

Signal-to-Noise, Resolution, Ensemble Averaging, Digital Smoothing

Introduction

Life is a compromise. Highly intelligent, capable, and educated people like your professors could be making the big money in industry schmoozing with top executives and working with up to date equipment. We have compromised those things for more personal and professional freedom. Using instrumentation of all kinds also involves compromises. This lab is designed to illustrate some of the compromises involved in performing measurements with spectroscopic or any other instrumentation: to demonstrate some of the “trading rules”.

Before performing tasks illustrating these concepts, let's briefly discuss these terms, which you can read more about in the text.

RESOLUTION: The most straight forward definition of resolution is in terms of the difference in frequency (wavenumber, cm^{-1}) or wavelength (nm) between two absorbance peaks that can be just separated by the instrument. All other factors being equal, the greater the resolution the better to detect signals as close together as possible.

SIGNAL-TO-NOISE: In the text, S/N is defined as $1/\text{RSD}$ (mean/standard dev.) of a recorded signal (D.A. Skoog et al. “Principles of Instrumental Analysis, 6th Edition” Section 5A, pp. 110). This can be thought of as the “root mean square” signal-to-noise ratio. All other factors being equal, the greater the S/N the greater the ability to detect the weakest absorbances, or absorbances at lower concentration levels.

SIGNAL AVERAGING: Also called ensemble averaging, it's a way to enhance the S/N ratio by acquiring multiple spectra and obtaining the average of the result (Skoog et al., Section 5C-2, pp. 117-119). The signal increases to the first power of the number of spectra averaged but the noise, being random, increases to the square root of the number of spectra averaged. Thus the signal increases faster than the noise as multiple spectra are averaged resulting in an increased signal-to-noise ratio.

SMOOTHING: Skoog et al. Section 5C-2, (pp. 119-123) calls this digital filtering, which is one way to smooth data that will be used in this laboratory. This is another way to enhance the S/N ratio of the instrumental output.

As you will see, there are trade-offs which must be considered when obtaining any instrumental analytical data. You always want a high S/N, but you need sufficient resolution to obtain a representative spectrum for your needs in a reasonable period of time. You must pick the appropriate conditions which best fulfill your needs, and the only way to accomplish this is to understand how S/N, resolution, smoothing and signal averaging interact to change the data acquisition time and instrumental output.

A Short Introduction to Spectroscopy

Although you will be using two spectroscopic methods, UV-visible and infrared spectroscopy, the concepts of S/N and the various trade-offs are inherent in any instrumental analytical measurement. Nevertheless, since spectroscopic methods will be used to generate the data in this laboratory, a short introduction to spectroscopic methods follows.

The absorption of electromagnetic radiation by ions and molecules serves as the basis for numerous analytical methods of analysis, both qualitative and quantitative. Studies of absorbance spectra provide knowledge concerning the formula, structure, and stability of many chemical species, as well as establish the most favorable conditions for analysis.

Because the energy of a molecule is the sum of many individual types of energy,

$$E_{\text{molecule}} = E_{\text{electronic}} + E_{\text{vibrational}} + E_{\text{rotational}} + E_{\text{translational}} + \text{others}$$

absorption of a photon can increase molecular energy in a variety of ways. In UV-visible spectroscopy, the energy of the photon corresponds with electronic energy transitions of molecules and ions. Thus UV-visible spectroscopy is a type of electronic absorption spectroscopy. In infrared spectroscopy, the energy of the photon corresponds with vibrational energy levels of molecules. Thus infrared spectroscopy is a type of vibrational spectroscopy. It is usually safe to say that more detailed structural information can be derived from vibrational spectroscopy than from electronic spectroscopy.

In UV-visible electronic absorption spectroscopy in liquids, the absorption peaks are very broad. The broad peaks result from a phenomenon called “collisional broadening”. In the liquid state, molecules are constantly colliding and interacting with one another. This causes a near continuum of vibrational and rotational energy levels superimposed on top of the electronic energy levels; resulting in broad absorbance bands over a range of wavelengths. These collisions are much less frequent in the gas phase, so one can see individual vibrational peaks superimposed on top of the electronic peaks in gas phase UV-Vis spectroscopy (assuming the instrumental conditions of obtaining the gas phase spectrum affords sufficient resolution). Similar phenomena occur in the infrared region. Relatively broad peaks are observed in the infrared spectra of liquids, because a near continuum of rotational energies are superimposed on vibrational energy levels. Collisional broadening in the liquid phase makes it impossible to detect the individual rotational transitions. In the gas phase, you will see both vibrational and rotational transitions occurring as a series of sharp peaks. You will see this for gas phase CO₂ in the atmosphere, and see changes in these gas phase spectra as you change the spectral resolution. You should conceptually understand what the implications are of spectral resolution in obtaining the gas phase spectra, and how the other variables of ensemble averaging, digital smoothing and resolution interact with one another. This makes it possible to decide on appropriate instrumental parameters in the acquisition of spectra for the information content desired.

This laboratory will use a combination of UV-visible and infrared spectroscopy. That is not because some concepts pertain to one instrumental method and other concepts do not. *It must be stressed that all issues addressed in this laboratory are common to all instrumental methods.* One or the other method will be used in various cases just for convenience. Since the FTIR (for collection of infrared spectra) is being used however, a few points which may otherwise cause confusion should be discussed.

Both instruments to be used, the CCD array UV-visible and FTIR spectrometers are single-beam instruments. The instrument components lab is designed to clearly show the difference between single-beam and double-beam instruments. A measurement of the % transmittance (from which absorbance can be calculated) actually requires 2 measurements.

$$\%T = (I/I_0) \times 100$$

where I = intensity of light at a given wavelength that the detector senses when the sample is in the source beam, and I_0 = intensity of light at the same wavelength that the detector senses when a “blank” is in the source beam. In a single beam instrument an absorbance or % transmittance spectrum is obtained by first making measurements on a blank to obtain I_0 as a function of wavelength or frequency. This is a single beam spectrum. Then a sample is put into the source beam and measurements obtained to obtain I as a function of wavelength or frequency. This is also a single beam spectrum. By taking the ratio of I/I_0 as a function of wavelength or frequency one obtains a transmittance spectrum.

With the FTIR, when you collect a background spectrum, you obtain a plot of I_0 as a function of frequency. Similarly, with the CCD array spectrometer, when you collect a reference spectrum you obtain a plot of I_0 as a function of wavelength. If you look at these spectra you will see that the y-axis is not %T or absorbance, but emittance (FTIR) or counts (UV-vis). This is simply a spectrum of instrument signal intensity as a function of frequency or wavelength. When you obtain a sample spectrum, you obtain a plot of I as a function of frequency. You obtain a transmittance or absorbance spectrum by performing the appropriate mathematics to both I and I_0 . In this lab you collect both single beam spectra I and I_0 under identical conditions; that is no sample in the source beam in either case. In the resulting transmittance spectrum the deviation from 100%T is a direct measurement of the random error or noise of the measurement, just as in the instrument components laboratory.

In steps 6 and 7 of the procedure you are plotting single beam spectra of CO_2 , a plot of I_0 as a function of frequency. You are able to do this because of CO_2 present in the air. Since what you are seeing here are single beam spectra, this is not a noise measurement (although noise always exists in the measurement). The differences you are seeing in steps 6 and 7 are primarily a result of differences that the instrument detects for the CO_2 signal. Do not interpret the results in steps 6 and 7 as coming from differences in the amount of noise in the spectra.

PROCEDURE

1. Beginning with the CCD array spectrometer, collect a transmittance spectrum with either distilled water in a cuvette or no cuvette at all. This is done by 1st blocking the light source and collecting a dark spectrum, then activating the correct for electrical dark box on the spectrum screen. Then with either distilled water or nothing in the sample beam store a reference single beam spectrum. This will make it possible to collect a transmittance spectrum by activating the [T] button in the software. Save the processed spectrum to calculate the signal-to-noise ratio between 600 and 700 nm.
2. Using a spreadsheet perform the following moving boxcar smoothing routines to this data: 9, 13, 17, 21 and 25 point. Calculate the signal-to-noise ratio over the same 600-700 nm spectral region. Tabulate your data and make a plot of S/N ratio versus number of smoothed points. *In your write-up, comment on the effect of smoothing on the S/N.* A plot of a few of these “spectra” (in quotations because the only spectral information present is that of noise) will visually illustrate what is occurring. Note that the CCD array spectrometer software also provides the capability of smoothing on the spectrum screen, and that the exact same exercise could be performed on the FTIR also with essentially identical results.
3. Exploring how signal averaging affects signal-to-noise ratio is also more conveniently done on the CCD array spectrometer. This is because a single spectrum can be obtained on the array spectrometer in a few microseconds, whereas FTIR requires a few seconds to acquire and transform the spectrum. Thus averaging 50 or more scans can take a significant amount of time using the FTIR, but not much more time with the array spectrometer. Collect transmittance spectra with the following number of scans: 1, 2, 4, 9, 25, and 64 scans. **It is important to obtain both the reference spectrum and the sample spectrum with the same number of scans, or the data will not come out right and it will be apparent in your report.** Export the data to a spreadsheet and calculate the signal-to-noise ratio for each spectrum between 600 and 700 nm. Plot signal-to-noise ratio on the y-axis vs. the number of spectra averaged on the x-axis. Fit this data to a power function. This is done in Excel by adding a trendline and choosing a power fit. Annotate your plot with the equation. *Discuss the functional form of this relationship, and how your result compares to the result which you would expect from theory.*

The remaining parts to this lab are more conveniently done with the FTIR because spectral resolution can be readily varied with this instrument, but spectral resolution cannot be varied with the array spectrometer. The sequence of this lab is unimportant, so if it is more convenient to perform the FTIR portion of the lab at the beginning that is no problem.

1. Your instructor will start up the Digilab FTIR spectrometer, and briefly go over the operation of the instrument and the use of the software with you. You shouldn't have to do anything with the instrument except type at the keyboard, save and plot spectra. Consult your instructor with any questions or problems.

2. With 1 background and sample scan perform experiments at the following resolutions: 0.5 cm^{-1} , 1 cm^{-1} , 2 cm^{-1} , 4 cm^{-1} , 8 cm^{-1} , 16 cm^{-1} and 32 cm^{-1} . You will need to run a background spectrum at each resolution for this to work. This is a lot of spectra, keep track of them in the spreadsheet by naming them as you go. Export and save these spectra as ASCII or txt files so a spreadsheet can import them to calculate the S/N of these spectra. The S/N should be calculated in the region between $2200\text{--}2000\text{ cm}^{-1}$. *Make a plot of spectral resolution (x-axis) vs. S/N ratio (y-axis). Discuss the results and also discuss how spectral acquisition time varies with resolution.*

The rest of this laboratory does not involved signal-to-noise ratio, but illustrates the effects of spectral resolution and boxcar averaging on a single beam spectrum which contains many spectral peaks which are close together.

3. Obtain a background spectrum by co-adding 8 scans at 0.25 cm^{-1} resolution. Display this spectrum between $2400 - 2200\text{ cm}^{-1}$. You see a series of vibrational/rotational transitions for gas phase CO_2 in the spectrometer from the atmosphere. If you look around in other regions of the spectrum, you will also see many sharp peaks for gas phase water around 3500 and 1600 cm^{-1} . Save this gas phase spectrum (this is not an absorbance or transmittance spectrum because it is a single beam spectrum) between 2400 and 2200 cm^{-1} . It would be wise to export the data also into a file type that a spreadsheet can read. Now repeat the smoothing routines which were done for the 100% T line in Step 2 of the CCD array portion of the lab. Don't calculate S/N of the smoothed spectra as before (why?), but observe what the smoothing does to your CO_2 spectra. *In your lab write-up discuss the affect of digital smoothing on the spectrum of gas phase CO_2 . In light of your results on smoothing earlier (Step 2) in terms of S/N, discuss the trade-offs and compromises involved in using digital smoothing. Plot appropriate spectra which illustrate what you observe.*

4. Obtain three more spectra of gas phase CO_2 at 1 , 8 , and 32 cm^{-1} resolution, respectively. *Plot and/or save to disk (in an exportable form) these spectra to illustrate how resolution affects the gas phase CO_2 spectrum. In your lab write-up, discuss the effect of resolution on the gas phase spectrum of CO_2 . In light of your results on resolution with respect to S/N and spectral acquisition time, discuss the various considerations one must take into account when choosing a suitable resolution to acquire a spectrum.*

For your report

Suggestions of what should be included in the report have been given throughout the procedure. Remember to always cross-reference figures and plots in your write-up.

Questions

Here are some issues which should probably be addressed directly in your write-up. Many of these have already been mentioned in the procedure section in italics. This list is not all inclusive.

1. What is the relationship between S/N and the number of scans co-added to obtain a spectrum? Why does this occur? Are there any disadvantages to co-addition?
2. What is the affect of smoothing and the number of smoothed points on S/N ratio?
3. What is the affect of smoothing on the gas phase spectrum of CO₂?
4. What is the affect of resolution on the gas phase spectrum of CO₂?

Most importantly, answering this question thoughtfully would make a fine conclusion to the lab write-up.

5. EXPLAIN THE VARIOUS TRADE-OFFS AND CONSIDERATIONS ONE MUST MAKE IN BALANCING S/N, RESOLUTION, ENSEMBLE AVERAGING, AND DIGITAL SMOOTHING WHEN CONSIDERING INSTRUMENTAL CONDITIONS FOR SPECTRAL ACQUISITION.