sensitivity increases the slope also increases and usually the ability to measure smaller concentrations of analyte also increases. Much research in modern instrumental analysis is involved with increasing sensitivity.

A typical linear response curve is shown in Figure 3-1.

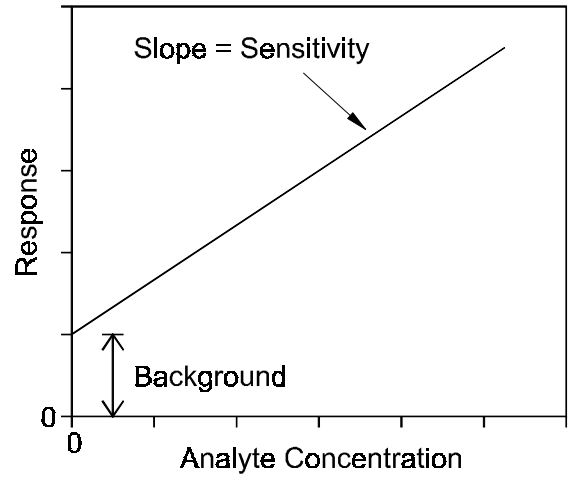

Figure 3-1

3.1.2 DEVIATIONS FROM LINEARITY

Almost all linear instrumental methods of analysis have a specific concentration range over which linearity is observed. In some methods this range may be as small as one order of magnitude in concentration, in others it may extend to more than six orders of magnitude. There are many reasons why nonlinearity might occur in techniques which should, in theory, be linear. These are best discussed along with the techniques to which they apply. Usually nonlinearity occurs in the high concentration region of the response curve. In other words the instrument response is linear at low concentrations and deviates from linearity as concentration increases. If the deviation from linearity appears as a decrease in slope (the curve bends toward the concentration axis, the concentration grows more rapidly than the response, or the sensitivity decreases with increasing concentration), the deviation is said to be *negative*. If the deviation appears as an increase in slope (the curve bends toward the response axis), it is called *positive*. These deviations are illustrated in Figure 3-2. Negative deviations are more commonly observed with instrumental methods.

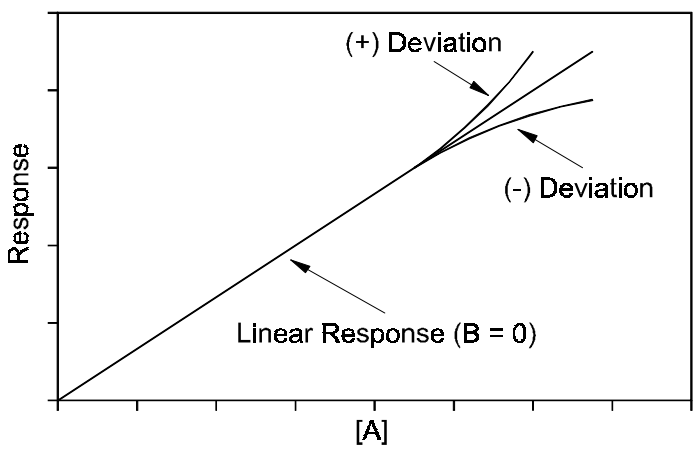

Figure 3-2

3.2 RELATING RESPONSE TO CONCENTRATION

Obtaining a response to a physical or chemical property of an analyte is of no use unless that response can be used to predict concentration accurately. This is often more complicated than it might first appear, depending on the steps taken in the analysis, the method of analysis, and the complexity of the sample. Thus there are several different methods commonly used to relate response to concentration. These will be discussed below.

3.2.1 ABSOLUTE METHOD

In principle, if a method provides a linear response, it should be possible to measure both the background, B, and the sensitivity, S, and use Eqn. (3-1) to relate concentration to response. In practice,

backgrounds vary so much from day to day and instrument to instrument that they cannot be accurately determined. Thus the absolute method is used only for techniques in which the background is zero or can be cancelled exactly. The sensitivity, *S*, is then measured using a *standard* or is obtained from the literature. A standard is a sample containing the analyte in a concentration known to the analyst. The response of the instrument to an unknown is then recorded and the response equation, Eqn. (3-2), is used to determine the analyte's concentration.

Polarography is an instrumental technique in which the current flowing through an electrochemical cell is measured. This current is linearly related to the concentration of material undergoing reaction in the cell. The cell current measured for a solution containing 1.00 ppm Pb(II) is 0.0282 μAmp. The cell current, measured in the same manner for an unknown, is 0.0426 μAmp. What is the concentration of Pb(II) in the unknown?

Again, since the instrument response (current) is linear in concentration (assuming that the background is zero),

$R = S(\text{conc. Pb(II)})$ or

$S = R/(\text{conc. Pb(II)}) = (0.0282\ \mu\text{Amp})/1.00\ \text{ppm} = 0.0282\ \mu\text{Amp/ppm}$

$\text{Conc. Pb(II)}_{unk} = R/S = (0.0426\ \mu\text{Amp})/0.0282\ \mu\text{Amp/ppm} = 1.51\ \text{ppm}$

25.0 mL of a colour-forming reagent are added to 25.0 mL of a sample containing iron and the sample is placed in an instrument capable of measuring the amount of light absorbed (a spectrophotometer). The absorbance (the instrument response), which is linearly related to concentration, is given by the instrument as 0.493. The sensitivity (which will be shown in the next chapter to be a combination of a constant called the molar absorptivity and the distance the light travels through the sample) is found to be 9754 L/mol from a handbook. What is the concentration of iron in the unknown?

Assuming a linear instrument response (with no background),

$R = S[\text{Fe(II)}]$ or $0.493 = (9754\ \text{L/mol})[\text{Fe(II)}]$

$[\text{Fe(II)}] = 0.493/9754\ \text{L/mol} = 5.05\text{x}10^{-5}\ \text{mol/L}$

Since the addition of the colour-forming reagent diluted the original sample, this must be taken into account in reporting the analyte concentration.

$[\text{Fe(II)}]_{unk} = (5.05\text{x}10^{-5}\ \text{M})(50.0\ \text{mL}/25.0\ \text{mL}) = 1.01\text{x}10^{-4}\ \text{M}$

In the above example the colour-forming reagent also contained a reducing agent which converted all the iron in the sample to Fe(II). This was necessary since Fe(III) and Fe(II) form different colours with most colour-forming reagents.

Note that one does not have to understand either spectrophotometry (the measurement of light intensity) or polarography to solve the above problems. All one needs to know is that instrument response is linear in concentration. However, one should never use an instrumental method without understanding the fundamentals behind the method.

The absolute method has the advantages of being simple and requiring, at most, only one standard. For this reason the absolute method is often called a one point calibration method. However, it is seldom used in practical analyses. There are several reasons for this. If the sensitivity, *S*, is taken from the literature, one is never sure that the conditions used in its determination are the same as those used in the analysis, since the determination and the analysis are done in different laboratories. This can be a major source of error. Even if the sensitivity is measured by the analyst (as in the second example above) linearity of response and absence of background must be assured before this method can be applied. In addition the absolute method is more sensitive to matrix effects (see below) and changes in instrumental parameters than other methods. Thus this method finds most use when rapid but less accurate results are needed.

3.2.2 CALIBRATION CURVES

Calibration curves are actually just the instrument response curves discussed above. These are prepared by measuring the instrument response to a set of standards containing various known concentrations of the analyte. These data are plotted to get the calibration graph. The instrument response to one or more unknowns is then measured and the graph is used to convert the response(s) to concentration(s).

An analyst prepared a set of standards containing a colour-forming reagent and various concentrations of Fe(II). The instrument response (absorbance) for these solutions was measured with a spectrophotometer (an instrument that measures light intensity) and the following results were obtained:

Response	Concentration
0.000	0
0.189	2.00×10^{-5} M
0.383	4.00×10^{-5} M
0.571	6.00×10^{-5} M
0.756	8.00×10^{-5} M
0.948	1.00×10^{-4} M

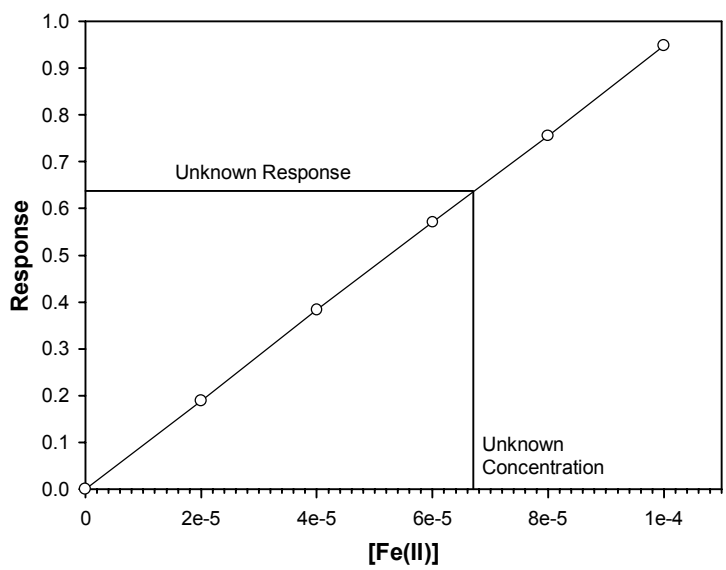

Figure 3-3

A calibration curve, as shown in Figure 3-3, was prepared from these data.

A sample containing Fe(II) was then measured and found to give a response of 0.634. From the calibration curve the concentration of Fe(II) in the measured sample was determined to be 6.68×10^{-5} M. If the original sample had been diluted to prepare the sample that was measured, this dilution would have to be taken into account in estimating the concentration in the original sample. The data could also have been fit to a first order equation using the method of least squares. This data set was used as the example of a least squares fit in Chapter 1, Section 1.3.1. The concentration of Fe(II) determined from the resulting equation is the same as that listed above.

The calibration curve requires the preparation and measurement of a number of standards and is thus more labour intensive than the absolute method. However, the curve, once prepared, can be used for more than one sample. The two major advantages of the calibration curve are: (1) the instrument response need not be linear to obtain accurate results and (2) the line used for the estimation of the analyte concentration is drawn to "average" the data points and thus the error in each datum point is somewhat "averaged out", provided that more than two points are used in the calibration. This can be done mathematically using the method of least squares, if the data are truly linear, as mentioned above.

3.2.3 MATRIX EFFECTS

A matrix effect is a change in the instrumental response for the analyte caused by the presence of other components in the sample. For example, magnesium can be obtained from sea water. Thus the refiners need to know accurately the magnesium content of their feedstock. It is found that the instrumental response for magnesium ion (as magnesium chloride) in distilled water is not the same as for magnesium ion in sea water, mainly due to the presence of high concentrations of sodium, chloride, and sulfate ions in sea water. Thus if the sensitivity for magnesium is measured with or the calibration curve is prepared from standards made using magnesium chloride in distilled water, accurate results for magnesium might be obtained in measurements on rain water but inaccurate results will be obtained on sea water samples. This is an example of a matrix effect

The sample matrix may affect the background (the intercept of the calibration curve), the sensitivity (the slope of the curve), or both. Matrix effects that change the background are the hardest to deal with. Less drastic measures can often be used to overcome matrix effects that change the sensitivity. In many methods of analysis matrix effects can cause large errors and therefore techniques for dealing with matrix effects must be considered. Some of the more useful methods are listed below.

1) If the matrix is known and reasonably constant, add the components of the matrix to all the standards used to prepare the calibration curve or measure the sensitivity. All measurements will then be made under the same circumstances (with the same matrix). This would be the case for sea water and this method could be used to avoid matrix problems in the example given above. In general, it is good practice to prepare standards for either the absolute or calibration curve methods that have a matrix as similar to the samples as possible, thus avoiding or reducing matrix effects.

If the matrix is unknown or varies to such an extent that standards cannot be prepared to duplicate the sample matrix, then one of the following methods should be employed:

2) Add materials to both the samples and the standards that will completely overpower the sample matrix. Again all measurements will be made under the same circumstances and the matrix problem will be avoided or reduced.

3) Use the sample itself to prepare the standard(s). In this way the sample provides the matrix and guarantees that the matrix effects are the same for all measurements. This is called the method of standard addition and is explained below.

4) Add a material similar to the analyte to each sample and standard and use the ratio of the responses of the two materials for quantitative purposes. This is known as the use of an internal standard and is also explained below.

5) If possible, destroy the sample matrix (but not the analyte).

6) If all else fails, separate the analyte from the other components of the sample and make the measurements in the absence of a matrix. Methods of separation (chromatography) are discussed in Chapter 6.

All of the above methods can be used to reduce types of matrix effects that change the sensitivity of an analytical technique. Unfortunately, methods (2), (3), and (4) are not usually effective with the types of matrix effects which alter the background in an analytical method.

3.2.4 THE METHOD OF STANDARD ADDITION

In the method of standard addition, the sample is made to act as both the standard and the unknown. This is done in such a manner that the matrix is similar for all solutions and certain kinds of matrix effects are minimized. In this method the instrument response must obey a specific relationship to the concentration of analyte, usually a linear relationship. However, in Chapter 5 an example of standard addition is given for a logarithmic response.

In the following it will be assumed that the analyte concentration in the sample is $[A_u]_o$ and that a standard is available, also containing the same analyte at concentration $[A_s]_o$. $[A_s]_o$ must be known by the analyst. A linear response with no background will also be assumed, thus $R = S[A]$. Two slightly different but common approaches to the method of standard addition are outlined below. The reader is cautioned that these are not the only approaches; the circumstances of the analysis will dictate how the method is done and how the mathematical solution is obtained. *Do not memorize the equations below; instead use the methods shown as examples of how to approach applying the technique of standard addition.*

Method 1

Measure the response, R, of the sample (which contains $[A_u]_o$). Then add a *known* volume of standard, V_s, to a *known* volume of the sample, V_u, and measure the new response, R'.

$$R = S[A_u]_o \tag{3-3}$$

$$R' = S[A_s] + S[A_u] \tag{3-4}$$

where $[A_s]_o$ and $[A_u]_o$ have been diluted to $[A_s]$ and $[A_u]$ respectively.

$$[A_s] = \frac{[A_s]_o V_s}{V_s + V_u} \tag{3-5}$$

and

$$[A_u] = \frac{[A_u]_o V_u}{V_s + V_u} \tag{3-6}$$

thus

$$R' = S\left\{\frac{[A_s]_o V_s}{V_s + V_u} + \frac{[A_u]_o V_u}{V_s + V_u}\right\} \tag{3-7}$$

There are now two equations, Eqns (3-3) and (3-7), and two unknowns, S and $[A_u]_o$. S can be eliminated by dividing (3-7) by (3-3).

$$\frac{R'}{R} = \frac{1}{[A_u]_o}\left\{\frac{[A_s]_o V_s}{V_s + V_u} + \frac{[A_u]_o V_u}{V_s + V_u}\right\} \tag{3-8}$$

Solving for $[A_u]_o$ one obtains

$$[A_u]_o\left(\frac{R'}{R} - \frac{V_u}{V_s + V_u}\right) = \frac{[A_s]_o V_s}{V_s + V_u} \tag{3-9}$$

or
$$[A_u]_o = \frac{R[A_s]_o V_s}{R'(V_s + V_u) - RV_u} \qquad (3\text{-}10)$$

Do not memorize Eqn. (3-10), rather learn how to derive it. As stated above the particular equation used in standard addition depends on the circumstances and may not be Eqn. (3-10) or any of the other equations presented below. However, *always remember* that standard addition *dilutes* both the *standard* and the *unknown*.

With this method one does not need to know S, one needs only one standard, and only two measurements are made. However, one must be certain that the instrument response is linear. Also if a large number of samples are to be run, then standard addition will take a larger number of measurements than the calibration curve method since each sample will require two measurements, rather than one.

The absorbance (spectrophotometric response) of a solution containing an unknown amount of iron and a large excess of thiocyanate ion is measured as 0.346. 1.00 mL of a 50.0 ppm iron standard is added to 10.00 mL of the unknown. The absorbance of this solution is measured as 0.415. Assuming that absorbance is linear in concentration, what is the iron concentration in the sample?

$0.346 = SC_u$

$0.415 = S\{C_u(10.00\text{ mL})/(11.00\text{ mL}) + (50.0\text{ ppm})(1.00\text{ mL})/(11.00\text{ mL})\}$

$0.415/0.346 = (10.00/11.00) + 50.0\text{ ppm}/11.0(C_u)$

$C_u = 50.0\text{ ppm}/(11.00)(0.415/0.346 - 10.00/11.00) = 15.7\text{ ppm}$

Graphical approach - Method 1

A graphical approach using several solutions is sometimes employed. In this case only one standard is still needed. The test solutions are prepared with the same known volume of sample, V_u, in each, but with differing known volumes of standard, V_s. V_s now becomes one of the variables. To see how this is treated, refer to Eqn. (3-7) above, but replace R' with R, response in general..

$$R = \frac{S[A_s]_o V_s}{V_s + V_u} + \frac{S[A_u]_o V_u}{V_s + V_u} \qquad (3\text{-}7)$$

or
$$R(V_s+V_u) = S[A_u]_o V_u + S[A_s]_o V_s \qquad (3\text{-}11)$$

Thus a plot of $R(V_s+V_u)$ *vs* V_s should be linear with a slope of $S[A_s]_o$ and an intercept of $S[A_u]_o V_u$. $[A_u]_o$ can be obtained from the slope and the intercept of this plot. There is an even easier method. Extrapolate the plot to the X intercept as shown in Figure 3-4. Determine the value of V_s at the X intercept, $(V_s)_x$. This value will be negative. At the X intercept the value of $Y = 0$.

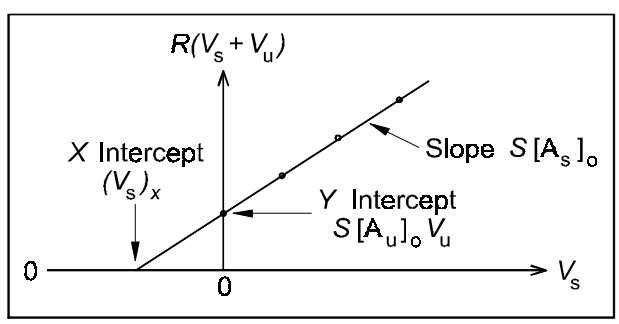

Figure 3-4

$$R(V_s+V_u) = 0 \qquad (3\text{-}12)$$

and thus $$S[A_s]_o(V_s)_x + S[A_u]_o V_u = 0 \tag{3-13}$$

or $$[A_u]_o = -\frac{[A_s]_o(V_s)_x}{V_u} \tag{3-14}$$

Note that since $(V_s)_x$ is negative, $[A_u]_o$ will be positive, as it must be. The graphical method has the advantage of "averaging" the error in the data and of indicating whether response is actually linear in concentration. However, it requires more solution preparation and more measurements.

Method 2

In this method a known volume of the sample, V_u, is *diluted* with solvent to a known final volume, V_t. Both V_u and V_t are constant in all solutions. The response of this solution, R, is then measured. Next the same volume of sample, V_u, and a known volume of standard, V_s, are diluted to the same final volume, V_t. The response, R', of this solution is also measured.

For both solutions

$$V_s + V_u \leq V_t \tag{3-15}$$

and $$[A_u] = \frac{[A_u]_o V_u}{V_t} \tag{3-16}$$

and for the second solution

$$[A_s] = \frac{[A_s]_o V_s}{V_t} \tag{3-17}$$

Again there are two equations (below) in two unknowns, $[A_u]$ and S.

$$R = S[A_u] \tag{3-18}$$

and $$R' = S\{[A_u] + [A_s]\} \tag{3-19}$$

Divide Eqn. (3-18) by (3-19), eliminating S, and solve for $[A_u]$.

$$\frac{R}{R'} = \frac{[A_u]}{[A_u]+[A_s]} \tag{3-20}$$

$$R[A_u] + R[A_s] = R'[A_u] \tag{3-21}$$

$$[A_u](R'-R) = R[A_s] \tag{3-22}$$

or $$[A_u] = \frac{[A_s]R}{R'-R} \tag{3-23}$$

On substitution for $[A_u]$ and $[A_s]$ in (3-23) using (3-16) and (3-17) the final mathematical solution is obtained.

$$\frac{[A_u]_o V_u}{V_t} = \frac{[A_s]_o V_s R}{V_t(R'-R)} \tag{3-24}$$

and $$[A_u]_o = \frac{[A_s]_o V_s R}{V_u(R'-R)} \tag{3-25}$$

Method 2 may seem somewhat simpler than Method 1, but it involves more solution manipulation. Again, as stated above, you should not memorize the final result, Eqn. (3-25), since this is not an

equation applicable to all cases. It is far better to know how to derive such an expression, depending on the particular circumstances of the problem.

The same sample containing iron thiocyanate and the same 50.0 ppm stock iron solution as in the example above are treated somewhat differently. 10.00 mL of the sample are diluted to 15.00 mL with distilled water. The absorbance (response) of this solution is 0.231. 10.00 mL of sample and 2.00 mL of stock iron solution are diluted to 15.00 mL with distilled water. This solution gives an absorbance reading of 0.378. What is the iron concentration in the unknown?

$0.231 = SC_u(10.00\text{ mL}/15.00\text{ mL})$

$0.378 = S\{C_u(10.00\text{ mL})/(15.00\text{ mL}) + (50.0\text{ ppm})(2.00\text{ mL})/(15.00\text{ mL})\}$

$0.378/0.231 = 1.00 + (50\text{ ppm})(2.00)/(10.00)C_u$

$C_u = (100\text{ ppm})/(10.00)(0.378/0.231 - 1.00) = 15.7\text{ ppm}$

Graphical approach - Method 2

As for Method 1, a graphical approach may be taken in which several solutions are prepared, each with the same V_u and V_t, but with different V_s (V_s now becomes a variable). Using Eqn. (3-19), but replacing *R'* with response, *R*, in general

$$R = S\{[A_u] + [A_s]\} \tag{3-19}$$

or

$$R = \frac{S[A_u]_o V_u}{V_t} + \frac{S[A_s]_o V_s}{V_t} \tag{3-26}$$

A plot of *R vs* V_s should be linear with a slope of $S[A_s]_o/V_t$ and an intercept of $S[A_u]_o V_u/V_t$. Thus $[A_u]_o$ can be determined from the slope and intercept. Again it is easier to use the *X* intercept. At the *X* intercept R = 0 and $V_s = (V_s)_x$.

$$0 = \frac{S}{V_t}\left\{[A_u]_o V_u + [A_s]_o (V_s)_x\right\} \tag{3-27}$$

or

$$[A_u]_o = -\frac{[A_s]_o (V_s)_x}{V_u} \tag{3-28}$$

$(V_s)_x$ is read directly from the graph (see Figure 3-5) and $[A_u]_o$ is calculated from Eqn. (3-28) above. The same comments regarding the graphical method apply to Method 2 as well as to Method 1.

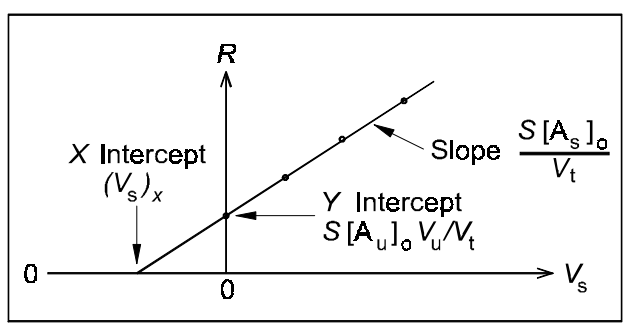

Figure 3-5

General comments

It might seem that Method 2 has the advantage of simpler mathematics and Method 1 requires less solution manipulation. Modern

computers tend to even out differences in mathematical complexity, thus Method 1 might seem to have the advantage. However, if the method of standard addition is being used to overcome sensitivity differences caused by matrix effects, note that Method 1 does not do this as well as Method 2. In Method 1 the "concentration" of the matrix (and of the unknown) is not the same in each solution, since the unknown is diluted to different extents, while in Method 2 it is the same in all solutions, since the unknown is always diluted to the same extent. Thus Method 2 will provide superior matrix correction and is the method of choice in this regard.

In all the above it was assumed that the background was zero. Therefore these methods, as presented, will correct for the types of matrix effects that affect sensitivity, but not the types that produce non-zero backgrounds. It might be thought that the method could be made more general by using the general response equation, $R = B + S[\mathrm{A}]$, rather than the simpler equation, $R = S[\mathrm{A}]$. In this case there would be three unknowns, B, S, and $[\mathrm{A}_u]_o$, assuming that B does not change from solution to solution. This would require three measurements to be made, one on the unknown alone and two with different concentrations of standard. However, when the mathematics are attempted, it is found that the three equations will not provide a solution for $[\mathrm{A}_u]_o$. In other words, the method of standard addition cannot tell the difference between background and additional concentration of the original analyte in the unknown. Thus this method corrects only for the type of matrix effect that changes the sensitivity of a measurement.

3.2.5 INTERNAL STANDARDS

An internal standard is a compound which is added to *all samples* and used to monitor the progress of an analysis. If a problem occurs, the change in its response can be used to correct the response of the unknown and circumvent the problem. The internal standard must not to be present originally in any of the samples, must not interfere in the analysis of the analyte, and must parallel the behaviour of the analyte as closely as possible. It is usually added in the same amount to all samples and standards. The response of the instrument to both the analyte and the internal standard is recorded. The ratio of these responses is then used, in either an absolute fashion or with a calibration curve, to calculate the concentration of analyte.

Assume that instrument response is linear for both the internal standard and analyte and that the background is zero. Then, using Eqn. (3-2),

$$R_A = S_A[\mathrm{A}] \qquad \text{for the analyte and}$$

$$R_I = S_I[\mathrm{I}] \qquad \text{for the internal standard}$$

where [I] is the concentration of internal standard in the sample. When the ratio of the responses is calculated

$$\frac{R_A}{R_I} = \frac{S_A[\mathrm{A}]}{S_I[\mathrm{I}]} = \frac{k[\mathrm{A}]}{[\mathrm{I}]} \tag{3-29}$$

where k is a constant (the ratio of two other constants). If the concentration of internal standard is always maintained constant, then the above expression becomes even simpler.

$$\frac{R_A}{R_I} = k'[\mathrm{A}] \tag{3-30}$$

This corrects for matrix effects in a more subtle manner than the method of standard addition. A matrix effect that acts upon sensitivity causes S_A to change from unknown to standard. If the internal

standard is chosen to behave like the analyte, then the matrix effect will change S_I to approximately the same extent as S_A. In this case k, the ratio of the two sensitivities, will change very little or not at all. Thus the equations above involving k or k' will be almost independent of matrix effects. The same can be said for changes in instrument characteristics that affect the sensitivities. If it is known that instrument characteristics vary, then calibrations must be performed often to avoid errors. The use of internal standards avoids this problem.

Internal standards exhibit advantages in another area. In many analytical procedures, there are steps which involve a large error or in which sample is lost. For example, some procedures require that the analyte be extracted from the original sample prior to instrumental analysis. This extraction is often incomplete and, worse, the percentage recovery varies considerably from sample to sample. If an internal standard is added to the original sample, and if the percentage recovery of the analyte is higher in one case than in another, so will be the percentage recovery of the internal standard. The ratio of responses will not be affected by this change. Likewise if a large error is made involving the analyte, say 20%, the same 20% error will be made involving the internal standard, but the ratio of the responses will not be affected. Thus an internal standard can be employed to cancel the effects of such problems.

When internal standards are used in a fashion similar to the absolute method, a standard solution is prepared containing both the analyte and the internal standard in known concentrations and k (or k') is determined from the measured responses of this standard. The internal standard is then added to the unknown, the responses measured, and the analyte concentration calculated using the value of k (or k'). In this case it is not necessary that the internal standard be added to all samples in the same concentration.

Cu(II) is to be used as an internal standard in the polarographic analysis of Pb(II). A standard solution containing 2.00 ppm of both Cu(II) and Pb(II) is prepared and, when analyzed, provides currents of 0.0505 and 0.0584 μAmp respectively. 25.0 mL of a 2.00 ppm solution of Cu(II) is added to 25.0 mL of an unknown containing Pb(II) but no Cu(II). The currents measured for the unknown are 0.0226 and 0.0393 μAmp for the Cu(II) and Pb(II) respectively. Assuming that measured current is linear in concentration, what is the concentration of Pb(II) in the original unknown?

$k = R_A[\text{Cu(II)}]/R_I[\text{Pb(II)}] = (0.0584\ \mu\text{Amp})(2.00\ \text{ppm})/(0.0505\ \mu\text{Amp})(2.00\ \text{ppm})$

$k = 1.16$

$[\text{Pb(II)}] = R_A[\text{Cu(II)}]/R_I k = (1.00\ \text{ppm})(0.0393\ \mu\text{Amp})/(0.0226\ \mu\text{Amp})(1.16)$

$[\text{Pb(II)}] = 1.50\ \text{ppm}$

Since the original unknown was diluted by addition of the internal standard, this must be taken into account.

$[\text{Pb(II)}]_{orig} = (1.50\ \text{ppm})(50.0\ \text{mL}/25.0\ \text{mL}) = 3.00\ \text{ppm}$

If the analysis had been performed by the absolute method, neglecting the information provided by the internal standard, the result would have been as follows:

$S_A = R_A/[\text{Pb(II)}]_{std} = (0.0584\ \mu\text{Amp})/2.00\ \text{ppm} = 0.0292\ \mu\text{Amp/ppm}$

$[\text{Pb(II)}]_{unk} = R_A/S_A = (0.0393\ \mu\text{Amp})/0.0292\ \mu\text{Amp/ppm} = 1.35\ \text{ppm}$

This last value would again have to be corrected for dilution to give a final answer of 2.69 ppm. Obviously, since the two results do not agree, some factor changed between the measurements on the standard and those on the unknown. This change will have affected both the response for Cu(II) and Pb(II). Since the

responses and the concentrations of the internal standard are known for both solutions, they can be used to correct those of the unknown and provide a more accurate result.

Internal standards can also be used to prepare calibration curves. A set of standard solutions, each containing the internal standard at the same concentration and the analyte at different known concentrations, are prepared and the responses measured. The ratio of responses, R_A/R_I, for these standards is then plotted *vs* the concentration of analyte, [A], to produce a graph such as that shown in Figure 3-6. The internal standard is then added to all unknowns, *in the same concentration as in the standards*, and the responses measured. The ratio of these responses is then calculated and the concentration of the analyte is determined from this ratio using the calibration graph in the usual manner.

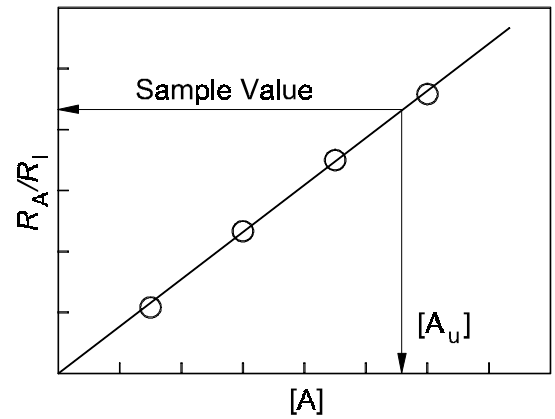

Figure 3-6

The calibration curve method has all the advantages of the use of internal standards plus the advantages that linearity of response is not required and some "averaging out" of the errors in the data is achieved. In terms of ability to reduce matrix problems, the method of standard addition is usually superior to the use of an internal standard, since in the former method the analyte itself is used as the standard. It is almost impossible to select an internal standard that reacts to the matrix in exactly the same fashion as the analyte itself. However, the use of internal standards can correct for effects other than matrix effects, as explained above, that cannot be corrected by standard addition.

3.2.6 PREPARATION OF SOLUTIONS FOR CALIBRATION CURVES

The data for calibration curves are normally obtained by measuring the instrument response to a set of solutions containing the analyte of interest at known concentrations. Such solutions are most easily prepared by a method known as serial dilution in which a solid is weighed and dissolved to form a stock solution. This stock solution is then diluted in a series of steps to prepare the calibration solutions with the desired concentrations. The concentrations of the final solutions prepared in this fashion usually differ one from another by simple ratios, *e.g.* by factors of two. The main difficulty with this method is that an error in any particular step will influence all successive steps .

On the surface it would seem to be a straight forward matter to prepare a set of such solutions. However, since a variety of factors must be considered to obtain good results, and since there may be several ways of preparing a satisfactory set of solutions, there is no single method which can be recommended for this task. Since this is a problem that faces all chemists doing analysis, it must be discussed in some detail. Before deciding on a method of preparation, at a minimum the following factors must be known: (1) the concentration range needed for the solutions, (2) the material to be used as a source of analyte, (3) the number of solutions needed (usually at least four or five), (4) the volumes of solution needed, and (5) the accuracy required for the concentrations in the solutions. Each of these factors is specific to the analytical method, the analyte, and the samples under investigation. The analyst must use chemical knowledge and common sense in deciding on these factors.

An example perhaps best illustrates the procedures involved in preparing a set of calibration solutions. Assume that you are asked to determine the sodium ion content of a set of fresh water samples which you know will probably contain between 2 and 4 ppm Na^+. Your calibration curve

should include this entire range, so you decide to prepare calibration solutions between 1 and 5 ppm Na^+ with an accuracy of about 1%. You decide to use NaCl as your source of sodium ion since it can be obtained pure and dry and because it can be easily weighed. The method of analysis chosen is flame emission spectrometry. This method provides a signal linear in the concentration of sodium ion and requires about 10 mL of solution for each reading. In order to have enough solution for many standardizations, you decide to prepare about 250 mL of each solution. Finally you decide to prepare five calibration solutions because you suspect that the calibration curve may not be quite linear (as sometimes happens with flame emission spectrometry).

You now have the choice as to whether to prepare concentrations that vary by a constant factor or that vary by a constant amount. If you select a constant factor of two (a common value) your concentrations will be 5, 2.5, 1.25, 0.625, and 0.3125 ppm. If you select a constant amount, say 1 ppm, your concentrations will be 5, 4, 3, 2, and 1 ppm. Common sense dictates the choice of the second method in this case for two reasons: (1) this method covers the range of concentrations in your sample (2 - 4 ppm) much better and (2) 250 mL volumes of each of the solutions are more easily prepared by the second method. Successive dilutions by a factor of two usually requires taking half of the first solution and diluting it by a factor of two to form the next solution, then performing the same operation on each new solution until the desired number of solutions has been prepared. To prepare 250 mL of each solution would require an original volume of 500 mL and a method to accurately measure half of this volume.

The next matter to decide is the mass of NaCl to be weighed. You might think that it would be simplest to weigh enough NaCl to prepare the solutions directly. The most concentrated of these solutions contains 5 mg/L of Na^+ or 1.25 mg in 250 mL. 1.25 mg of Na is contained in (1.25)(58.5/23) or 3.18 mg of NaCl. Can this amount be easily weighed? Not easily and not on conventional analytical balances which are accurate to about 0.0002g. To maintain an overall accuracy of about 1%, each step in the preparation procedure must be accurate to somewhat better than 1%. This requires that you weigh out a larger mass, prepare a more concentrated solution, and dilute this one or more times to prepare the final solutions. Dilution by a factor of ten to form a final volume of 250 mL is easily done, so a stock solution of 50 ppm Na would be convenient. This would require 31.8 mg of NaCl dissolved in 250 mL or 127.2 mg/L. This latter amount is easily weighed to better than 1% accuracy. Thus you decide to weigh about 127.2 mg of NaCl and dissolve it in 1.000 L of distilled water to make the 50 ppm solution. Dilute calibration solutions are almost always prepared by weighing enough material to prepare more concentrated stock solutions and then diluting the stock solutions.

The 5 ppm calibration solution is next prepared by pipetting 25.00 mL of the 50 ppm stock solution into a 250 mL volumetric flask and diluting to the mark (dilution by a factor of ten). When calculating the dilution factors, keep in mind that the total number of moles in a dilution remains constant. Thus

$$C_1V_1 = C_2V_2 \qquad (1\text{-}43)$$

where the Cs are concentrations and the Vs are volumes. This equation can be solved for any of the variables. In the case of serial dilution, V_1, the volume of stock solution to be diluted, is usually the variable of interest.

The method of preparation of the remaining calibration solutions depends on the type of volumetric glassware available. A volume of stock solution of (4 ppm/50 ppm)(250 mL) = 20 mL when diluted to 250 mL will provide a concentration of 4 ppm. Likewise volumes of 15, 10, and 5 mL will be needed for the 3, 2, and 1 ppm solutions respectively. Twenty and 15 mL pipettes are available, but are not common, whereas 10 and 5 mL pipettes are quite common. If all pipette sizes are available, simply use

these to prepare the 4, 3, 2, and 1 ppm calibration solutions just as the 25 mL pipette was used to prepare the 5 ppm calibration solution. If only 10 and 5 mL pipettes are available, use the 10 mL pipette twice to measure the 20.00 mL for the 4 ppm solution and use the 10 and 5 mL pipettes once each to measure the 15 mL for the 3 ppm solution.

Another alternative exists. A buret is capable of accurately delivering various amounts of solutions. Simply fill a buret with the 50 ppm stock solution and use it to measure the volumes required to prepare the calibration solutions.

One final question remains, can each of these steps be performed to an accuracy of better than 1%? A 250 mL volumetric flask can be filled to an accuracy of better than 1% and the pipettes listed above can all be used, with care, to better than 1%. However, if you had decided to use a 1 mL pipette in one of your steps, it would doubtful that it could be used to 1% accuracy. You would have to revise your procedure to avoid the use of such a small pipette. All steps in a serial dilution procedure must be checked to insure that the overall accuracy is maintained. A buret can be read to about ±0.02 mL so a volume can be measured to about ±0.03 mL (two buret readings). Therefore all volumes above about 3 mL can be measured to better than 1%. Thus any of the methods outlined above will prepare the calibration solutions as desired.

3.3 DETECTION LIMITS

One of the major goals of analytical chemistry is to be able to make ever more sensitive measurements, to detect materials at ever decreasing concentrations. Thus the question "What determines the smallest concentration that a technique can measure?" must be addressed. At first it might seem that the sensitivity of the instrument will determine the detection limit. However, with modern electronics, it is usually possible to increase this sensitivity whenever necessary. In practice it is the noise present in all measurements that usually limits the ability to detect any compound. The noise is simply random fluctuations in the response of the instrument. This is normally seen by the analyst as fluctuations in the meter reading or recorder trace presented by the instrument. When the signal from the analyte falls below a certain level, it becomes buried in the noise and is no longer observable. Thus when one discusses the ability to detect the presence of a compound, one must discuss the *signal-to-noise ratio* (*S/N*). The noise in a measurement may arise as some form of electronic noise in the instrument itself or it may arise from the method of sampling or sample background, in which case it is sometimes called chemical noise. The origin and magnitude of the noise varies depending on the technique in use.

The definition of the limit of detection is somewhat arbitrary. However, many today agree on the following definition. If the signal from an analyte is three times the magnitude of the standard deviation of the noise ($S/N = 3$), then the analyte can be said to be detected in the sample. However, just because a compound can be detected does not mean that it can be accurately quantified. The precision of measurement will not be very good if the signal is only three times the noise. Thus a second question must be considered "At what concentration can the analyte be accurately quantified?" Again this level is somewhat arbitrary, since, up to a point, the higher the concentration, the better the measurement precision. However, it is not unreasonable to suggest that for good quantification the analyte must provide a signal or response equal to ten times the standard deviation of the noise ($S/N = 10$).

Decreasing the limits of detection often has as much to do with decreasing the noise in a measurement system as with increasing its sensitivity.

The response equation for the polarographic determination of Zn(II) is found to be

$R = (5.35\text{x}10^{-3}\ (\text{amp})\text{L/mol})[\text{Zn(II)}]$

and the standard deviation of the noise in the polarographic measurement is measured as 1.9 nAmp ($1.9\text{x}10^{-9}$ Amp). What are the limits of detection and quantification for the determination of zinc by polarography?

At the detection limit $R = 3N = 3(1.9\text{x}10^{-9}\ \text{Amp}) = 5.7\text{x}10^{-9}$ Amp.

$[\text{Zn(II)}] = (5.7\text{x}10^{-9}\ \text{Amp})/(5.35\text{x}10^{-3}\ (\text{Amp})\text{L/mol}) = 1.1\text{x}10^{-6}$ mol/L

At the limit of quantification $R = 10N = 1.9\text{x}10^{-8}$ Amp

$[\text{Zn(II)}] = (1.9\text{x}10^{-8}\ \text{Amp})/(5.35\text{x}10^{-3}\ (\text{Amp})\text{L/mol}) = 3.6\text{x}10^{-6}$ mol/L

Thus for zinc the limits of detection and quantification are $1.1\text{x}10^{-6}$ M and $3.6\text{x}10^{-6}$ M respectively.

3.4 PROBLEMS

Sections 3.1and 3.2: Instrument Response and Calibration Curves

3-1) Flame photometry is a technique in which the light given off by metal atoms in a flame is measured. The light intensity is usually linear in the concentration of atoms in the flame and thus also linear in the concentration of the metal ions aspirated into the flame. The flame photometer reading from a sample containing no Li was adjusted to zero. When a 1.00 ppm Li solution was aspirated, the reading was 139.0 and when an unknown was aspirated, the reading was 34.2. What is the concentration of lithium in the unknown? Ans: 0.246 ppm.

3-2) Irv Gratch, a former student of analytical chemistry, decides to establish a commercial analysis laboratory. He fails to obtain an ACOA grant and has to buy an inexpensive flame photometer without a zero adjustment (see Prob. 3-1 above). When he aspirates a sample containing no Li into his instrument, the reading produced is 13.6. A 2.00 ppm lithium solution and an unknown read 47.6 and 61.9 respectively. What should Irv report (assuming constant background) as the concentration of Li in the unknown? Ans: 2.84 ppm.

3-3) In fluorescence the light emitted by a molecule is linear in concentration at low concentrations. The amount of quinine in tonic water is determined by fluorescence as follows: A fluorometer is set to zero with distilled water. A $5.0\text{x}10^{-6}$ M solution of pure quinine sulfate in distilled water reads 78.6 on the instrument. A sample of tonic water reads 69.5 on the instrument. 20.0 mL of the tonic water are treated with 5.0 mL of a reagent that destroys the quinine but does not change any other physical or chemical property of the solution. This solution reads 11.8 in the fluorometer. Assuming a linear response and constant sensitivity, what is the concentration of quinine in the original sample? Ans: $3.5\text{x}10^{-6}$ M.

3-4) Sodium is often determined by flame photometry (see Prob. 3-1). A flame photometer is zeroed with distilled water and the following readings taken with standard solutions.

Concentration of Na^+	Response
5 ppm	43.4
10 ppm	78.6
15 ppm	104.0
20 ppm	121.0
25 ppm	132.9

What is the concentration of sodium in a river water sample that reads 115.0? Ans: 17.9 ppm.

3-5) Cu(II) is naturally coloured and the colour intensity is increased in the presence of a large excess of NH_3. The following copper standards were prepared in 1 M NH_3 and their absorbance measured in a spectrophotometer.

[Cu(II)]	Absorbance
1.00×10^{-3} M	0.158
2.00×10^{-3} M	0.320
3.00×10^{-3} M	0.475
4.00×10^{-3} M	0.638

1.00 mL of 10 M NH_3 is placed in a 10.0 mL volumetric flask and diluted to the mark with an unknown. This solution (after mixing) produces an absorbance of 0.385 in the spectrophotometer. What is the concentration of Cu(II) in the original unknown? Ans: 2.69×10^{-3} M.

Section 3.2: Standard Addition Methods

3-6) Irvina Gratch (Irv's sister) has just purchased a house in a suburb of Halifax and finds that her new white porcelain plumbing is turning brown. She gives a sample of her well water to Irv for analysis. To 10.0 mL of her water sample he adds 10.0 mL of a commercial colour-forming reagent and measures the absorbance in a spectrophotometer as 0.592. To a second 10.0 mL aliquot of her sample he adds 10.0 mL of colour-forming reagent and 5.00 mL of 5.00 ppm Fe(II). This solution reads 0.635 absorbance units. Assuming that absorbance is linear in concentration, what is the concentration of iron in Irvina's well water? Ans: 7.37 ppm.

3-7) At concentrations below 2 ppm, the light intensity given off by potassium in a flame is linear in concentration provided that matrix effects are taken into account. 10.0 mL of an aqueous sample containing potassium are placed in a 25.0 mL volumetric flask and diluted to the mark with distilled water. This solution reads 86.3 when introduced into the flame photometer. 10.0 mL of the sample and 2.00 mL of a 2.00 ppm solution of K^+ are placed in a second 25.0 mL volumetric flask and diluted to the mark with distilled water. This solution reads 98.4. What is the concentration of potassium ion in the sample? Ans: 2.85 ppm (why can the response still be considered linear with this result?).

3-8) The flame photometric detector for sulfur measures the light given off by sulfur-containing molecules in a cool hydrogen/air flame. The response of this detector follows the law (under certain circumstances)

$$R = S([RS]^2)$$

where RS represents a molecule containing a sulfur atom. Thiophene is a sulfur-containing impurity often found in benzene. A 2.00 mL benzene sample is diluted to 10.0 mL with hexane, injected into the detector, and reads 42.3. A second 2.00 mL sample of benzene is added to 2.00 mL of a 0.0100 M solution of thiophene in hexane and diluted to 10.0 mL with pure hexane. When injected into the

detector, it reads 68.4. What is the concentration of thiophene in the benzene sample, assuming that the response equation above applies? Ans: 3.68×10^{-2} M.

3-9) The response of Ca^{2+} in a flame photometer is known to be linear over a wide concentration range. A series of solutions each with 10.0 mL of a sample (containing Ca^{2+}) and varying volumes of a standard containing 20.0 ppm Ca^{2+} is subjected to flame photometry. The response, multiplied by (10.0 mL + V_s) is plotted *vs* V_s. The resulting straight line has a slope of 77.2, a *y* intercept of 610 mL, and an *x* intercept of -7.90 mL. What is the concentration of Ca^{2+} in the sample? Ans: 15.8 ppm.

3-10) A Ca^{2+} sample is treated somewhat differently than described above in Prob. 3-9. 10.0 mL of the sample and differing amounts of a 20.0 ppm Ca^{2+} standard are mixed and always diluted to 25.0 mL. The flame photometer readings for these solutions are:

Response	Vol. of 20 ppm Ca^{2+}
53.0	3.00 mL
62.2	6.00 mL
71.5	9.00 mL
80.8	12.00 mL

What is the concentration of Ca^{2+} in the sample? Ans: 28.3 ppm.

Sections 3.2 and 3.3: Internal Standards and Detection Limits

3-11) Indium is seldom found in lead-containing samples and thus makes a good internal standard for lead analyses. 10.0 mL of a 1.00×10^{-4} M Pb(II) solution are added to 10.0 mL of a 1.00×10^{-4} M solution of In(III). The resulting currents, measured polarographically, are 0.485 and 0.873 μA for Pb(II) and In(III) respectively. 2.00 mL of a 2.00×10^{-4} M In(III) solution are added to 10.0 mL of a sample and the currents for Pb(II) and In(III) are 0.186 and 0.504 μA respectively. Assuming a linear response between current and concentration, calculate the concentration of Pb(II) in the sample a) neglecting the information provided by the internal standard and b) taking the internal standard into account. Ans: a) 2.30×10^{-5} M, b) 2.66×10^{-5} M.

3-12) Manual sampling in the technique of gas chromatography is rather imprecise since a very small sample size is produced with a μL syringe. The use of an internal standard might be useful to avoid the imprecision of sampling. The following data are available:

Run #	Response for C_6H_{12}	$[C_6H_{12}]$	Response for C_6H_{14}	$[C_6H_{14}]$
1	275	0.100 M	162	0.0500 M
2	248	0.100 M	298	0.100 M
3	213	0.100 M	506	0.200 M

Would cyclohexane (C_6H_{12}) make a good internal standard for hexane (C_6H_{14})? Why? 1.00 mL of a 0.500 M solution of cyclohexane is added to 4.00 mL of a sample (containing no cyclohexane) and the resulting solution was analyzed chromatographically. The responses for cyclohexane and hexane were 261 and 483 respectively. What is the concentration of hexane in the sample? Ans: Yes, 0.194 M.

3-13) Stripping voltammetry is a trace electrochemical technique in which the current is linearly proportional to concentration. A solution of 1.0×10^{-7} M Pb(II) gave a current of 0.139 μA. If the noise is found to be 0.00362 μA, what is the detection limit for the determination of Pb(II) and what is the limit for reasonable quantification? Ans: 7.8×10^{-9} M, 2.6×10^{-8} M.