B. SELECTING A METHOD FOR LEAD ANALYSIS

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The measurement of lead is based on similar chemistry to that which made lead so useful to humanity. There are many insoluble salts of lead, leading to the earliest known methods for determination of lead: precipitation based reactions. Because of it's easily accessible redox states of 0, 2^+, and 4^+, lead can be changed from a cation to a metal. Lead can be electrolytically deposited as the metal or as the metal oxide onto an electrode for subsequent gravimetric (precipitation) based measurements.

Lead forms alloys of metals and metal sulfides easily. Lead can be reduced from the divalent state to the metal and solubilized in mercury, or even into or on a gold surface for an electrochemical determination known as anodic or cathodic stripping voltammetry. The alloy of PbS with Ag_2S, which can also be considered a precipitation reaction, can be used to develop voltages dependent upon the lead concentration. Other voltage measurements can be devised based upon the chelation of aqueous lead to a nonaqueous carrier in which lead is partially transported into a membrane. The voltage across the membrane, just as the voltage across a cell membrane, will be altered by the extent of lead transport.

The valence shell electron configuration of metallic lead is $s^2d^{10}p^2$. That of the divalent lead is $s^2d^{10}p^0$. The fact that the d orbital is filled makes the divalent cation "silent" to light in the visible region, a surprising fact, since lead is a major component in two of the three primary colors, red and yellow. The color in the pigments lies in a charge transfer band, (an internal oxidation/reduction of the oxide compound) as opposed to a perturbation of d electrons from a filled lower state to an unfilled higher energy state. As a result the most popular method for analysis, colorimetric, is the most difficult to create. Colorimetric methods rely upon the charge transfer reaction (PbI_2) or upon the chelation of the lead ion in which the chelate undergoes color change with reaction with lead. The electron promotion occurs within the chelate's valence shell electrons from the highest occupied molecular orbital to it's lowest unoccupied molecular orbital (HOMO to LUMO). This method therefore relies upon chelation for selectivity, something that is particularly difficult to achieve.

Better success is achieved with manipulating the s and p valence electrons or the inner shell electrons. The s and d electronic transitions can be accessed through atomic absorption spectroscopy, atomic emission spectroscopy or atomic fluorescence. The most commonly used methods are those of atomic absorption spectroscopy using electrothermal gas phase production, and that of atomic emission spectroscopy using a plasma source for the gas phase production and excitation of the electrons. Internal shell electrons can be probed using X-ray fluorescence methods.

Success is also obtained by probing the electrons within the bonds, by checking for bond strength via a test of the bond motion in vibrational spectroscopies. Traditional infrared techniques have been used to understand the structure of the charge transfer based pigments. Non-destructive vibrational methods involving reflection of light that are very sensitive and very accurate have recently been introduced for art analysis using Raman spectroscopy.

Manipulation of the lead into the gas phase and separation based upon weight (gas diffusion, or acceleration) provide a method for isotopic analysis. Although in some senses this is a weight based measurement, it falls under the general category of spectroscopic analysis, since a spectrum of isotopes is scanned.

Table 1: Limits of Detection

Acronym	~Dates	Method	LOD ppb = ng/g = µg/L
Gravimetric	1823	Lead Sulfide spot tests	
	1823-1920	Lead Chromate precipitation	
UV-Vis	1920-1960	Complexation with Dithizone	20-500 ppb
EP	<1980	Erythrocyte Protoporphyrin Fluorescence Assay	>250 ppb
ASV	1960-present	Anodic Stripping Voltammetry	20-50 ppb
AAS	1960-1970	Flame Atomic Absorption Spectroscopy	20-50 ppb
AFS	1960-present	Flame Atomic Fluorescence Spectroscopy	500
ISE		PbS/Ag_2S ion selective membranes	10^{-5}M (2 ppm)
GFAA	1970-1985	Graphite Furnace Atomic Absorption	50 ppb
GFAA+ preconc.		Graphite Furnace AA	0.05 ppb
XRD		X-ray diffraction	Qualitative
IR		Infrared	Qualitatitve
XRF		X-ray Fluorescence, house paint	0.9 mg/cm^2
		M. Sperling, X. Yin, B. Wetz., Frenz. J. Anal. Chem. 1992, 343, 754.	
NMR	1970s on	Nuclear Magnetic Resonance	1%
Raman	1980s		Qualitative
LEAF	1980s	Laser Excited Atomic Fluorescence	0.2 to 9 ppt
IEC		Ion Exchange Chromatography	50
		M. A. Bolsho, C. F. Boutron, A. V. Zybin, Anal. Chem., 1989, 61,1758.	
ICP-AES	1980s	Inductively Coupled Plasma Atomic Emission	20
ICP-MS	1990s	Inductively Coupled Plasma Mass Spectroscopy	1-10 ppt
EXAFS	1990s	Extended Xray Absorption Fine Structure	Qualitative
Optodes	1990	UV-Vis methods coupled to Fiber Optics	sub ppt
Immonoassays	2000s	Monoclonal Antibodies will be raised to chelates of lead	sub ppt

Separation based methods in which a flowing stream of gas or liquid is carried past a stationary support and the relative time for exit from the stationary phase is used to separate the lead can also be used. The most common method is that of gas chromatography for the determination of the organolead compounds. Liquid based methods (useful for divalent lead or a chelate of divalent lead) have been reported but are not among the most common or validated methods.

The use of biotechniques (enzyme assays, monoclonal antibody assays) is in its infancy.

Among all of these possible methods how does one determine the **right** one? To select a method a variety of **critical choices** must be made (Figure 1). Often during a teaching lab students are exposed to a single set of choices (calibrate the instrument). In real life the analyst must not only calibrate the instrument, he/she must choose an instrument.

The first criteria for determining the right method is to ascertain if a qualitative or quantitative answer is required. It may be that the issue is not the quantity of lead, but the type of

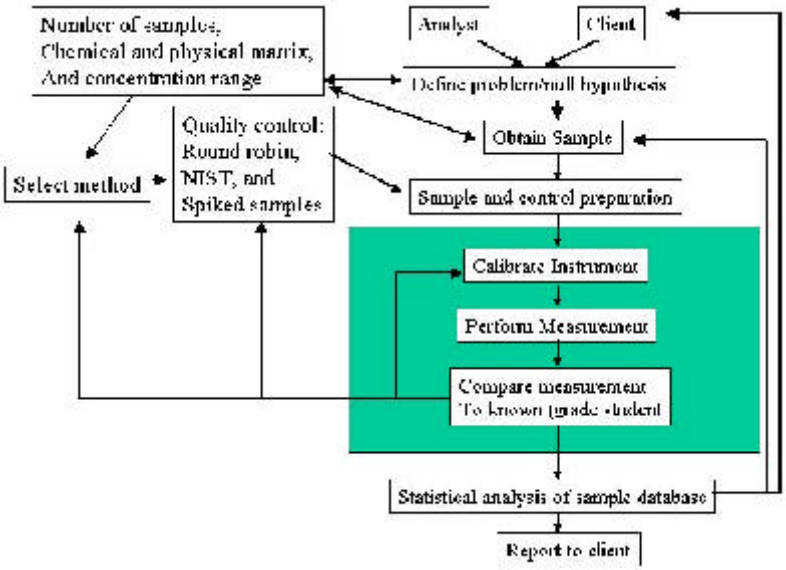

Figure 1: The feedback pathway used by students in most instrumental analysis classes is shown in the shaded box The full analytical process is more complex requiring the analyst to **choose** an appropriate method of analysis based both on instrumental capabilities and the type of sample and amount of sample to be analyzed. Further, an appropriate interaction with the "client" and the design of quality control measures are required.

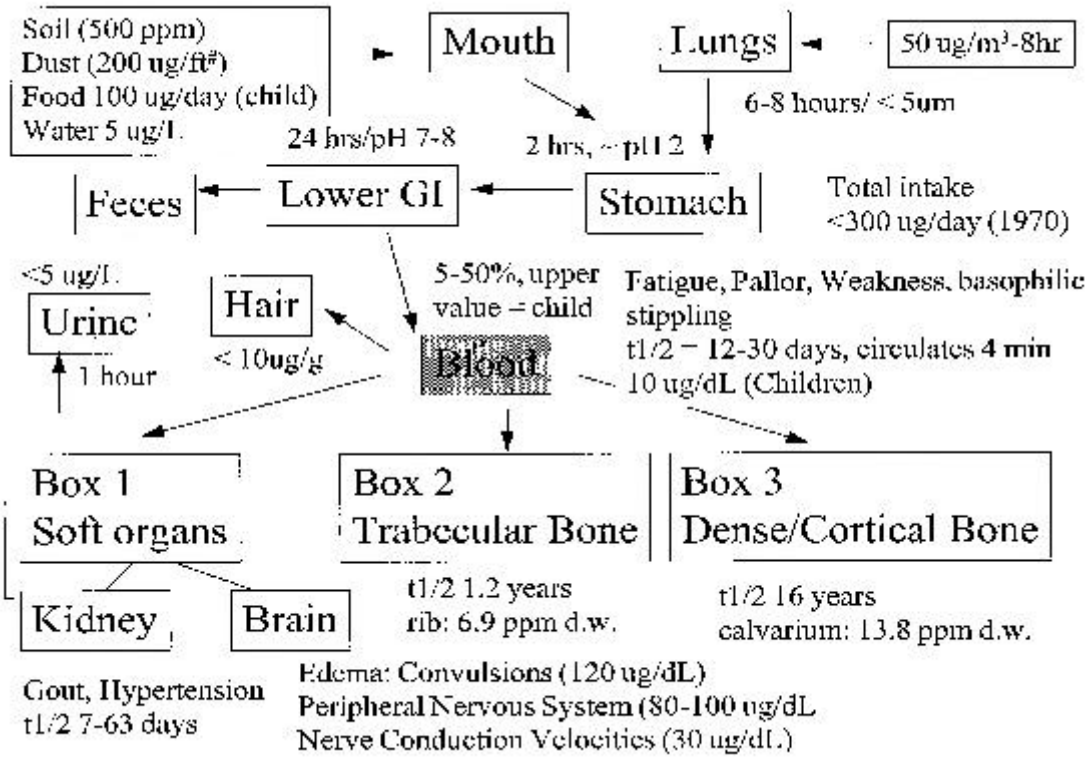

Figure 2: Lead moves from a variety of sources in the environment through several pathways into the body where it is stored in different tissues or excreted. Based on multiple studies on the uptake and storage of lead allowable limits of lead have been set. Values listed for physiological response represent average concentrations resulting in certain clinical responses.

lead present. The toxicity of lead in soil depends upon the solubility of the lead containing compound. A limit of 500 ppm lead may be conservative or not depending upon the compound. A large piece of galena, PbS, may contribute to a soil lead of 1000 ppm but be unlikely to be tracked as dust into the house and to be digested within the stomach. Another case might be the tracing pigments for forgeries. The exact type of lead pigment is the required information, not the quantity.

If a quantitative answer is required the method selected depends, in part, upon the sample and the amount of work it will take to prepare it (dust, bronze, blood, bone, archaeological bone, hair, soil). Some samples can **not** be prepared (rare art, human remains of certain ethnic groups, live human bone). The allowable chemical preparation will in part determine the method, since the preparation may be integral to the application of the instrument.

The total number of samples to be run will determine how routine the analysis must become, which will determine how cost effective a particular method will be. Whethor or not the sample is be determined on site (rare paintings) or in a centralized lab will determine how exotic the method should be.

Limits of Detection.

A principle factor in method selection is the sensitivity required. The allowed limits of lead (Figure 2) will determine what degree of sensitivity is required. A method for lead in soil with an allowed limit of 500 ppm may not work for dust with an allowed limit of 5 ppm, or for blood, with an allowed limit of 5 ppb.

The detection limits of the various methods generally drop with the date of introduction. The lowest detection limits are obtained with graphite furnace atomic absorption, inductively coupled plasma-mass spectrometry, and stripping voltammetry as shown in Table 1. Lower detection limits can be obtained with several advanced techniques.

Figure 3 illustrates that, roughly speaking, the detection limits vary with time in a log-log manner. The first method for lead analysis was based on the separation of silver from lead during the metallurgical process, cupellation. By the Roman Empire knowledge about the oxidation and reduction potentials of lead with respect to the known metals was codified within the days of the week. The first "real" analytical chemistry begins with Acum's text (1820) which describes a PbS method for lead analysis. By the beginning of the 1900s German organic chemists had devised a colorimetric method lead based on dithizone complexation, a method used until the 1960s.

How are Limits of Detection Determined?

The limit of detection for a **method** depends upon the random error introduced in sampling, chemical preparation, and the instrument and on the response of the **method** to a change in the lead concentration:

A measurement can be considered to be an average of a small sample from a population of an infinite series of measurements (a population) which are influenced by the measurement process in

random and non-random fashions. Thus one's "true" weight may be at 145 lbs but wishful thinking and a squinting eye (**determinate** error) sets the weight at 140 lbs. If one weighs

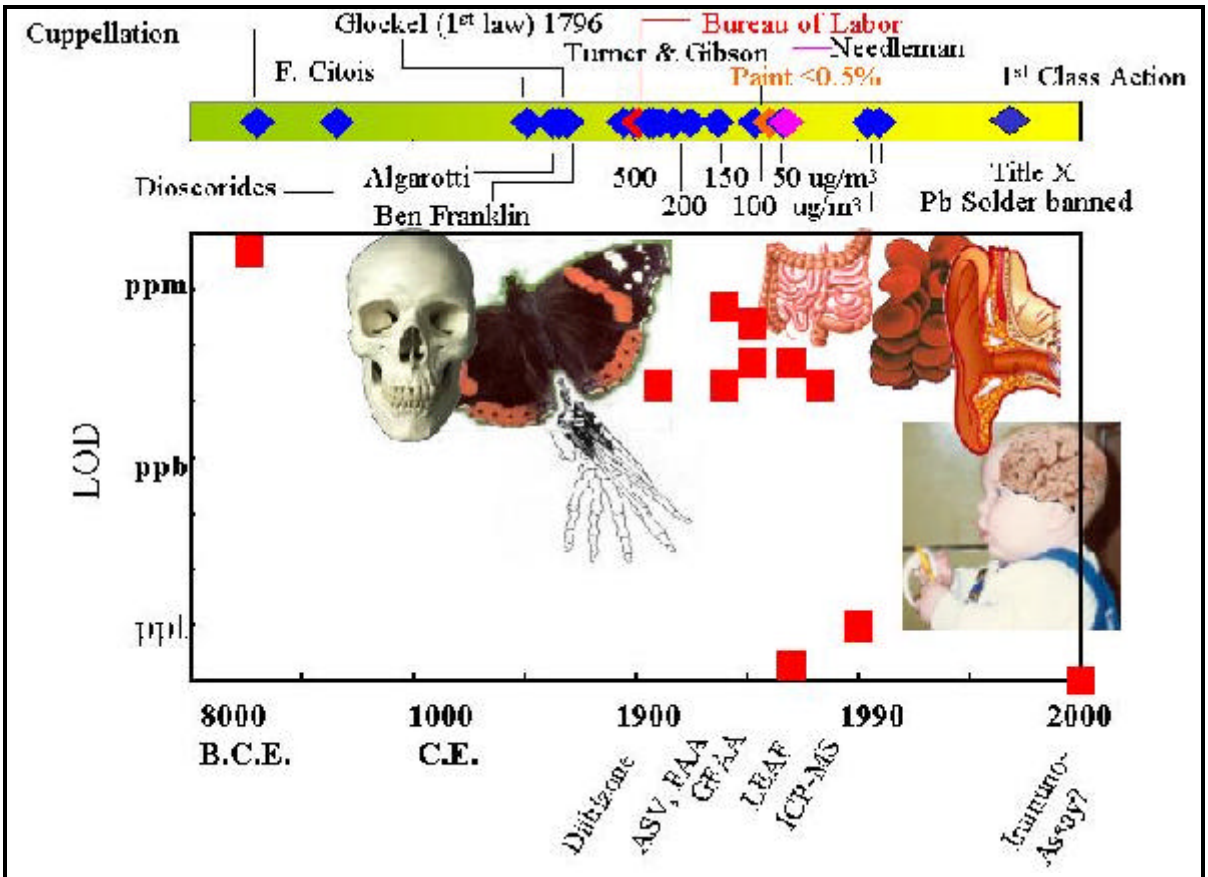

Figure 3: The Limit of Detection has fallen in a roughly log-log fashion with time since lead was discovered and manipulated. The early analysis of lead centered around removal of silver (cuppellation) which could be accomplished to approximately 99% lead purity. The relative oxidative reactivity of lead compared to other known metals was established and codified by the time of the Roman empire (days of the week). Gravimetric methods were introduced in 1820 in Acum's analytical text. Shown in the figure are various methods (x axis) and the range of symptoms policy makers worried about (images). The upper portion of the figure gives a few important social/scientific breakthroughs in lead toxicity. It should be noted that concern over lead poisoning centers first on death, then on occupational health issues and finally, as the LOD has dropped, on developmental toxicity.

oneself everyday (Figure 4) for one year, a plot of those daily measurements will show some fluctuation about the "true" value. A histogram is also a measure of the fluctuation (Figure 5). The histogram represents a Gaussian or normal population that has not been sampled adequately. A “normal” curve is described by the function:

[1]

$$f(x) = \frac{1}{\sigma\sqrt{2\pi}} e^{\frac{-(x-\bar{x})^2}{2\sigma^2}}$$

where f(x) is the probability of observing the number (number of observations expected), ó is the variance which is **estimated** by the standard deviation, s, x is the value of x and $\bar{x}$ is the mean value of

Table 2: Statistical Moments

Moment	function	common name	formula
0	f(x)	Probability	$f(x) = \frac{1}{s\sqrt{2p}} e^{-\frac{1}{2}\left(\frac{x-m}{s}\right)^2}$
1	xbar	mean or average	$m = \int_{-\infty}^{\infty} x\left[f(x)\right]dx$
2	s	standard deviation	$s = \left[\int_{-\infty}^{\infty} x^2\left[f(x)dx\right]\right] - m^2$
3	skew	shape	$skew = \left[\int_{-\infty}^{\infty} x^3\left[f(x)\right]dx\right] + ms^2 + m^2$

x. Physicists and mathematicians refer to this function as the zeroth moment. The normal error curve is shown in Figure 6.

This curve has the following characteristics:

a. There is a peak at x = µ
b. The peak is symmetric
c. There are inflection points on either side of the peak which define ó, which accounts for 68.3% of the measurements.

The first and second derivatives of the error curve are also shown. The first derivative crosses the x axis at the mean of the population. The mean is the first moment of the population and can be calculated by equation 2:

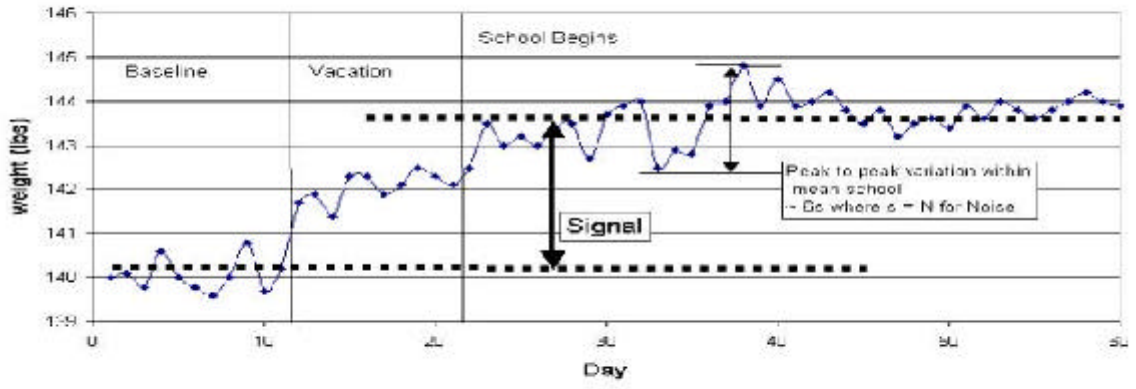

Figure 4 My weight over summer plotted as a function of pounds vs days. The first 11 days represent average summer weight (baseline). The next 10 days represent weight gain during vacation, and the final section represents weight gain on the beginning of the semester. The line marked peak to peak variation represents the maximum and minimum weights measured during the beginning of the school year.

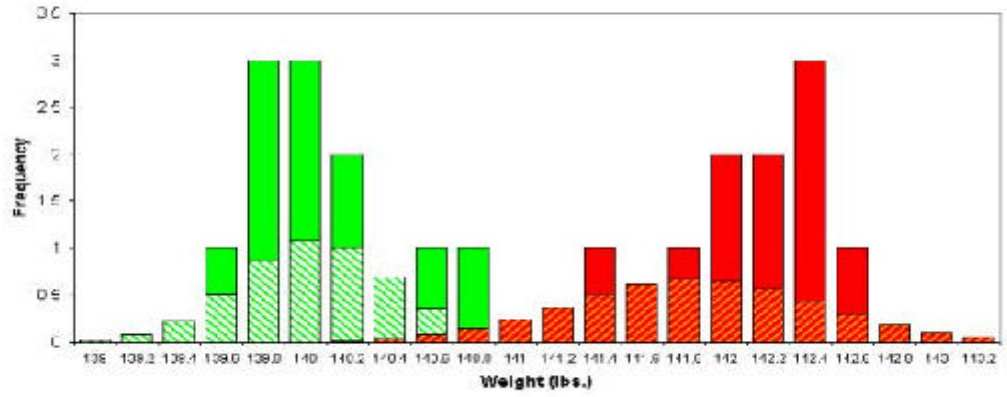

Figure 5 Histogram or frequency plot of the weight data from above figure for the "baseline" and "vacation" portions of the data. The striped areas represent the fit normal curve to the data (solid) based on the calculated mean and standard deviations. Note that the "real" data is not well approximated by the normal data with this population size, and that the estimated populations signficantly overlap.

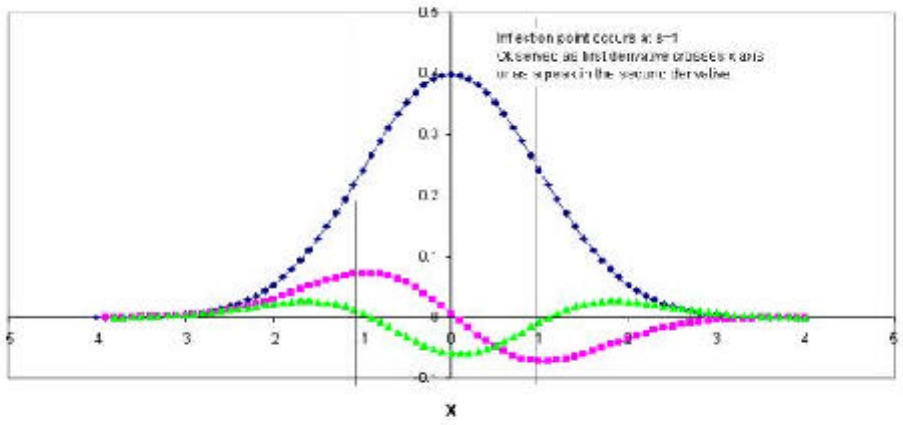

Figure 6 The standard (s=1, μ=0) error curve, with its first and second derivatives showing that the curve has inflection points at s ± 1. The inflection points are observed in the first derivative as the points at which the curve crosses the x axis and in the second derivative as peaks.

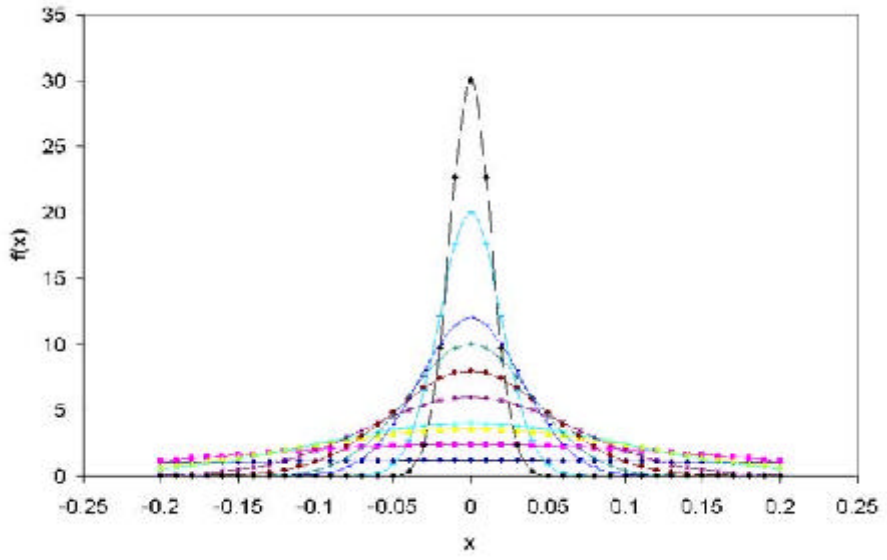

Figure 7 A Normal or Gaussian or error curve for samples of an N-1,000,000 member true population with a population standard deviation of 1. The sample size, n, increases from 3, 6, 9, 10, 15, 20, 25, 30, 50, to 75. As the sample size increases the standard deviation of the sample decreases while the peak associated with the mean increases.

[2] $$\bar{x} = \sum_{i=1}^{n} \frac{x_i}{n}$$

The second moment is the standard deviation of the population, a measure of the width of the normal curve. The standard deviation, s, is calculated:

[3] $$s^2 = \sum_{1}^{n} \frac{(x - \bar{x})^2}{(n - 1)}$$

If we consider a theoretical population consisting of a large number of individuals, N, with a population standard deviation of s_{pop} of which we only sample a few members, n, then the standard deviation of the sample, s_{sam}, scales with the number of individuals sampled:

[4] $$s^2_{sample} = \left(1 - \frac{n}{N}\right) \frac{s^s_{population}}{n}$$

If N is very large (10,000) compared to the sample size (1-100) then equation 4 reduces to

[5] $$s_{sample} = \frac{s_{population}}{\sqrt{n_{sample}}}$$

Figure 7 shows how the error curve varies as the population size is increased (N= 1,000,000) from n=3 to 70. Note that the standard deviation decreases and the population height increases. Equation ([5]) tells us that the standard deviation decreases with 1/√n (Figure 8). Consequently our goal is to increase the sample size in order to reduce the standard deviation.

For a population following a normal error distribution 68% of the area under the curve falls between ± 1s; 95.4% of the area under the curve falls between ± 2s, and 99.7% of the area falls between ± 3s (Figure 9). Another way to state this is that 99.7 % of all the measurements made for a population will fall between -3 and +3 standard deviations.

Two rules of thumb derive from this type of curve. The first rule of thumb can be derived from the observation that 99.7% of the measurements are encompassed by ±3s. If you imagine that the data has been acquired as a time stream (like my weight in Figure 4) then the data fluctuates about the mean. The maximum and minimum data points will be observed as the largest positive and negative going peaks in the time stream. These points correspond roughly to the maximum outliers in the bell curve, from which we get a useful rule of thumb:

[6] $pp \sim 6s$

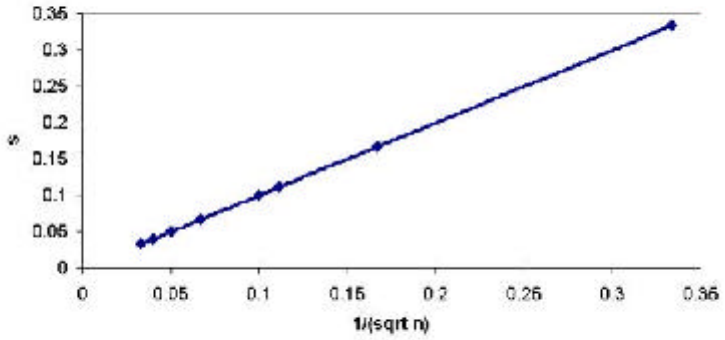

Figure 8 The standard deviation decreases as the sample population increases, as observed by a linear plot of s vs 1/√n for the data shown in Figure 7.

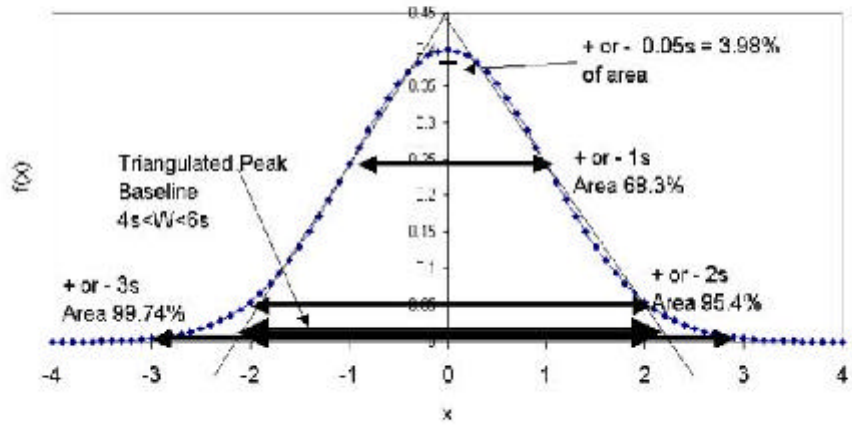

Figure 9 A normal error curve contains 68.3% of the measurements between ±1s ; 95.4% of the measurements (area under curve) between ± 2s; and 99.74% of the measurements between ±3s. Triangulation of the peak (drawing a line along each side of the curve through the inflection point, estimates the peak base width between ±2s (4s total) and ±3s (6s total). The top of the peak (between ±0.05s) accounts for 3.98% of the population of measurements.

where pp represents the peak to peak distance between the largest positive and negative going peaks.

The second rule of thumb is that the area under a very narrow segment at the peak contains a fixed proportion of the population (for example ±0.05s contains 3.98% of all the individuals within the population) (Figure 9). Therefore as the population size increases the peak height will scale also (e.g. 3.98% of n= 100 is 3.98 and of n=1000 is 39.8). For this reason the peak height is often used to measure the intensity of a signal, assuming that the signal has a normal error shape.

To scale for the peak height the Gaussian equation is modified by a **pre-exponential** factor, A, which is the peak height.

[6A] $$f(x) = A\frac{1}{s\sqrt{2p}}e^{-\frac{1}{2}\left(\frac{x-m}{s}\right)^2}$$

The rule of thumb, peak height proportional to area under peak, fails when the population is **skewed**.

The third moment (Table 2) tells whether or not the population is skewed, or, in fact, an ideal, random population. The formula for the third moment is given as:

[7] $$skew = \sum_{1}^{n}\frac{(x-\bar{x})^3}{ns^3}$$

A positive skew means that the population has a tail at the higher values of x. A negative skew means that the population has a tail at lower values of x. A variant of this number is often used in chromatography to determine the **tailing** of a peak. In this case an asymmetry factor is calculated

[8] $$A.F. = \frac{b}{a}$$

as shown in Figure 10. When a peak is asymmetric peak heights do not correlate with the area under the curve and it is better to use an area based measurement as opposed to a peak height measurement.

We are now ready to examine the question of resolution and the related question of limits of detection and S/N. To do so let us consider two normal curves that are adjacent to each other (Figure 11) Resolution embodies our ability to distinguish one population of measurements from another.

[9] $$R = \frac{\overline{x_b} - \overline{x_a}}{\frac{W_a}{2} + \frac{W_b}{2}}$$

In general R must be greater than 1 in order to **resolve** the peaks. If the peaks have similar shapes and standard deviations then the baseline widths are equal ($W_a = W_b$) and equation 9 can be simplified:

[10] $$R \cong \frac{\overline{x_b} - \overline{x_a}}{W}$$

How much do the populations overlap when R = 1? This can be estimated by recalling the rule of thumb (Figure 9) that the triangulated base of a normal curve is between 4s and 6s.

[11] $$\overline{x_b} - \overline{x_a} = 6s \qquad \overline{x_b} - \overline{x_a} = 4s$$

By substituting equation [11] into equation [10] equation [10B] can be obtained:

[10B] $$R \cong \frac{\overline{x_b} - \overline{x_a}}{6s} \quad \text{or} \quad R \cong \frac{\overline{x_b} - \overline{x_a}}{4s}$$

In this class we generally use the first of equations [10B].

If the means are 6s apart then each population "touches" when 3s has been covered. The population that extends beyond 3s is (100-99.7)/2 = 0.15%. Thus 0.15% of the population of A lies under the bell curve for the population of B and vice versa. If the means are really only 4s apart then population A touches population B at 2s from the mean of A. The population that extends beyond 2s is (100-95.4)/2 = 2.3%. Thus a resolution of 1 means that between 0.15 and 2.3% of the population could fall in the second bell curve.

For our example involving vacation weights the resolution (using equation 10B) of the vacation (not school!!) weight from the baseline weight is R = (141.9-140)/6(.329) = 0.96. Since R <1 we have not completely resolved the vacation weight from the baseline weight. Resolution could be accomplished by either having me gain more weight during vacation (change the slope of the curve) or by having the standard deviation decreased (only weigh myself in the same clothes every day).

The limit of detection is similar to resolution as it is also based on the idea of overlapping bell curves. Analytical chemists consider that one population can be **detected** when the mean signal for the population lies 3 standard deviations away from the mean signal for the blank or baseline:

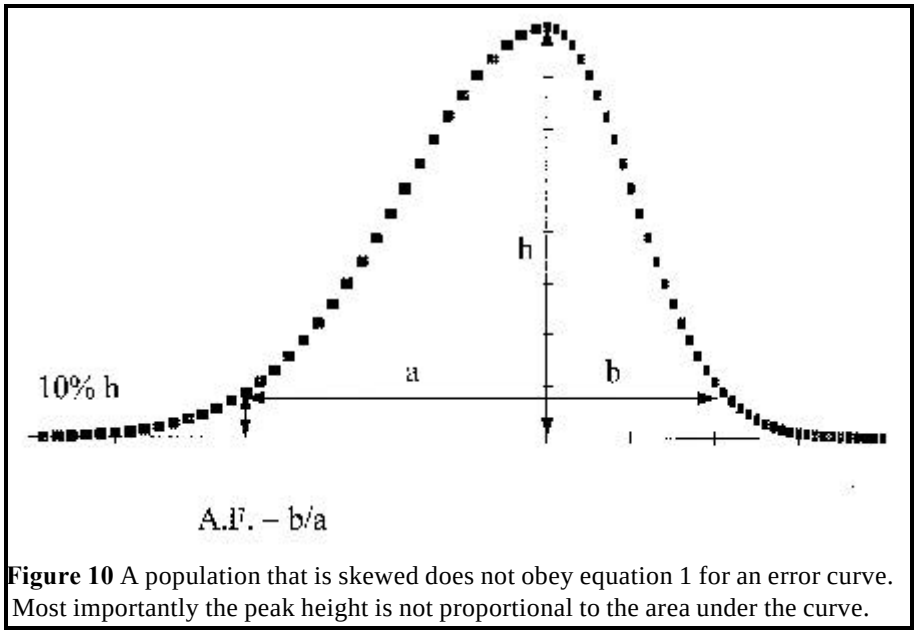

Figure 10 A population that is skewed does not obey equation 1 for an error curve. Most importantly the peak height is not proportional to the area under the curve.

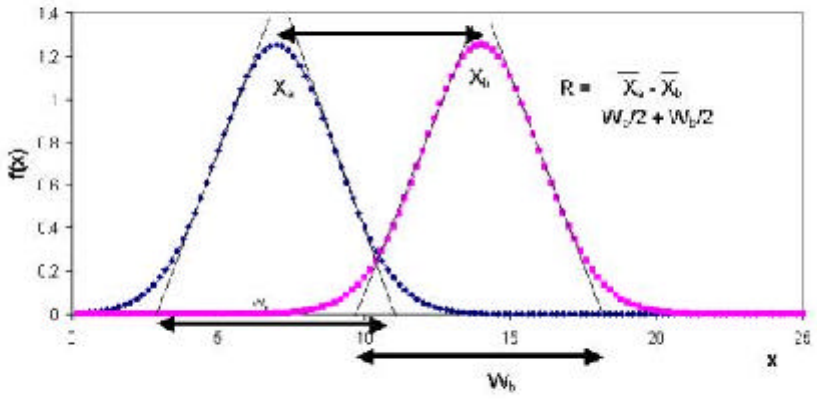

Figure 11 Resolution is computed from two overlapping normal populations. The triangulated baseline width is used for each peak.

[12] $$\overline{x_{LOD}} = \overline{x_{blank}} + 3s_{blank}$$

The population of the detectable signal and the blank touch at 3s/2populations=1.5s per population. The percent of the population that resides beyond 1.5s from the mean is 6.68%. We falsely attribute 6.68 percent of the measurements from the blank as part of the population of interest. For our example (**Vacation** vs Baseline weights) the limit of detection is 140 + 3(0.329) = 140.977 lbs. Since the vacation weight is 141.9 lbs, we can clearly detect a weight change.

The **limit of quantification** is more stringent.

[13] $$\overline{x_{LOQ}} = \overline{x_{blank}} + 9s_{blank}$$

In our example (**Vacation** vs baseline weights) the limit of quantification will be 140 + 6(0.329) = 141.97. The weight change during vacation was 141.9 lbs so that we are not able to quantify the weight change.

A very similar measurement is termed the **signal to noise ratio S/N**. In this number the signal, S, is the difference between the mean of the signal and the mean of the blank, while the noise, N, is the standard deviation, s, of the blank:

[14] $$\frac{S}{N} = \frac{\overline{x_{signal}} - \overline{x_{blank}}}{s_{blank}}$$

Referring to the time based plot of the weight gain (Figure 4) we note that there are fluctuations of weight around a mean in the area marked baseline and the area marked vacation. The distance from the maximum to the minimum is termed the peak to peak fluctuation and was noted as approximating 6s (equation 6). From this plot we can determine the S/N of the vacation weight as (140.977-140)/(142.8-141.2)/6) = 3.66.

Analysis of Variance

Suppose that the resolution between populations is small enough that it is not immediately visually apparent, i.e. baseline resolution is not observed and the peaks overlap to the extent that they appear as a single large "lumpy" peak. How is this situation handled?

An example is to look at the histogram of weights in Figure 5 and apply a statistical test to "resolve" the populations. The first step is to assume that the bar graph in Figure 5 represents a random homogeneous population. A random population assumption allows us to make use of normal or Gaussian mathematics.

In order to assign confidence in our data we first **assume** that they can be described by the normal curve formulas (equations 1-3). We then compute the mean and standard deviation (width) of

our population, use these numbers to compute the expected number of observations, and plot this curve as an overlay of the histogram of real data. If the match is good (as measured by deviations from the histogram to the expected observations) then we accept the computation. If the match is poor then we must check to see if we really have two or more populations. (An examination of the Figure 5 shows that the error curves estimated from the sample population do not actually match the data very well).

As an example let's determine if the vacation weight is part of the random fluctuations in weight with time or is truly a separation weight population. We will perform an **analysis of variance** on the system as shown in Figure 12. The actual computation for an analysis of variance is rote (or by formula) but the concept behind the analysis of variance is to find a theoretical population curve or curves that best matches the histogram (shown visually in Figure 5). The tool by which a population is judged to fit a model of a single or a multiple population is the F statistic. This statistic computes the expected mismatch between acquired data and the predicted population based on the population size and the uncertainity one is prepared to expect. When the calculated F value is greater than the expected F value there is too much variation in the attempt to match the data to a single population. The alternative hypothesis, that the data is better described by two sample populations, is correct.

The best way to learn to do an ANOVA (analysis of variance) is to follow the example

Sample	Normal"	Vacation	Totals
i=16n	j = 1	j = 2	j =16k
1	140	141.7	
2	140.1	141.9	
3	139.8	141.4	
4	140.6	142.3	
5	140	142.3	
6	139.8	140.6	
7	139.6	142.3	
8	140	142.7	
9	140.8	141.7	
10	139.7	141.6	
11	140.2		
n_j	11	10	n = 11+10=21
mean	140.05	141.85	
s	0.36705	0.5961	
s^2	0.1347	0.3561	
$T_j = \sum_i^n x_{ij}$	1.5406×10^3	1.4185×10^3	$T = \sum T_j = 2959.1$
$\sum(x_{ij})^2$	215769×10^5	2.01217.4	SS =416986.8

TSS = total sum squares = $SS-(T^2/n) = 21.4381$

BSSS = between samples sum squares = $\sum_{j=1}^{k}(T_j^2/n_j) - (T^2/n) = 16.885$

BSMS = between sample mean square = BSSS/(k-1) = 16.885/(2-1) = 16.885

R = residual error = TSS-BSSS = 21.438-16.885 = 4.55

RMS = residual mean square = R/(n-k) = 4.55/(21-2) = 0.2395

$F_{calculated}$ > predicted at 5% probability of randomness then difference is significant
=BSMS/RMS = 16.885/0.2395 = 70.477
$F_{k-1,n-1,á} = F_{1,20,0.05} = 4.35$ (from table)

Since BSMS/RMS = 70.5> F@5% (4.35) probability the result is significant. We note that random variability will not account for the differences observed. We are 95% certain that I gained weight.

Figure 12: An example calculation of analysis of variance (ANOVA) using the data from Figure 4. The value for the theoretical F number is found in the appendix**.**

step by step. An ANOVA can also be calculated on a spread sheet such as Excel.

Calibration Curve.

Each measurement we make of the signal for a concentration consists of a histogram of measurements centered around a mean signal with a standard deviation associated with the measurement. We can construct a calibration curve using these variable measurements. For the sake of argument, let us assume that the baseline weight (Figure 4) was associated with eating 1500 calories/day, the vacation weight gain was associated with eating 4000/day and the semester weight gain with eating 6000 calories /day. Using all the data shown we can construct a calibration curve (Figure 13).

The "calibration" curve is constructed with two sets of data. The first includes all the weight measurements and illustrates the "scatter" of data, similar to the baseline of a Gaussian curve. The second set of data is constructed from the mean of the scattered data and the standard deviation associated with that mean for each calorie/day value. This data has been used to create a regression curve which projects backward to the weight associated with zero calories per day (how easy it is to "misrepresent" with statistics!!!).

The errors associated with calibration curves are computed in a fashion similar to the ANOVA. The variability of all the measurements is the sum of the variability in the x population, the variability in the y population and the variability along the line:

[15] $$s_q^2 = \left(\frac{\partial q}{\partial x}\right) s_x^2 + \left(\frac{\partial q}{\partial y}\right) s_y^2 + \left(\frac{\partial q}{\partial_x}\right)\left(\frac{\partial q}{\partial y}\right) s_{xy}$$

where σ_{xy} is the variability along the line:

[16] $$s_{xy} = \frac{1}{N}\sum_{i=1}^{N}(x_i - x_{line})(y_i - y_{line})$$

The differences between the measurement and the line should be randomly distributed in positive and negative directions, therefore the sum of the differences should go to zero. In this case the variance associated with an individual measurement should approach the randomness in the x and y populations:

[17] $$r^2 = \frac{\left(\frac{\partial q}{\partial x}\right)^2 s_x^2 + \left(\frac{\partial q}{\partial y}\right)^2 s_y^2}{s_q^2 - 1}$$

What this equation states is that a good fit (r^2 goes to 1) occurs when the variance along the x axis

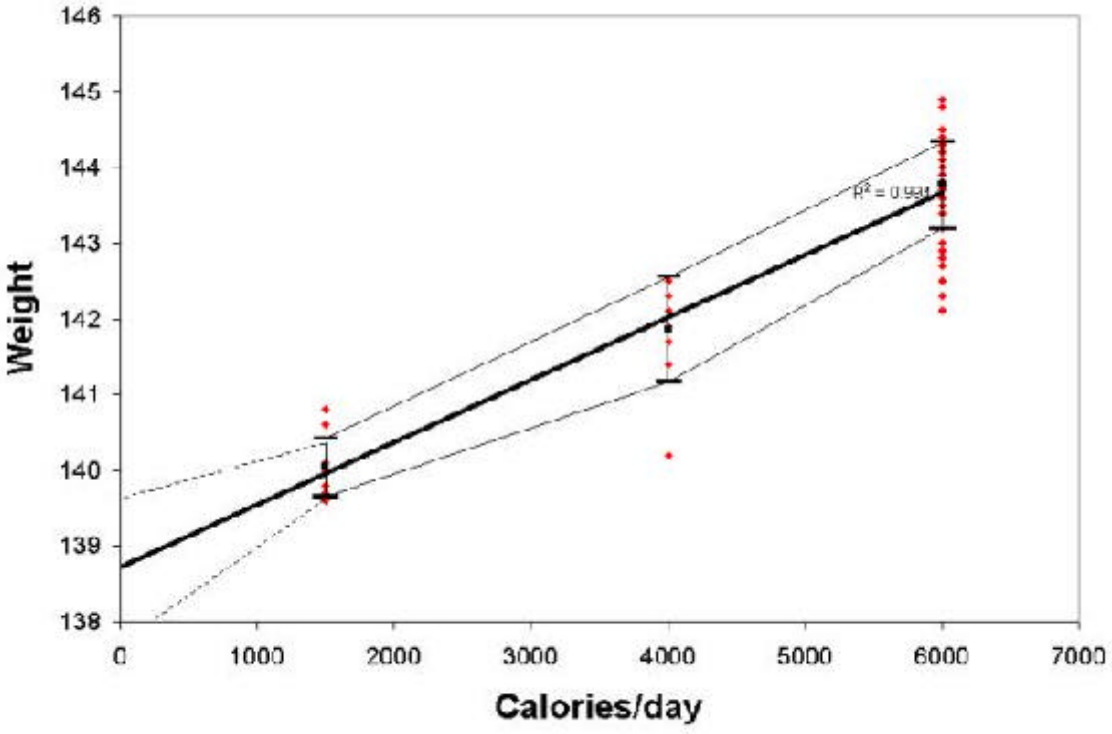

Figure 13 A calibration curve of the data from Figure 4 assuming the weight change is "calibrated" or associated with calories/day. All of the data is plotted (small red dots), as is the mean weight associated with each calorie count and the standard deviations. From this plot it should be apparent that each individual point in the calibration curve represents a population which, if random, or Gaussian, is described by an s and a mean. A regression line is fit through the means, but does not, in fact, pass through any of the three means. The regression line is described by residual error, r^2, of 0.994. The regression line is projected back toward a blank value, weight associated with zero calories per day!!!. The error associated with the line fit is used to project error associated with the intercept (dashed lines).

summed to the variance along the y axis is close to or equal to the total variability of the data. We expect r to be >0.9 for a good linear fit to the data.

How good is good for a given population size? If we report an r value of 1 for a two point line we have zero confidence in the value of r = 1, because we can always fit a straight line through two points. If r = 1 on a line with 100 points we are much more confident in the value of r. A line of three points with an r=0.9 would have a random chance of such a correlation 29% of the time. Thus we are only 71% confident that the line is the appropriate fit through the data. If we have a five point line with an r=0.9 we have a 3.7% change of random events giving us the line, or we are 96.3% confident that the r value is accurate.

Another useful piece of information can be obtained by an analysis of the variability of the data in the regression. A backwards projection of the magnitude of error along the regression line can be

used to get an estimate of the variability associated with the intercept (blank). This is useful in cases where the analyst has fouled up and forgotten to obtain the requisite 3 measurements of the blank for determination of the standard deviation of the blank. An estimate of the standard deviation of the blank can be obtained by error analysis of the intercept. Most standard statistical packages performing linear regressions will give intercept error estimates. The error associated with the means and with the fit to the line are used to also project backward the error bars associated with the blank (see Figure 13).

Chemistry and/or **instrumentation** comes into play with the calibration curve by setting the distance along the x axis. The **slope** of the calibration curve or the **sensitivity** of the method is measured in the x axis.

[18] $$\bar{x}_{signal} = \bar{x}_{blank} + b[conc.]$$

where b is the slope. At the **concentration limit of detection** equation 18 becomes:

[19] $$\bar{x}_{LOD} = \bar{x}_{blank} + b[conc.LOD]$$

By setting equations 19 and 12 equivalent to each other we find that

[20] $$[conc.LOD] = \frac{3s_{blank}}{b}$$

Equation 20 tells us that the concentration limit of detection is determined by a ratio of the precision of the measurement to the sensitivity of the instrument. If we want to lower the limit of detection we must either increase precision (lower s) and/or increase selectivity or sensitivity. One of the easiest ways to lower s of the blank is to increase the number of measurements (equation 5).

When comparing various instruments the measurement of interest is the concentration LOD, not the signal LOD.

Not only are LOD and sensitivity of the method important, but also the **linear range**. The linear range is the concentration range over which a single slope "b" applies. There are very few instrumental methods which have large linear ranges coupled to low limits of detection. Figure 14 illustrates a typical instrumental calibration curve with the linear range denoted.

Instrumental Considerations

Many of the limits of detection that are reported are for the **instrument** and not for the **complete method**. This may be because the instrument is the one thing that the analyst can control. One of the factors affecting instrumental response is **rise time** and **electronic noise**.

The **rise time** is a measure of the time it takes the instrument to move from 10% to 90% of the full signal. This is related to the frequency response of the instrument. Any signal

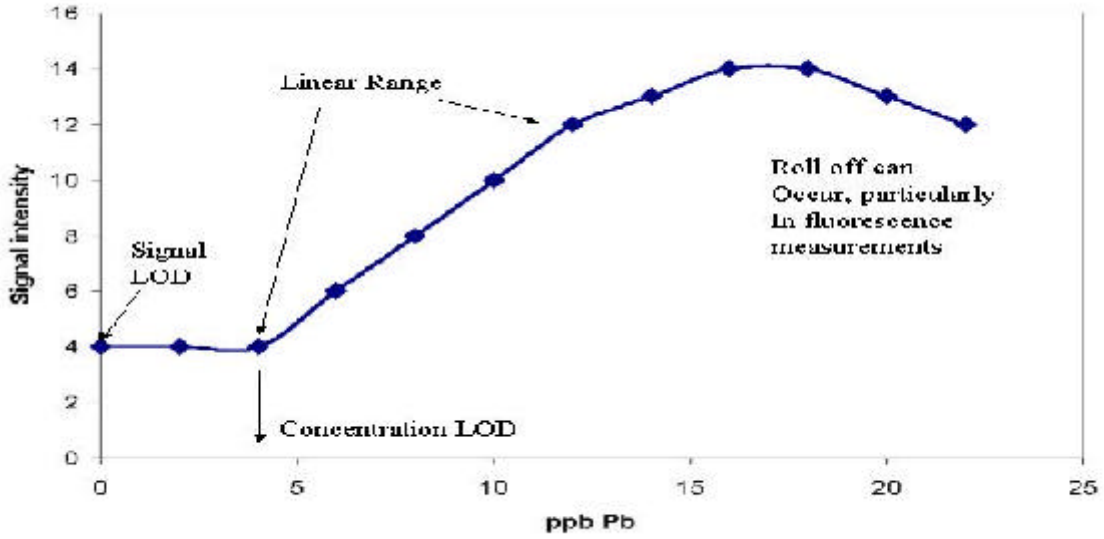

Figure 14 A calaibration curve is often linear over a specific range. The lower end is terminated with the LOD, often a function of the standard deviation of the blank. The upper end of the linear range may sometimes exhibit a decrease in signal intensity with increased concentration. This is particularly the case for some fluorescence measurements.

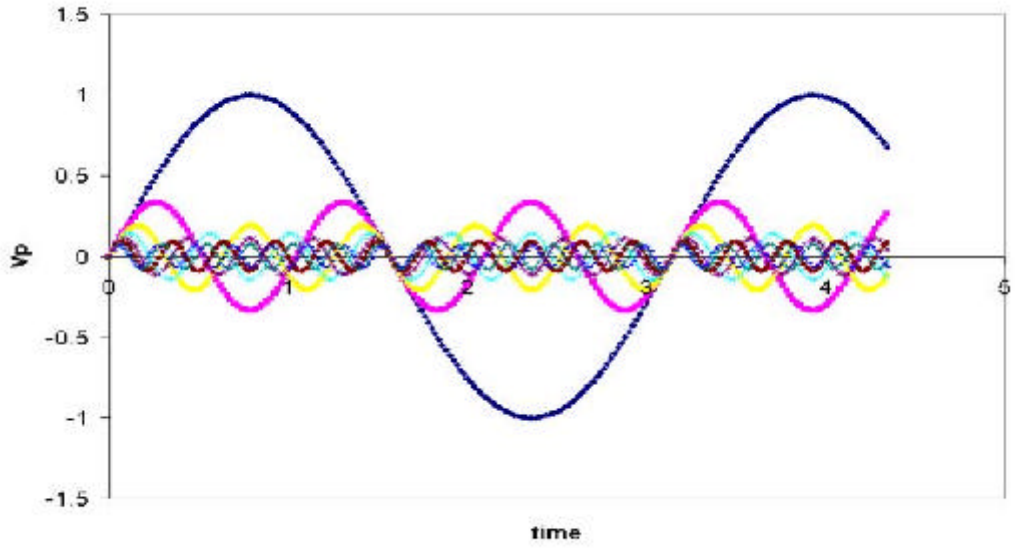

Figure 15 The sin waves shown in this figure represent a "base" frequency of amplitude 1, a frequency tripled wave of amplitude 1/3, a 5xbase frequency of amplitude 1/5 and so forth to 16xbase frequency. When these sin waves sum they will add in the region shown and will subtract or destructively sum in the region near the peak of the base frequency.

generated by an instrument in response to some chemical measurement can be thought of as a voltage vs time plot. As an example consider the square wave. A reasonable square wave can be generated by summing a base frequency, f_1, sin wave with sin waves of decreasing amplitude and increasing frequency. The summed sin waves are odd number multiples of the base frequency in order to ensure that **constructive** interference leads to an increase in the voltage at the leading and falling edges of the sin wave with out causing an overall increase in the amplitude of the signal. Figure 15 shows the sin waves to be summed and indicates where constructive summation will occur. Figure 16 shows the various "square" waves obtained when an increasing number of frequencies are summed. Figure 17 is an expansion of Figure 16 at the leading edge of the square wave.

[21] $$V_t = \sum_{1}^{n} \frac{V_1}{n} \sin\left(2pt\left(nf_1\right)\right)\Big|_{n=1,3,5,7,9,\ldots\ldots}$$

As shown in Figure 16 the rise time between 10 and 90% of the signal decreases as the summed frequencies increase from 1 (the base sin wave) to 17 where a reasonable approximation of a square wave appears. The rise time is expressed as:

[22] $$t_r = \frac{1}{3\left(f_2 - f_1\right)} = \frac{1}{3\Delta f} \approx \frac{1}{3f_2}$$

where bandwidth Äf is the difference between the high frequency at which amplitudes begins to roll off (f_2) and the low frequency at which the amplitude begins to fall (f_1). (See also Figure 20.) For modern **op amp** based instruments there is no low frequency roll off so that t_r is defined only by the high frequency roll off point.

In general, any time varying signal can be considered to be some summation of sin waves, expressed as a Fourier series:

[23] $$f(x) = \int_0^{\infty} \left\{ A(a)\cos ax + B(a)\sin ax \right\} dx$$

where the amplitudes A(á) and B(á) are themselves integrals of sin waves. Figure 18 shows the amplitudes of the frequencies which sum to make a reasonable square wave. The plot shown is called a frequency domain plot and can be obtained by taking a Fourier Transform of the time varying data in equation 23

[24] $$\Im\{f(x)\} = F(x) = \int_{\infty}^{\infty} f(x)e^{-iax}dx$$

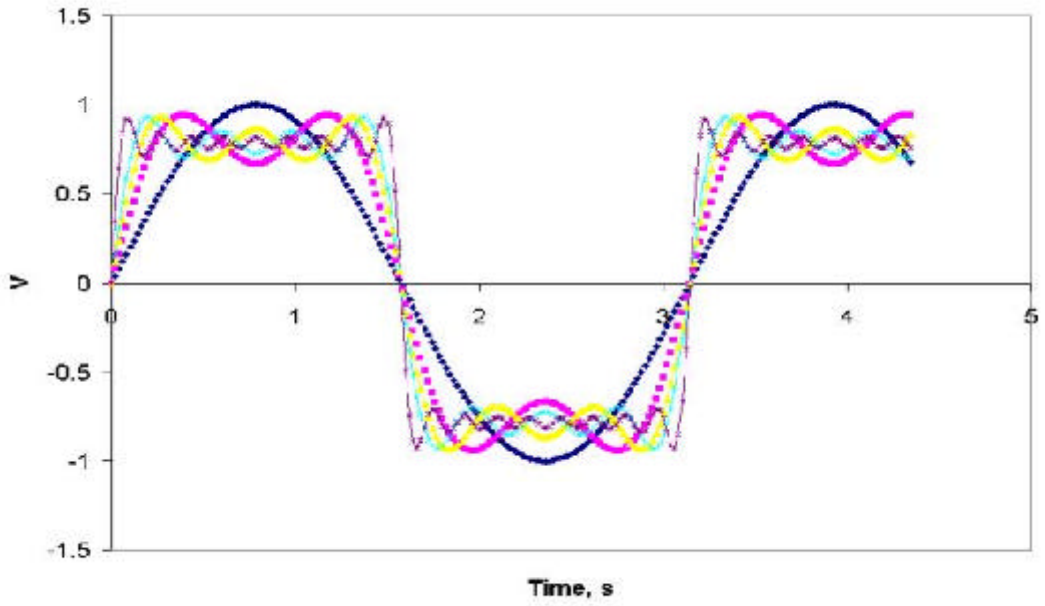

Figure 16 Plots of "square" waves obtained by summing multiple sin waves shown in fthe above figure. Note the ripple at the square wave that developes around the center of the base sin wave. The ripple decreases as more frequencies are summed.

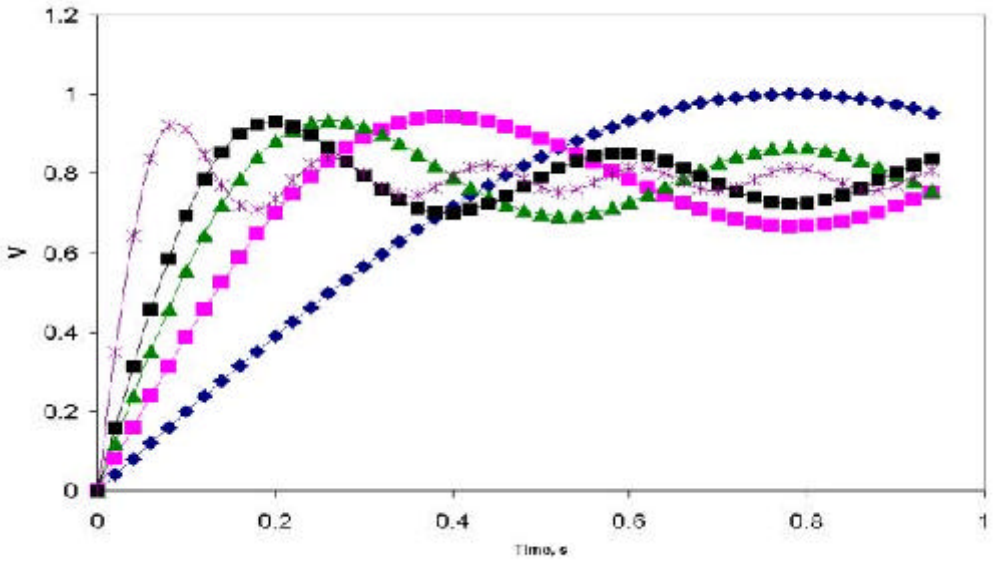

Figure 17 The "square" wave as a function of number of summed higher frequencies is plotted. As the number of higher order summed frequencies is added the time for the signal to rise from 10 to 90% of the full amount decreases.

The Fourier Transform can be discretized as

[25] $$F(x) = \sum_{x=1}^{N} f(x) W^{nm}, m = 1,2,\ldots,N$$

Solution of N^2 multiplication operations are required which made it an undesirable application. However performing the operation as a matrix set of operations results in a fast Fourier transform (FFT) which can be used even within fairly simple computational packages including Excel.

This is very useful because an analog signal converted to a digital set of data within the computer can be manipulated by the FFT transform to derive a frequency spectrum of the signal. If the source of noise is known it can be removed from the frequency spectrum and the time domain signal reformed minus noise.

Not all instruments are capable of tracking higher frequency signals. In many cases an instrument may distort a square wave by rounding off the leading and falling edges. This is due to unintentional internal RC filtering of the analog components of the instrument.

Intentional filtering can be accomplished either computationally or by analog (electronic) circuits. Analog filtering is based on passing the electronic signal containing the signal and reducing signal of the blank or background discriminating against the two by the frequency at which the signal and background are found at.

A low pass circuit is shown in Figure 19. The output of the low pass circuit can be described by:

[26] $$\frac{V_o}{V_i} = \frac{1}{\sqrt{1 + (2pfRC)^2}}$$

where R is the magnitude of the resistor, C is the magnitude of the capacitor, and f is the frequency in Hertz of an ac signal. A plot of equation 26 is shown in Figure 20 as a **Bode** plot. When RC = 1/2ðf the equation simplifies to:

[27] $$\frac{V_o}{V_i} = \frac{1}{\sqrt{1 + 1^2}} = \frac{1}{\sqrt{2}} = 0.707$$

This particular ratio of the output frequency to the input frequency is called the **cutoff frequency** and represents a way of ranking various RC circuits.

As an example we can consider the situation where the signal of interest arising from the chemical sample is of low frequency which during measurement picks up a high frequency noise (from an adjacent instrument) (Figure 21). Passing the acquired signal (signal plus noise) through a low pass filter results in recovery of the noise free sample signal.

The amplitude of the high frequency component often constitutes noise. This noise can be

reduced by making use of equation 5, increasing the number of measurements. One

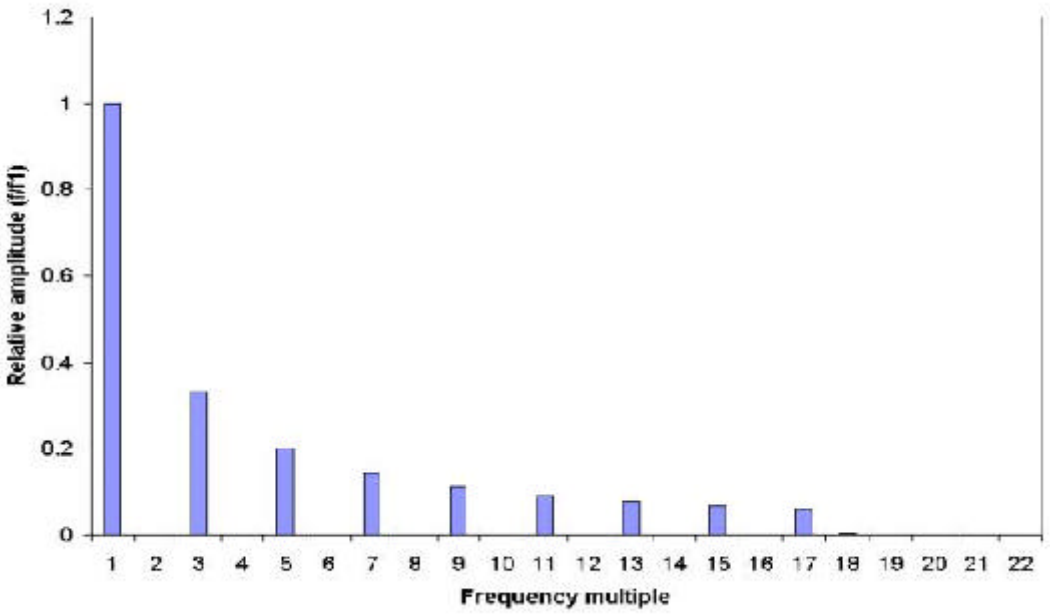

Figure 18 A fast Fourier transform of the square wave obtained by summing to the 17x base frequency is shown. The plot represents the amplitude of each frequency as a function of the frequency used in the summation. This FT was obtained using a simple spread sheet tool pack analysis.

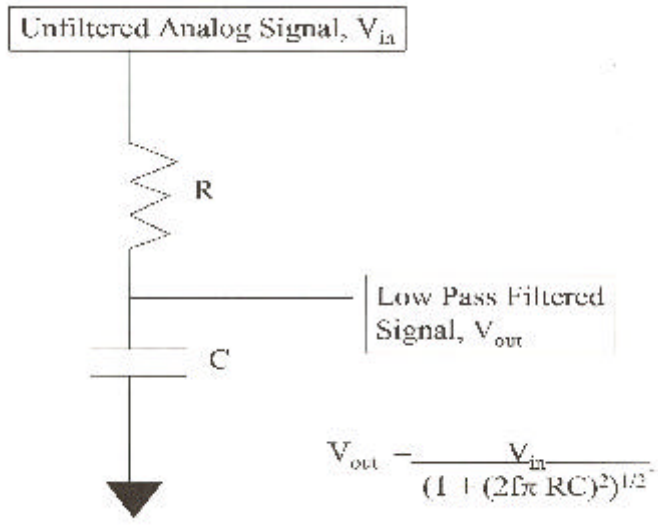

Figure 19 A low pass analog filter consits of a resistor in series with a capacitor tied to ground. The output voltage is sample between the resister and capacitor.

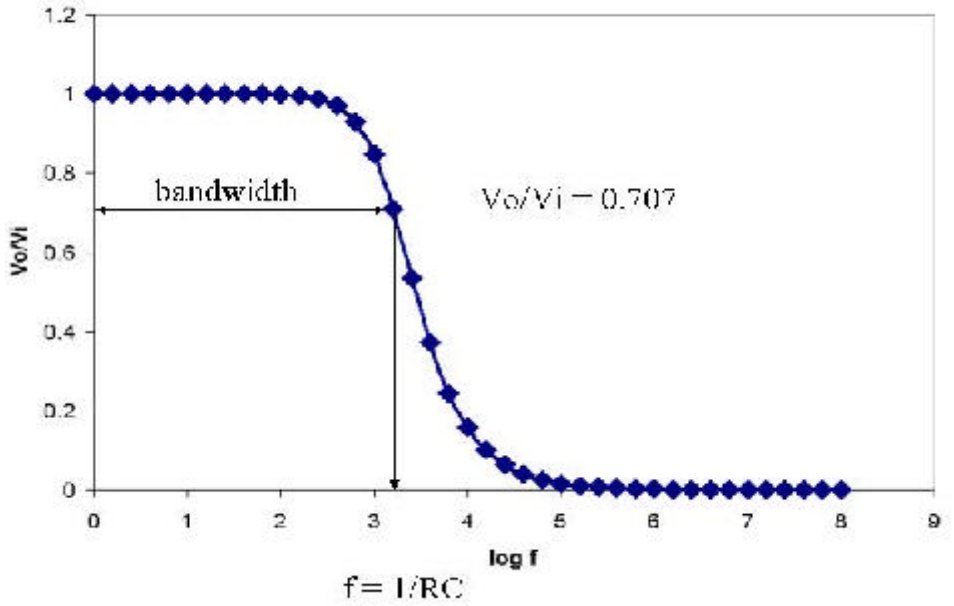

Figure 20 A Bode plot is a plot of signal attenuation vs frequency. The frequency at which the signal declines to 0.707 of its initial value is known as the cutoff frequency. It is also a rough estimate of f_2, the frequency used to characterize the bandwidth of an op amp based instrument.

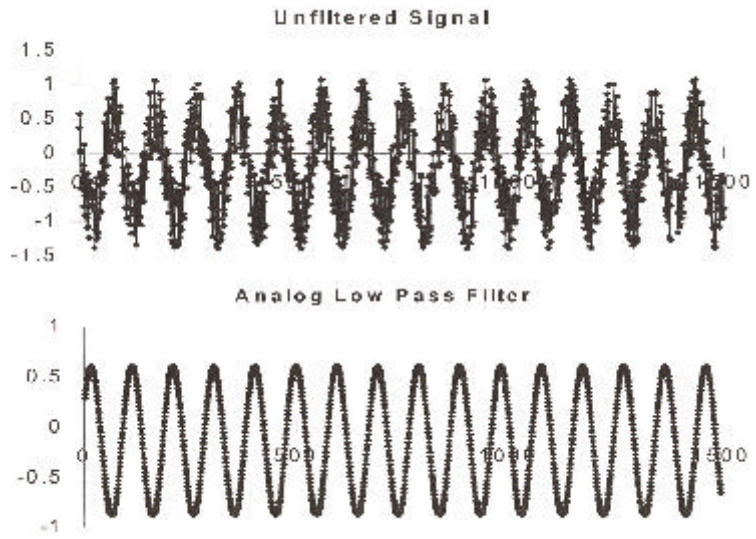

Figure 21 A noisy voltage vs time signal (upper trace) when passed through a low pass filter can be enhanced as shown in the lower trace. The low pass filter attenuates the amplitude of the high frequency components while passing the low frequency component unattenuated.

way to do this is to sum a waveforms obtained. In order to do this the **phase angle** of the "identical" waveforms to be summed must be considered. Sin waves can be identical in amplitude, V_p, and in frequency, f, and differ in their offset or phase angle, È (Figure 22):

[21] $$V_i = V_p \sin(2pf + q_i)$$

When sin waves with identical È are summed the effect is to increase the amplitude of the sin wave. This effect is termed **constructive interference**. When sin waves are 180º out of phase the effect is to sum the amplitude to zero, an effect termed **destructive interference** (Figures 23-25).

Because noise is random, it can be thought of as incoherent, therefore destructive interference is likely to occur on summation. The signal, on the other hand, is coherent, and therefore sums constructively, thus increasing it's amplitude. As repetitive waveforms are summed the result is to increase the relative amplitude of the signal compared to the noise. S/N increases (Figure 26). The difficulty of this form of filtering is that it requires absolute precision in initiating the waveforms. If the waveforms of the base signal are at all out of phase they will **destructively** sum and the signal is lost (Figure 27) or greatly distorted.

The second way of enhancing the signal (decrease the standard deviation of the signal) is to perform a **boxcar** filter. Here we assume that over a short time range of the waveform the signal magnitude has not changed greatly and that variation in the measured signal is due to a high frequency random perturbation (noise). By summing several of the "noise" points destructive interference will result in a loss of the high frequency noise. The summed points are averaged to give the estimated value for the signal, with a decreased contribution from the noise. The boxcar can be of any particular size (3 points, 5 points, 10 points) just so long as the length of the box is small enough that the assumption that the signal is not varying is valid. One problem with boxcar filtering is that data is lost at the front and rear end of the waveform, and that the number of data points is reduced by the averaging process (Figure 28). In a moving (sliding) boxcar, the data is treated as a string of points arranged in order of acquisition. For a five-point boxcar we start with the first five points (original data points 1-5, Figure 28), average the value of those points and store as point number 3. The boxcar is slid to the next five points (original data points 2-6), averaged and stored as point number four. This process is followed sequentially with the last five points (n-5 to n) averaged and stored as the n-2 point. Filtering efficiency increase with the size of the box and the number of points averaged (Figure 28).

The coupling of such electronic and digital filtration to an increased sampling has lowered the magnitude of the blank, lowered the standard deviation of the blank, thus lowering the limit of detection (equation 12). A concrete example of this is the drop in the limit of detection for lead from 0.5 ppm in the 1920 to 1 ppt in the 1990s, or by nearly 5 orders of magnitude.

Acquiring the "Physical" Sample

To estimate the randomness of, for example, lead in a soil, the physical sampling of the soil must be randomized and of sufficiently large number of individual "grabs" that a normalized

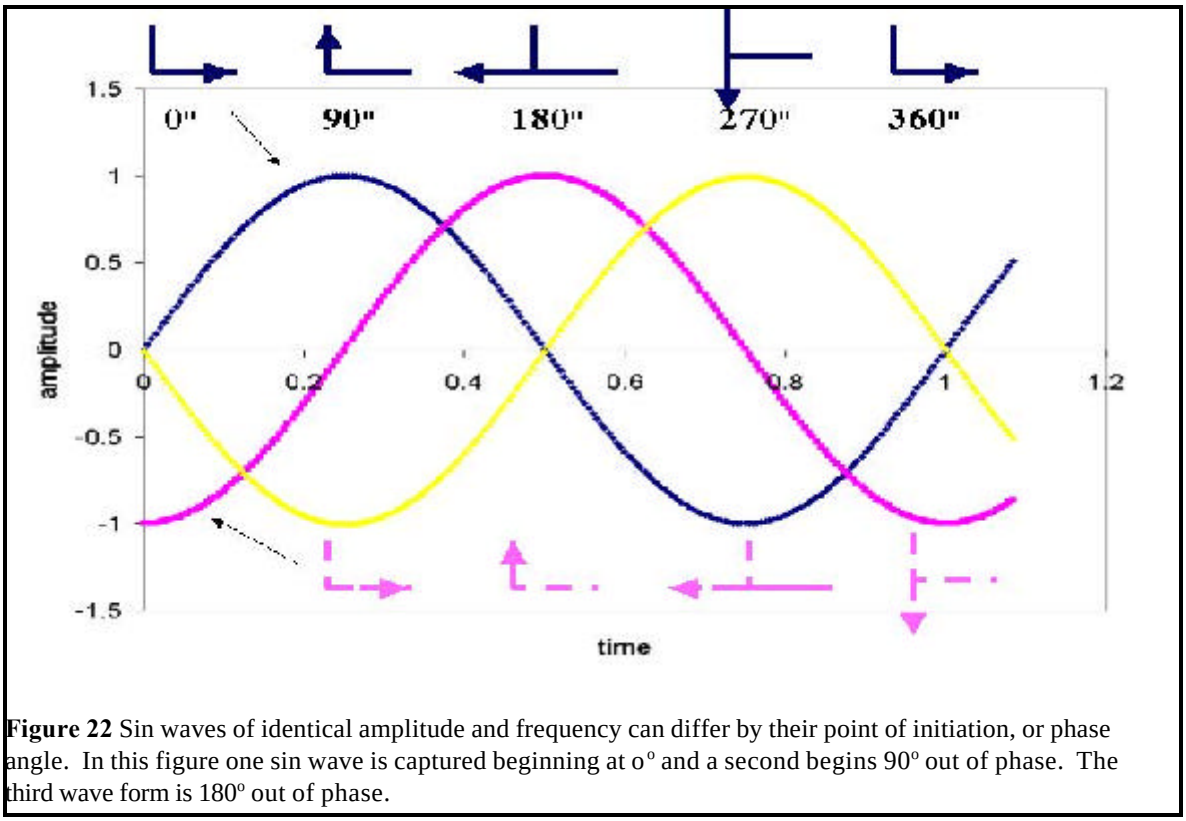

Figure 22 Sin waves of identical amplitude and frequency can differ by their point of initiation, or phase angle. In this figure one sin wave is captured beginning at o ^{o} and a second begins 90^{o} out of phase. The third wave form is 180^{o} out of phase.

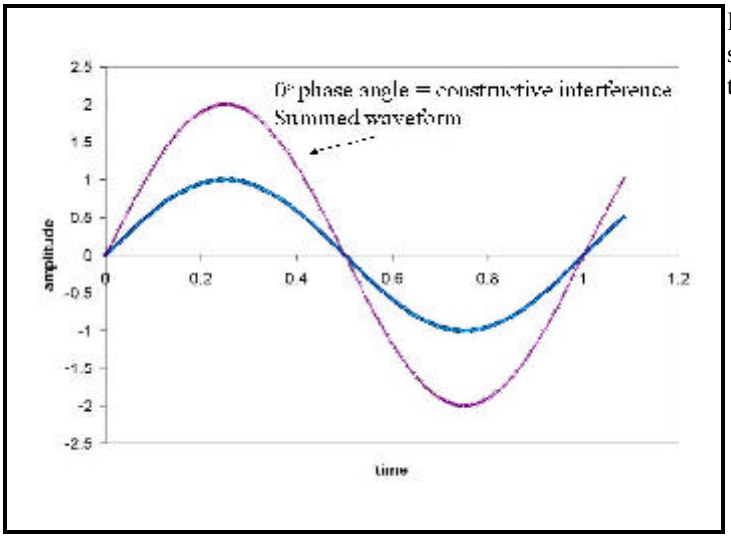

Figure 24: Waveforms in phase sum constructively to increase the amplitude.

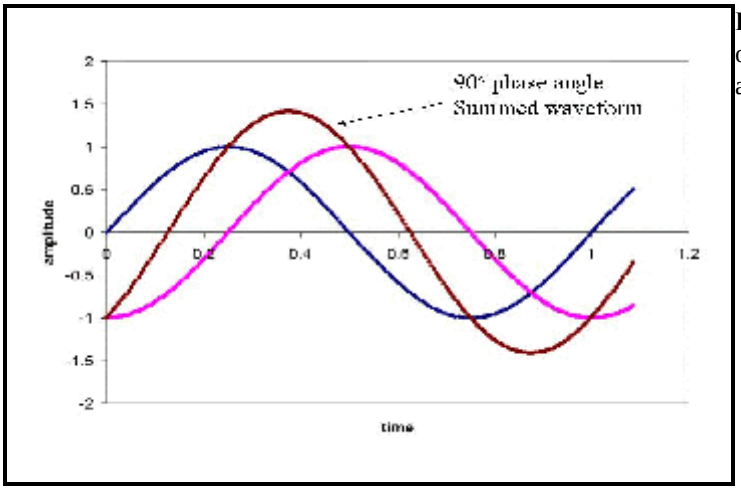

Figure 23: Waveforms that are 90º out of phase sum both constructively and destructively.

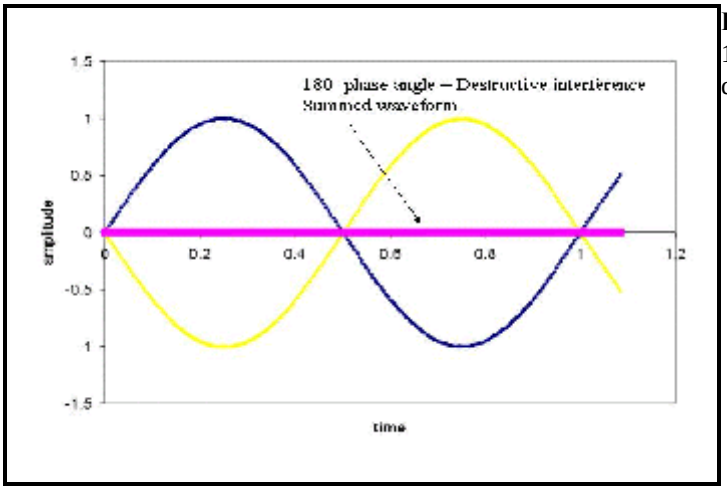

Figure 25 Waveforms that are 180 º out of phase will sum destructively.

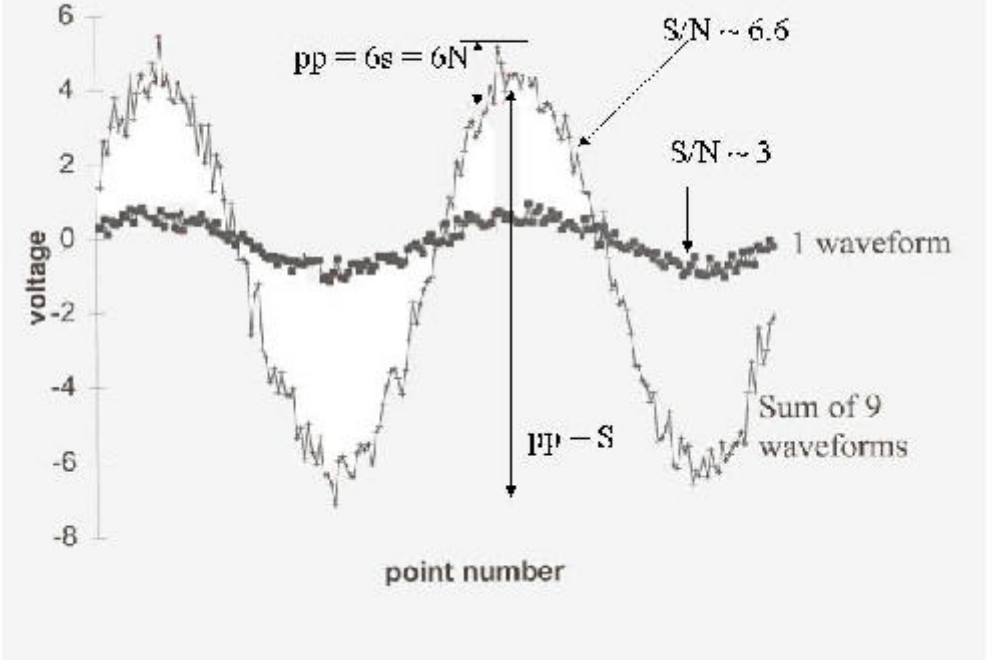

Figure 26 Summing a series of captured signals will increase the S/N if the noise component is random due to destructive interference of the noise component and constructive interference of the underlying signal.

error curve associated with sampling the soil can be approximated. That is, the reliability of the estimate of lead in the soil goes up with the number of samples taken.

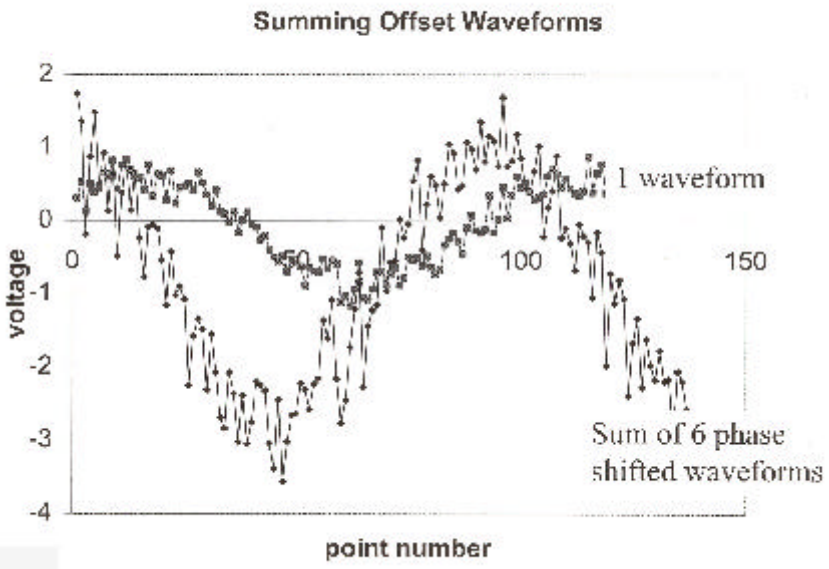

Figure 27 Summing offset or out of phase or incoherent waveforms distorts the signal.

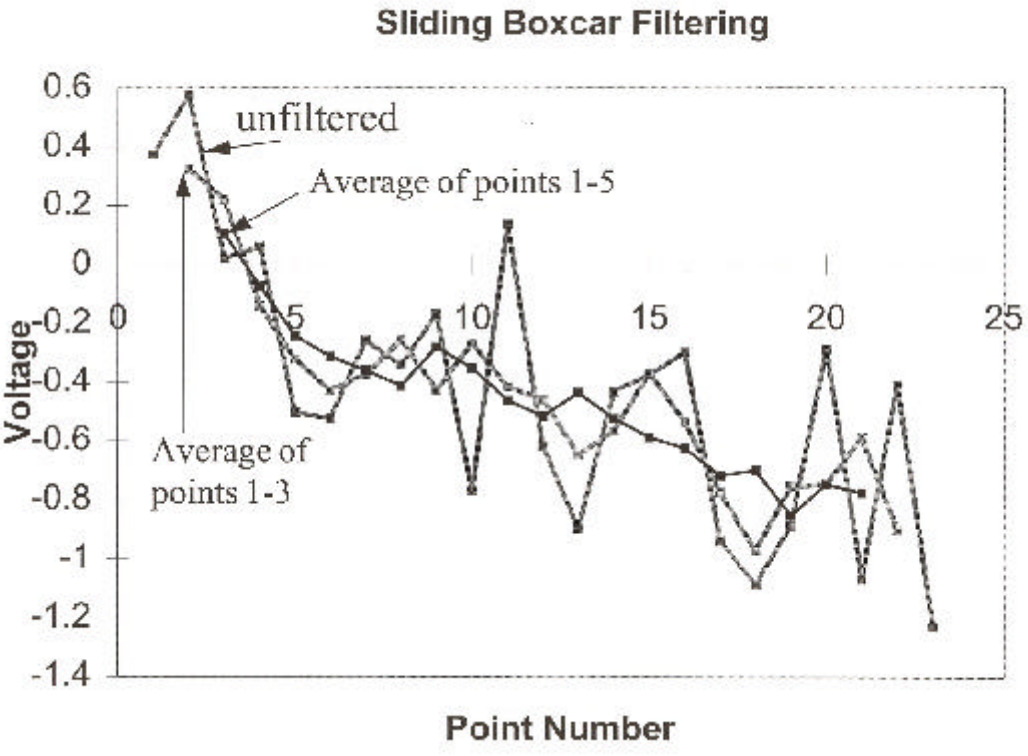

Figure 28 Voltage vs time (or point number) for an unfiltered signal and for signals filtered by boxcar averaging 3 and 5 points. The larger the number of points in the box , the more the "noise" component decreases. The data string shortens as con be observed by the loss of points at the beginning and end of the data string.