Quantitation

- the absorbance of an analyte is proportional to its concentration \((A \propto C)\)
- the absorbance of an analyte is proportional to the amount of sol’n it passes through \((A \propto b)\)
- the absorbance of an analyte is proportional to its absorption properties \((A \propto \varepsilon)\)

\[ A = \varepsilon b C \]  
(Beer’s Law)

where \(C\) = concentration  
\(b\) = path length  
\(\varepsilon\) = molar absorptivity

Analysis of Mixtures

- the concentration of several analytes in a mixture can be determined
- for a mixture of \(N\) components, choose \(N\) wavelengths
- absorption spectra must differ significantly at each wavelength
- uncertainties increase as the number of components increase

Analysis of Mixtures Lab

1) obtain pure substances and measure absorption spectra
2) 3 analytes, so pick 3 wavelengths \((\lambda_{\text{max}}\) for each)
3) obtain the absorbance at each wavelength

Analysis of Mixture Example

1) measure the absorption spectrum of the mixture at the \(\lambda_{\text{max}}\)'s
2) set up the matrix or algebraic solution

\[ \text{Abs}_{1} = \varepsilon_{A1} C_{A} + \varepsilon_{P1} C_{P} + \varepsilon_{C1} C_{C} \]
\[ \text{Abs}_{2} = \varepsilon_{A2} C_{A} + \varepsilon_{P2} C_{P} + \varepsilon_{C2} C_{C} \]
\[ \text{Abs}_{3} = \varepsilon_{A3} C_{A} + \varepsilon_{P3} C_{P} + \varepsilon_{C3} C_{C} \]
\[ \Rightarrow \text{Abs}_i = \varepsilon_i \]

where is an \(\varepsilon^{-1}\) inverse matrix

see example in your textbook
**Definitions**

- **signal**: carries information about the analyte
- **noise**: extraneous information that degrades the accuracy and precision of the analysis and the lower limit of detection

**Types of Instrumental Noise**

- **Thermal or Johnson or white noise**: thermal agitation of electrons in the electronic transducer and associated equipment
  - a random phenomenon, so it contains a broad range of frequencies (like white light)
  - can be reduced by temp ↓ or resistance ↓ or bandwidth ↓
  - \[ V_{Th} = \sqrt{4kTR\Delta f} \]
  - \( k \) = the Boltzmann constant
  - \( T \) = temperature
  - \( R \) = resistance
  - \( \Delta f \) = bandwidth of the instrument (how fast is the instrument)

- **Shot or Quantum or Schottky noise**: quantum mechanical uncertainty when electrons cross a junction (photocells, transistors, diode, vacuum tubes, electrodes, etc.)
  - it also has characteristics of white noise (all frequencies)
  - this type of noise is small in magnitude and only observed in low current signals
  - can be reduced by bandwidth ↓ or increase the current \( I \) because the signal goes up faster
  - \[ i_{q} = \sqrt{2Ie\Delta f} \]
  - \( I \) = the magnitude of the current
  - \( e \) = electron charge
  - \( \Delta f \) = bandwidth of the instrument (how fast is the instrument)

- **Flicker or 1/f noise**: a not-very-well-understood noise that appears at low frequencies
  - the best example is the long term drift of a baseline
  - can be reduced by making the measurement at higher frequencies (usually above 100 Hz)

- **Environmental noise**: noise that comes from in specific frequencies from various sources
  - examples are TV, radio, cell phone signals, earthquakes, AC powerlines (60 Hz and harmonics), etc.
  - can be removed by shielding or post-acquisition processing

**Types of Noise**

- **Signal to Noise Ratio (SNR)**
  - since the signal level says nothing about the overall quality of the measurement, a SNR is needed
  - it is an estimate of how trustworthy the results are
  - calculate noise: measure the peak to trough (AC signals) or peak to baseline (DC) height at a 99% confidence limit
Calculating Noise (assuming signal level is unknown)

- find a flat spot in the measurement where no signal occurs
- calc method 1: calculate the standard deviation
  - use a spreadsheet or calculator and lots of data points
  - avoid this region because of the increasing baseline

- calc method 1:
  \[ s_{\text{noise}} = 1711 \]
  elements 2200 - 4000

- there is a difference: \[ s_{\text{noise}} = 1711 \text{ vs. } 2725 \text{ vs. } 1963 \]
  - method 1 & 3 are only 15% difference
  - method 1 is preferred

- there is some variability in the S/N calc (it is noise after all!)
  - the best we can do is estimate

- there is an obvious difference:
  
  \[ s_{\text{noise}} = 1711 \text{ vs. } 2725 \text{ vs. } 1963 \]
  - method 1 & 3 are only 15% difference
  - method 1 is preferred

- there is some variability in the S/N calc (it is noise after all!)
  - the best we can do is estimate
Calculating Signal

- locate the signal peak
- determine the average baseline (use same range as noise) by estimating with a ruler or a spreadsheet
- signal = peak height – baseline = 6221

Signal-to-Noise Ratio (SNR)

- signal = 6221
- noise = 1711 (method 1)
- SNR = 6221/1711 = 3.6
- express with two sig figs max, maybe only one

Error Sig Figs

- sig figs are meant to communicate uncertainty in a number
- a buret reading of 20.14 mL
  - certain of the 20.1 mL
  - uncertain of the 0.04 mL
- if a measurement is 20.14 (±1.2) mL
  - the 0.04 mL is meaningless (so is the 0.2 mL, really)
  - it is OK to use 2 sig figs of uncertainty
  - 20.1 (±1.2) mL or 20 (±1) mL is OK

Signal-to-Noise Ratio (SNR) (assuming signal level is known)

- use population standard deviation (σ)
- all else is the same
- example: a brass calibration weight measured with an electronic balance

Table 1: Observed masses

<table>
<thead>
<tr>
<th>Mass (g)</th>
<th>Signal (g)</th>
<th>Noise (g)</th>
<th>SNR</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5217</td>
<td>1.0000</td>
<td>1.2985</td>
<td>0.77</td>
</tr>
<tr>
<td>0.1924</td>
<td>1.2985</td>
<td>0.3159</td>
<td>0.77</td>
</tr>
<tr>
<td>0.952</td>
<td>4.3598</td>
<td>-0.5412</td>
<td>-2.5</td>
</tr>
</tbody>
</table>

Decibels: $\text{dB} = 20 \times \log(x)$

SNR – How many more measurements do I need?

- Since more measurements decreases the standard deviation (noise), the SNR also improves with more measurements

$$SNR\text{ }\text{thenoretical}\text{ }l = \sqrt{N} \times (SNR\text{ }\text{actual})$$

$$N = \left( \frac{SNR\text{ }\text{thenoretical}\text{ }l}{SNR\text{ }\text{actual}} \right)^2$$

$$\frac{SNR\text{ }\text{thenoretical}\text{ }l}{SNR\text{ }\text{actual}} \equiv \text{improvement}$$
What SNR means

- it is an estimate of how trustworthy the results are
- there are no rules of interpretation carved in stone; much is left to interpretation
- in general⁶
  - no usable signal is present if SNR<3 (Limit of Detection or LOD or qualitative LOD)
  - in simple solutions concentrations of 5 to 10 times the LOD are required for quantitative measurement (LOQ)
  - in complex solutions concentrations of 20 to 100 times the LOD are required for quantitative measurement
- our book uses 3 for LOD and 10 for LOQ


Complex Signals

- this sine wave has a single frequency
- how can you determine it?
  - 1 cycle/0.02 sec = 50 Hz
  - Spectrum analyzer
- this sine wave has multiple frequencies
- how can you determine them?
- Fourier transform!

Fourier Transforms

- transforms from time to frequency (seconds to seconds⁻¹)

Fourier Transform

- forward transform \( F(\nu) = \int_{-\infty}^{\infty} f(t)e^{-i(2\pi\nu)t} \, dt \)
- inverse transform \( f(t) = \int_{-\infty}^{\infty} F(\nu)e^{i(2\pi\nu)t} \, 2\pi dt \)
- transforms are mathematically difficult
  - spreadsheets and other software do this
  - they use the Fast Fourier Transform (FFT)
- with FT, complex signals can be separated by their frequency spectrum

Memorize the Following

- common waveforms have recognizable FT pairs
- time and frequency domains are interchangeable
Gaussian and Lorentzian distributions

Gaussian
\[ F(x) = \frac{1}{\sigma \sqrt{2\pi}} e^{-\left(\frac{(x-\mu)^2}{2\sigma^2}\right)} \]
- has a finite area under the curve
- drops off exponentially

Lorentzian
\[ F(x) = \frac{1}{\pi \sigma^2} \left(1 + \left(\frac{x-\mu}{\Gamma/2}\right)^2\right) \]
- has an infinite area under the curve
- drops off as \(1/x^2\)

Strategy behind FT Digital Filtering

Fourier transform \(\rightarrow\) digital filter (apodization) \(\rightarrow\) inverse Fourier transform

Spectral Analysis

time domain signals \(\rightarrow\) frequency domain signals
- high frequency noise added
- white noise added

Apodization or Windowing

- in the frequency domain the signal and the noise are (often) distinguishable
- apodization truncates or attenuates the amplitude of the noise while leaving the signal (relatively) unaffected
- several functions for apodization can be seen below

Figure 5.12: Digital filtering with the Fourier transform. (a) noise spectral peak. (b) the frequency-domain spectrum of a resulting from the Fourier transformation. (c) low-pass digital filter function. (d) product of (b) times (c). (e) the inverse Fourier transform of (d) with most of the high-frequency noise removed.
Apodization or Windowing

- time domain signals
- frequency domain signals

**High Frequency Noise Example:** Truncate the high frequencies

**White Noise Example:** Truncate the high frequencies

- time domain signals
- frequency domain signals

- **Apodization or Windowing**
  - Truncation in the frequency domain often causes ringing (Gibbs oscillation) because the FT of a square wave is a sinc function
  - To prevent this, other apodization functions are used

**Square Wave**
- Amplitude

**Sinc Function**
- Amplitude

**Apodization or Windowing**
- Exponential apodization of white noise
- Exponential apodization of high frequency noise

- **Apodization or Windowing**
  - Time domain signals
  - Frequency domain signals

**Apodization or Windowing**
- **Exponential Apodization of White Noise**
- **Exponential Apodization of High Frequency Noise**
Distortion

- exponential apodization causes peaks to become Lorentzian

- truncation causes ringing

**Take-Home Quiz**

- Use the FTFilter.xls spreadsheet to answer the quiz questions

**Numerical Digital Filters**

- Running average
- least squares polynomial smoothing
  - quartic smoothing
  - 1st derivative cubic smoothing
  - 2nd derivative quartic smoothing

- all these methods improve S/N ratio at the cost of distortion
- most of these are computationally intensive and are present in many software packages
An Example: Running Average

- basis for numerical filters:
  - white noise changes randomly while the signal does not
  - high freq. noise oscillates over the size of the filter while the signal does not

Other SNR Enhancement Methods

- instead of getting rid of the noise after the signal has been acquired, some methods filter noise as the signal is collected
- analog filtering: using RC filters, pre-amps, etc.
- modulation or chopping: converts a DC signal to an AC signal
- lock-in amplification: can be used with choppers
  - require a known reference signal of the same frequency and known phase difference as the analyte

Chopping and Lock-in Amplification

Tools of the Trade: Spectrum Analyzer

- a spectrum analyzer takes an FT of the signal to show the frequencies of the signal and noise

Spectrum Analyzer

- chopper at 625 Hz
  - the odd harmonics are intense

The Nyquist Sampling Theorem
The Nyquist Sampling Theorem

- the sampling frequency must be at least 2x faster than the highest signal frequency
- Nyquist criterion = \( \frac{1}{2} \times \text{sampling freq.} = \text{highest frequency signal that can be accurately measured} \)
- usually the sampling frequency is up to 10x the highest signal frequency
- any signals > Nyquist criterion will appear at an aliased frequency
- these signals are said to be undersampled or aliased

Aliasing Examples

\[
\text{observed freq.} = | \text{signal freq.} - n \times \text{sampling freq.} |
\]
also observed freq. must be \( \leq \) Nyquist criterion

- Sampling freq. = 100 Hz
  - signal 1 = 20 Hz
  - signal 2 = 50 Hz
  - signal 3 = 80 Hz
  - signal 4 = 160 Hz
  - What is observed
    - signal 1 = 20 Hz (n = 0)
    - signal 2 = 50 Hz (n = 0)
    - signal 3 = 20 Hz (n = 1)
    - signal 4 = 40 Hz (n = 2)