

Chem466 Lecture Notes

Spring, 2004

Overview of the course:

Many of you will use instruments for chemical analyses in lab. settings. Some of you will go into careers (medicine, pharmacology, forensic science, environmental monitoring & remediation) which requires a working knowledge of instrumental analysis. The most widely used instrumental methods in these career paths are separation methods (gas & liquid chromatography) and mass spectrometry (often joined with chromatography in one instrument). The next tier of importance includes UV-VIS absorption spectroscopy, IR spectroscopy, fluorescence spectroscopy, and atomic analysis methods (absorption and emission). All of these methods will be covered in this course.

The order of topics (see the syllabus) is designed to provide essential information as it is needed. Consequently, spectroscopic methods are covered first and separation methods second because these methods are used as detectors in separation science methods. Mass spectroscopy follows separation science methods because it is easier to discuss hyphenated methods (e.g., gas chromatography-mass spectrometry or GC-MS) after discussing the separation methods. If there is time, the section on capillary electrophoresis, an increasingly important separation method, will be covered.

In order to allow discussion of many methods, there will be material in the lecture notes which will not be covered in lecture but for which you will be responsible. These will be clearly indicated. At the end of each section, there is a brief review of the important definitions, concepts and calculations on which you will be tested. A few topics are left in which will not appear in lecture or on tests. They are for your benefit.

Additional reading assignments are from Skoog, Holler and Nieman, *Principles of Instrumental Analysis*, 5th edition. The SH&N reading assignments are NOT required; they are for the instructor's benefit, since this course was originally tailored to that textbook.

I. DEFINITIONS.

Analyte - the substance being identified or quantified.

Sample - the mixture containing the analyte. Also known as the matrix.

Qualitative analysis - identification of the analyte.

Quantitative analysis - measurement of the amount or concentration of the analyte in the sample.

Signal - the output of the instrument (usually a voltage or a readout).

Blank Signal - the measured signal for a sample containing no analyte (the sample should be similar to a sample containing the analyte)

For most instrumental methods (exception: potentiometry), the signal is linear with respect to the concentration of the analyte over a range of concentrations:

$$S = mC + S_{bl}$$

where C = conc. of analyte; S = signal of instrument; m = sensitivity; S_{bl} = blank signal. The units of m depend on the instrument, but include reciprocal concentration.

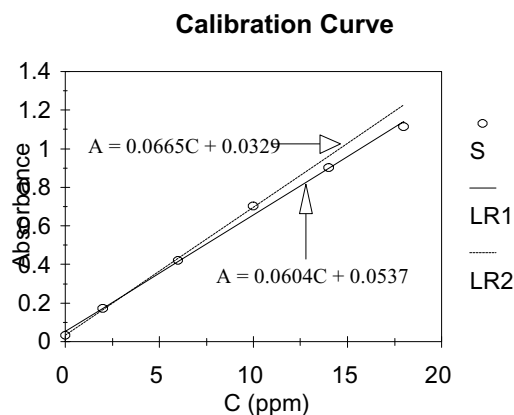
A standard (a.k.a. control) is a sample with known conc. of analyte which is otherwise similar to composition of unknown samples. A blank is one type of a standard. The words “standards” and “blanks” often refer to the signals generated by these types of samples.

The standard method of checking the above equation and defining the sensitivity of an instrumental method is to obtain a calibration curve - a plot of signal vs conc. for a set of standards. Calibration curves are often nonlinear at high and low concentrations, and linear at intermediate concentrations. The linear part of plot is the dynamic range. Often linear regression (method of least squares) is used to find the best straight line through experimental data points. **The slope of the linear part is the sensitivity m .**

Ex. Absorption spectroscopy

<u>C (ppm)</u>	<u>A (absorbance)</u>
0.00	0.031
2.00	0.173
6.00	0.422
10.00	0.702
14.00	0.901
18.00	1.113
[C?]	0.501

The first row is the measurement of the blank; the next 5 rows are data obtained from standards; the last row is an unknown. Questions: What is the linear dynamic range? What is the sensitivity? What is [C?]?



To find the linear dynamic range, plot the data and inspect it for a linear domain. In the preceding graph, the entire data set appears to be roughly linear. First approximation: the linear dynamic range is 0-18 ppm. Fitting all data to a linear regression line (LR1) yields a sensitivity of 0.0604 ppm^{-1} (slope of the LR line). However, closer inspection reveals that the data points show curvature. In particular, the highest 2 data points appear to be lower than expected based on the other data points. Second approximation: the linear dynamic range is 0-10 ppm. Fitting the first 4 data points to a linear regression line (LR2) produces a much better fit of data to the LR line. The sensitivity is 0.0665 ppm^{-1} . To calculate [C?], invert the equation:

$$A = 0.501 = 0.0665[C?] + 0.0329; [C?] = (0.501 - 0.0329)/0.0665 = 7.04 \text{ ppm}$$

Variation: Often, the blank signal is subtracted from all other data signals. The graph of the blank-corrected data ($S - S_{bl}$) should have an intercept near zero; i.e. $S_{corr} = mC$.

Note: on tests, be able to calculate the sensitivity given just two data sets. You can use linear regression, or set up two equations in two unknowns:

Equations: $S_1 = mC_1 + S_{bl}$ and $S_2 = mC_2 + S_{bl}$ (solve for m and S_{bl})

If the signal of the unknown falls between the two data sets, then you can use linear interpolation to calculate the concentration of the unknown.

Review:

Definitions: analyte; sample; blank; standard; sensitivity; dynamic range;

Calculations: Given calibration data, calculate sensitivity and the concentration of an analyte.

II. STATISTICS OF MEASUREMENT.

Precision - reproducibility of replicate measurements on a single sample.

Accuracy - agreement between measured conc. and true conc. (often not known).

Error - actual difference between measured conc. and true conc.

Ex. Target shooting.

Two types of error:

1. Random error (indeterminate error) - unpredictable and non-correctable changes in signal for replicate measurements.
2. Systematic error (determinate error)- predictable and usually correctable changes in signal from true value. Also known as bias.

Random error affects precision, systematic error affects accuracy.

Measurement of precision: make N replicate measurements ($x_1, x_2, \dots x_N$) on the same sample. x can be the signal S or the calculated conc. C .

$$\text{mean} = \langle x \rangle = (\sum x_i)/N$$

$$s \text{ (estimated standard deviation)} = [(\sum (x_i - \langle x \rangle)^2)/f]^{1/2}$$

where $f = \# \text{ degrees of freedom} = N-1$ for a single set of n measurements. s is the measure of precision.
RSD = relative standard deviation = $s/\langle x \rangle$ (often expressed as a percentage, which is the coefficient of variation); variance = s^2 . Note: You will not be tested on these formulas. Standard deviation can be calculated with built-in functions on most scientific calculators and spreadsheets.

The limit of detection (LOD) is the conc. at which one is 95% confident the analyte is present in the sample. The LOD is affected by the precision of the measurements and by the magnitude of the blanks. From multiple measurements of blanks, determine the standard deviation of the blank signal s_{bl} .

Then $LOD = 3s_{bl}/m$ where m is the sensitivity.

However, precision at the LOD is poor. The limit of quantitation LOQ is the smallest conc. at which a reasonable precision can be obtained (as expressed by s). The LOQ is obtained by substituting 10 for 3 in the above equation; i.e., $LOQ = 10s_{bl}/m$.

Ex. In the earlier example of absorption spectroscopy, the standard deviation of the blank absorbance for 10 measurements was 0.0079. What is the LOD and LOQ?

$$s_{bl} = 0.0079; m = 0.0665 \text{ ppm}^{-1}; LOD = 3(0.0079)/(0.0665 \text{ ppm}^{-1}) = 0.36 \text{ ppm}$$
$$LOQ = 10(0.0079)/(0.0665 \text{ ppm}^{-1}) = 1.2 \text{ ppm}$$

Review:

Definitions: precision; accuracy; random and systematic error; limit of detection (LOD); limit of quantitation (LOQ)

Calculations: From replicate measurements of one sample, calculate the mean, standard deviation, RSD and S/N. Given sensitivity and the standard deviation of the blank, calculate LOD and LOQ.

III. MEASUREMENT METHODS.

A major problem in measurement of analyte conc. in samples is the matrix effect. The matrix of the sample interferes with the measurement. For example, a solid sample can be non-uniform. Then light is scattered by the sample, and the measured absorbance is higher than the true absorbance.

Elements, ions, or compounds that specifically interfere with the measurement of a particular analyte are called interferences.

Techniques for reducing matrix effects include:

1. Matrix substitution - dissolving sample into liquid or gas solution, grinding sample with KBr powder.
2. Separation - using chromatography, solvent extraction, etc. to isolate analyte from complex matrix.
3. Preconcentration - collecting the analyte from sample into a much smaller volume to raise its concentration.
4. Derivatization - chemically modifying the analyte to improve volatility, light absorption, complex formation, etc., so that the instrument can more easily measure concentration.
5. Masking - modifying interferences so that they are no longer detected by the instrument.

An absolute method requires no calibration to calculate concentration from the output of the instrument. The sensitivity can be obtained from theoretical equations. Example: coulometry or coulometric titration.

All other methods require calibration to determine the sensitivity. There are 3 procedures for extracting concentration from instrument output.

- (a) Calibration curve (a.k.a. working curve) (see section I) is a plot of signal S vs. conc. C created by measuring a series of standards for a well-defined set of conditions. It is the best method if matrix effects are small and are independent of conc. of the analyte. The least error in measured conc. occurs when the sample signal is bracketed by standards (higher and lower signals for standards).
- (b) Standard addition (a.k.a. spiking) consists of at least three steps. First, measure the signal from the sample; second, add a known conc. of analyte (the spike) to sample; third, remeasure the

signal. With only one spike, you must assume linear response of the signal with conc. Additional spiking improves the precision and proves that the signal is linear with conc. Standard addition is especially useful when matrix effects are severe or when concentrations are near the detection limit. For the best precision, the first spike should at least double the analyte conc. in the sample.

For this course, be able to calculate via equations the conc. of an analyte given a single spike. Assumption: the data are blank-corrected (blank signal subtracted). There are 2 variations to this problem. Each involves writing 2 equations in 2 unknowns. The differences are the dilution factors (ratio of volumes).

Variation 1: A known volume of sample (V_x) is placed in one flask and diluted to the mark (V_t). The same volume of sample plus a known volume (V_s) of a standard (conc. C_s) is placed in a 2nd flask and diluted to the mark. The signal is measured on both solutions. Calculate C_x .

Equations: $S_1 = mC_x(V_x/V_t)$ and $S_2 = m(C_x(V_x/V_t) + C_s(V_s/V_t))$

Variation 2: The sample of known volume (V_x) is measured. To the sample is added a known volume (V_s) of a standard (C_s) and the measurement is repeated. Calculate C_x .

Equations: $S_1 = mC_x$ and $S_2 = m(C_x V_x/(V_s + V_x) + C_s V_s/(V_s + V_x))$

Examples will be given in the first homework.

- (c) Internal standard is a substance added to all samples, blanks and standards so that its concentration is fixed and known. The signal due to the internal standard (S_{is}) is measured at nearly the same time as the signal due to the analyte. The ratio S/S_{is} is plotted vs conc. of standards as in calibration curves above. This method is useful when the sensitivity of the instrumental method fluctuates or drifts with time, and when matrix effects are severe.

Review:

Definitions: matrix effects.

Concepts: What are the 5 techniques for reducing matrix effects? What is an absolute method? What are calibration curves? What is standard addition?

Calculations: Calculate concentration of an analyte from calibration curve data or standard addition data for any method for which the signal is linear in concentration; be able to state any necessary assumptions needed to do the calculations.

IV. INSTRUMENTAL NOISE.

Reading: Harris, ch. 20, pp. 487-488

SH&N, ch. 5, pp. 99-108 (omit difference and instrumentation amplifiers)

Assumptions: the instrument signal is monitored with time, and the signal is converted to the digital domain. The resulting binary numbers correspond to a specified voltage range of the instrument signal.

Most instruments these days convert the analog signal (a voltage or a current) to a digital signal using an ADC (analog-to-digital converter). The ADC samples the signal over a short time and convert it to a binary number. The output is a string of binary numbers representing the signal at evenly spaced intervals of time. Resolution and the minimum noise is determined by the # of bits of the ADC.

Ex. a 12-bit ADC has a resolution of $1 \text{ in } 2^{12} = 1 \text{ in } 4096$; consequently, the resolution and minimum noise (and RSD) cannot be smaller than 1 part in 4096.

Ex. A 12-bit ADC will generate binary numbers between 0 and 4095. This represents a signal between -5 and $+5 \text{ V}$. What is the resolution of the transformed signal? What is its minimum noise?

Binary ADC output	0	...	4095
Transformed input	-5	...	+5 V

The input range is $\text{max.} - \text{min.}$, i.e. $\{(+5 \text{ V}) - (-5 \text{ V})\} = 10 \text{ V}$. This range is divided into 4096 parts by the ADC, so the resolution of the signal is $(10 \text{ V})/4096 = 0.0024 \text{ V}$ or 2.4 mV . The minimum noise is the same as the resolution (2.4 mV).

The sampling frequency f_s of an ADC is the number of times per second the ADC samples the analog signal and converts it to a binary number. The reciprocal of sampling frequency is the sampling period τ_s .

For quantitative analysis, usually a sample with a constant analyte conc. is measured for a period of time. The signal should be constant but is not, due to noise. Example: $\rightarrow$

The data points are obtained at a frequency of 100 Hz (sampling period $\tau = 0.01 \text{ s}$).

For qualitative analysis, usually some property of the sample is measured as a function of a variable (e.g., wavelength of light) which changes linearly with time. Ex. an absorption spectrum with a linear wavelength scan ($\lambda \propto t$). $\rightarrow$
($f = 100 \text{ Hz}$, $\tau = 0.01 \text{ s}$)

The data contain noise (random fluctuations in signal). Where the signal should be constant (quant. anal. figure), noise is defined quantitatively as the standard deviation of the signal:

$$s = [(\sum(S_i - \langle S \rangle)^2)/f]^{1/2}$$

Ex. the noise in the Quant. Anal. Signal figure is 1.14. The mean value is 10.17 (50 points).

Define signal-to-noise (S/N) = $\langle S \rangle / s = 1/(\text{RSD})$

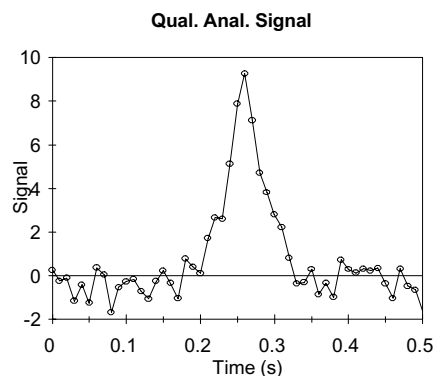
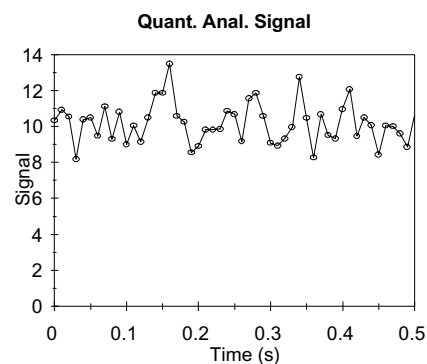
(a useful measure for data or instrument performance; higher S/N is desirable)

Ex. S/N in the Quant. Anal. figure = $10.17/1.14 = 8.92$

Where the signal is not constant (Qual. Anal. Figure), noise is obtained as the standard deviation from a region of relatively constant signal. Ex. the noise in the Quant. Anal. Signal figure between 0 and 0.1 s is 0.65.

S/N is usually defined for a peak value. Ex. S/N at the peak in the Qual. Anal. figure = $9.3/0.65 = 14$.

Types of instrument noise:



1. Environmental noise - includes power line noise at 60 Hz, harmonics (120 Hz, 180 Hz, ...) and sub-harmonics (30 Hz).
2. Johnson noise - random fluctuations of electrons in a conductor. "White" noise (present at all frequencies). Voltage noise proportional to square root of temperature (in Kelvin).
3. Shot noise - random variations of charged particles crossing an interface (present in most light detectors and diodes). "White" noise; current noise proportional to square root of current.
4. Flicker noise - origin not always clear. "Pink" noise; amplitude increases as frequency decreases. Includes drift - slow changes in instrument baseline and sensitivity.

Strategies to enhance S/N: Either enhance signal (discussed for various instrumental methods) or decrease the noise.

Methods for decreasing noise:

1. Shield the experiment, i.e., wrap it in a metal box connected to instrument ground. Removes noise induced by electrical fields (power line noise).
2. Cool the detector (frequently used for light detectors). Reduces Johnson noise.
3. Synchronous detection - modulate the "signal" (e.g. light beam, electrical voltage) at a fixed frequency. The amplitude of the periodic "signal" is changed by sample. Using special electronic equipment, isolate the signal at the fixed frequency and extract amplitude information. Reduces all types of noise, especially flicker noise.
4. Digitize the signal and use computer methods to decrease noise.
 - (a) For a DC signal (quant. anal.), digitize the signal N times and add the numbers in computer memory. Signal and baseline increase N times, but random noise only increases $\sqrt{N}$ times, so S/N increases by a factor of $\sqrt{N}$.

Ex. The first 5 data points of a quantitative measurement are: 5.55, 5.41, 6.26, 6.09, 5.48. Calculate the S/N. How many additional measurements are needed to increase the S/N to 30?

$$\text{mean} = (5.55 + 5.41 + 6.26 + 6.09 + 5.48)/5 = 5.76; \quad s = 0.39; \quad S/N = 5.76/0.39 = 15$$

$$S/N = k\sqrt{N}; \quad k = (S/N)/(\sqrt{N}) = 15/\sqrt{5} = 30/\sqrt{N}; \quad \sqrt{N} = (30/15)\sqrt{5}; \quad N = (4)(5) = 20$$

- (b) For a time-dependent signal (qual. anal. spectra), use ensemble averaging. Trigger the measurement repeatedly, digitize the signal at fixed time intervals, and add the binary numbers into separate memory locations, one location for each timer interval after the trigger. Alternately, use a multichannel detector (found mainly in optical spectroscopy) with each channel being digitized and stored in separate memory locations. Again S/N improves by the factor of $\sqrt{N}$.

Review

Definitions: signal-to-noise.

Concepts: What are the 4 types of instrument noise? What strategies can be used to enhance S/N?

Calculations: Calculate the change in S/N with N (# of measurements averaged).