

Course Overview

General Information

Structure of the Course

- Lab meets in HCAMS 316/318.
- First half of semester: Students complete one lab per week with a group (typically one partner).
- Second half of semester: Groups (typically partners) complete experiments of their own design to address a client's problem.

What you can expect from the instructor/TAs:

- Prepared for lab.
- Accessible to students.

What the instructor expects from you:

- Come on time and prepared for lab, including reading the lab handouts, supporting information, and preparing your lab notebook.
- Ask questions/seek help when you are uncertain about various topics
- That everything you turn in for a grade represents your best work.
- Work cooperatively with your group (typically partners), and complete group contract.

Graded Course Components

Assignment	n	pts	total	percent
Rotational Experiment Reports	7	70	490	49
Lab Notebook	1	10	10	1
Client-Based Project	1	400	400	40
Lab Citizenship	1	100	100	10
TOTAL			1000	100

Rotational Experiment Reports

You will be completing the experiments and writing the lab reports as a group (typically partners). The focus of each lab report, for the most part, will be presentation and evaluation of the results that you collect on the various instrumentation. Each week, you will rotate who will in your group take the lead on the writing. Your reports will be due, via Canvas submission, on the Friday of the week after you complete the wet-lab portion of the experiment. See the experimental protocols for required sections.

Lab reports will be graded for quality of writing, description of instrumental methods employed, quality of figures, and quality of results and discussion. Figures should be made with the utmost care (e.g., axes labels including measurement units and error bars). Each lab will have guiding questions to address in the results and discussion of your reports.

Laboratory Notebook

You will be expected to keep a lab notebook throughout your experiments. This will be particularly important during your independent project, where the notebook is the main vehicle for documenting the experiments you perform that are not specified in laboratory protocols. You can choose as a group to maintain your own individual lab notebook or a group notebook. It can be paper or electronic. This will be checked at the end of experimentation for quality/thoroughness. The notebook should be maintained throughout the semester and include experiment information that might not be recorded in a data file (e.g., calculations/concentrations of standards).

Client-based Independent Projects

In preparation for this course, the instructor has established a relationship with six different clients that have analytical chemistry problems. As a group, you will design and carry out a project aimed at solving one of these clients' analytical problems. Project assignment is finalized through a meeting between the instructor and each group to discuss the group contract and determine the client project for the group.

There are four graded components of this project: (i) methods proposal, (ii) client report, (iii) client presentation, and (iv) peer review. The methodology will be due before spring break (see calendar for specific dates) to allow ample time for feedback and for ordering materials. Your reports and presentations will be due / occur during finals week. Clients will be invited to the presentations. Details and rubrics are in the experimental protocols.

Lab Citizen Points

These points are awarded by the TA and instructor at the end of the semester. The TA and instructor will observe timeliness, contributions to group work in the lab, and safety, among other things. Additionally, peer-evaluations will occur 1-2 times during the semester and will be taken into account in the lab citizen points.

Policies (see syllabus for full list)

Attendance

Attendance for lab completion is required. Students are responsible for announcements and all materials covered in lab.

Appropriate Student Use of Class Notes

Taking notes is a means of recording information but more importantly of personally absorbing and integrating the educational experience. However, broadly disseminating class notes (or lab reports), or accepting compensation for taking and distributing lab reports, undermines instructor interests in their intellectual work product while not substantially furthering instructor and student interests in effective learning. Find additional info at:
<http://d.umn.edu/academic-affairs/academic-policies/classroom-policies/course-notes-and-materials>

Groups

You will form into groups of two or three. Groups are determined in the first week.

Group Contract

4243 - Group Contract

Group letter (as designated by instructor): _____

Each team member should sign to indicate that they agree to these expectations and intend to fulfill them.

Explicitly stating your group's expectations is for your benefit. The details of your contract will not be graded, and no feedback will be given (unless you ask). Be sure your expectations are thorough, but realistic. For example, setting the expectation that "Everyone will have the homework done before class." or "We will meet every weekend in sickness or in health." are probably unfair. However, "Everyone will do the reading before class." or "We will help anyone who misses class for good reason to catch up." are better rules.

Group Guidelines

Members of the above group agree to:

1. Work to the best of each individual's ability.
2. Follow the guidelines specified by the instructor including
 1. Designating and rotating roles for each person.
 2. Decide on what each member should do before lab and then follow through.
3. Communicate clearly with other group member(s), particularly about absences.
4. Consult with the instructor if you encounter problems that cannot be resolved within the group. Group members agree to abide by the Quitting/Firing policy specified in the syllabus

Other points agreed upon by group (go onto back of page if necessary):

Names of Group Members, Signatures, and Date

Most groups can work out differences in skill levels or work ethics simply by communicating. It often works best if you use your first meeting to discuss your expectations of one another. In this discussion:

1. Designate roles for each person (e.g., lead on writing). Rotate these roles each report to spread the workload out.
2. Decide what each member should have done before lab. Then, be sure to follow through!
3. Absence of an individual. A group member will not be given credit for the group's work if they are absent. The person submitting the work is responsible for reporting which group members worked on the activity when they submit their report on their team's performance.
4. Consult with the instructor if you encounter problems that cannot be resolved within the group. The instructor will meet with your team to seek out a resolution. If and only if a resolution still cannot be reached, then the following actions can be taken:
 - a. Quitting - You may quit your group if you find yourself consistently doing all of the work. You must first notify your team (copying the instructor) in writing that you intend to quit. If there is no improvement, you may quit the group. You must notify your group (and the instructor) in writing that you are officially quitting.
 - b. Firing - You may fire a group member who consistently does not contribute. You must first notify your group member (copying the instructor) in writing that they are in danger of being fired. If there is no improvement, you must notify the person (and the instructor) in writing that they are no longer in the group. The fired student should then meet with the instructor to discuss options. If you quit or are fired, it is your responsibility to find another group. The instructor will help, but you must take the initiative and go to the instructor.

Below, write your name, sign, and date to indicate your agreement to this contract.

Introduction to Signal Processing - Signal Averaging and the Fourier Transform

1. Learning Outcomes

1. Relate digital signal processing to S/N, including explaining the impact of noise on the content/quality of the signal
 2. Evaluate how digital signal processing changes the signal observed
 3. Describe how various digital filters impact the signal
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2. Pre-Experiment Reading

- [Chapter 5: Signals and Noise](#) — discusses signal-to-noise concepts and noise sources in instrumentation.
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3. Goals

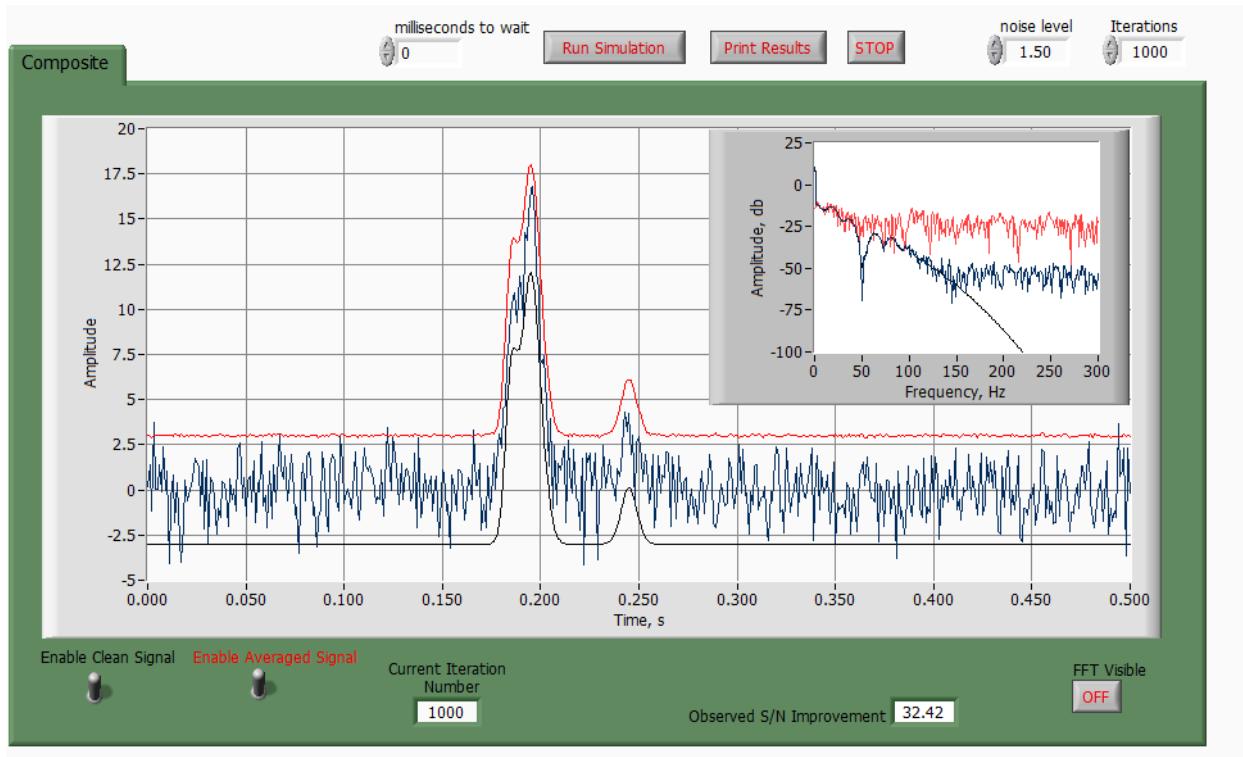
In this exercise you will explore the signal processing techniques of signal averaging and the Fourier transform through the use of software simulators written in LabVIEW. *An important component of this exercise* is for you to take control of the experiments which you carry out, and to think about designing them to evaluate the utility of signal averaging and the FFT as digital signal processing tools. An effective scientist is curious by nature! *Be curious and translate your curiosity into experimental protocol that answers questions that your curious nature raises.* In addition to these you will also familiarize yourselves with digital filtering techniques using an implementation based on the inverse Chebyshev algorithm. This and other digital filters will be encountered throughout your use of instrumentation, so it's important to know what's actually happening. Your observations will be enhanced and reinforced by virtue of using signals which are limited to the audio frequency range (20 Hz – 20 kHz) that can be not only viewed graphically in both the time and frequency domain, but also heard as sound output from the computer. Lastly, you will briefly characterize the human ear as a sound transducer, by loosely quantifying its frequency response or sensitivity, as a function of frequency throughout this range.

4. Background and Theory

Three simulation programs will be used to explore the techniques of signal averaging and the FFT. These are: *Signal Averaging*, *Intro FFT* and *FFT Sound Check*. Each is a compiled (executable) Program. **These programs are loaded onto the computers in HCAM 316.** When you double click on these shortcuts the programs will launch and start running. Each of these programs is briefly described below.

4.1. Signal Averaging

This program simulates the application of signal averaging to the improvement of S/N for a spectrum containing three features. Recall statistics of averaging predict that signal to noise ratio should improve with the square root of n, where n is the number of spectra averaged. Recall also that the experiment must be reproducible to prevent artifacts from the averaging process. Also keep in mind that there are various figures of merit or desired outcomes from the application of signal averaging such as overall improvements in the quality of the result as reflected in improvements in the ability to discern overlapped spectral features, as well as quantify peak height and/or area. The front panel of this simulator is shown in the following figure:



There are front panel controls to set the parameters of the simulation. *Milliseconds to wait* controls the time delay between successive scans of the “spectrum”. The Fourier transform of the wavelength dependent spectrum is shown as an inset in the plot. This feature can be activated using the control in the lower right-hand corner of the front panel. In both cases the black traces correspond to the noise-free spectrum, while the red are for the accumulated (averaged) spectrum and the blue are for the current single scan. The three buttons to the right control the primary modes of operation. Pressing *STOP* causes the program to cease execution and return to the operating system. The program can be restarted by clicking the mouse over the arrow in the upper right-hand corner of the window containing the front panel which may be resized or minimized, as with most windows applications.



The control to the immediate right of the *STOP* button (*noise level*) allows you to set the noise level (random white noise) which is superimposed on the synthetic spectrum. The control to its right (*iterations*) allows you to set the number of iterations or averages to be simulated. In the lower left corner of the panel are two controls which allow you to set whether or not the clean signal (black) is plotted, and likewise the averaged response (red). These are displayed when the switches are in the up position. Manipulation of these is accomplished by clicking the mouse when it is positioned in the vicinity of the corresponding switch. The raw data from a single scan (blue) is always displayed on the plot.

4.2. Intro FFT

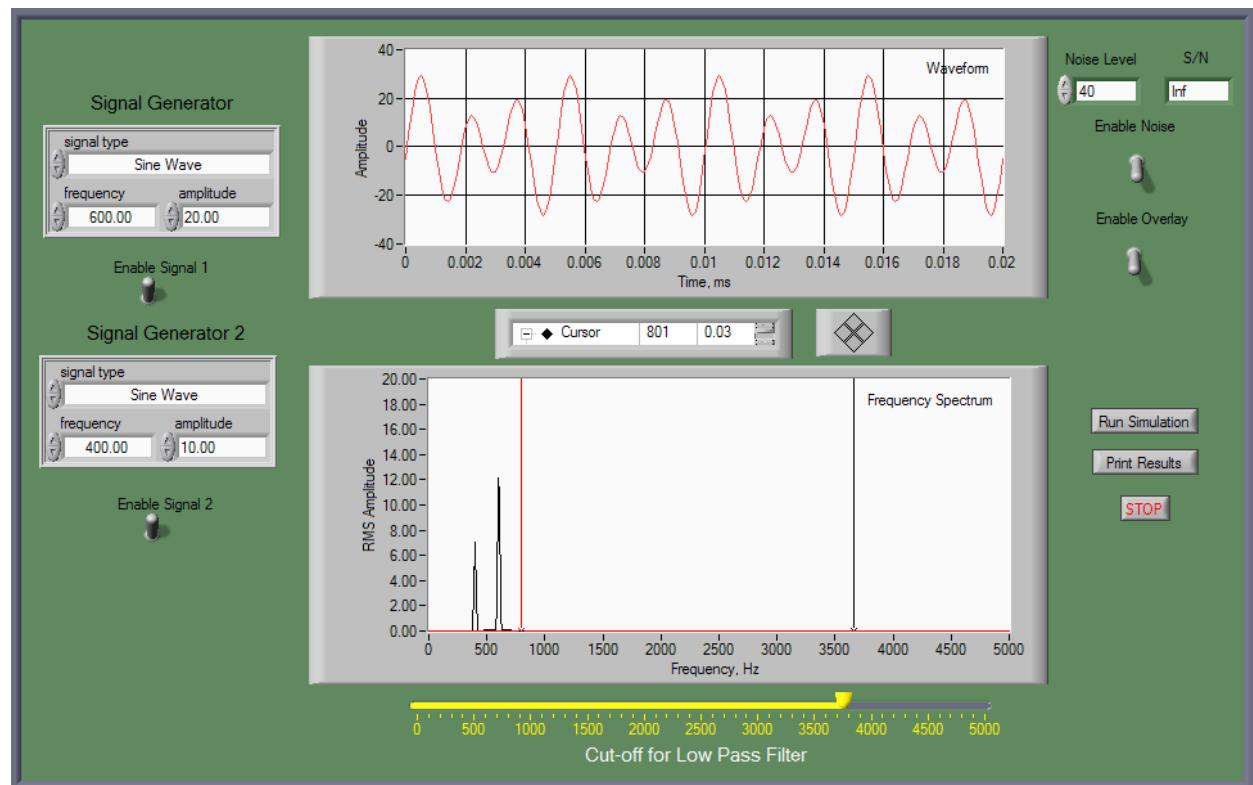
Below, we will introduce the front panel for the *Intro FFT* simulator and explain its components. First however, please take a moment and read this introduction. A key concept to understand for this simulator is the concept of harmonics. Harmonics are integer multiples of a base (fundamental) frequency. A pure sine wave contains only the fundamental, but real-world and synthetic waveforms with sharp features (like square, triangle, or sawtooth waves) are built from the fundamental plus additional harmonics. The mix of harmonics determines the waveform shape and

what it sounds like: more high-frequency harmonics generally means a brighter or harsher sound. In the frequency-domain plots you will see the fundamental and its harmonics as discrete peaks, and filtering changes the sound by reducing or removing specific harmonic components.

For periodic signals, like square, triangle, or saw waves, the Fourier transform shows that a waveform can be represented as a sum of sine/cosine components at discrete frequencies. If your base (fundamental) frequency is f_0 , then the harmonics occur at $n \cdot f_0$ where $n = 1, 2, 3, \dots$ is the harmonic number. For this experiment, you do not need to derive Fourier series coefficients, but you do need the expected pattern of which harmonics appear and how their amplitudes decrease with harmonic number:

Waveform	Harmonics present	Expected harmonic amplitude trend (relative)
Square	Odd only ($n = 1, 3, 5, \dots$)	Amplitude $\propto 1/n$ (odd n only)
Triangle	Odd only ($n = 1, 3, 5, \dots$)	Amplitude $\propto 1/n^2$ (odd n only)
Sawtooth	Odd and even ($n = 1, 2, 3, \dots$)	Amplitude $\propto 1/n$ (all n)

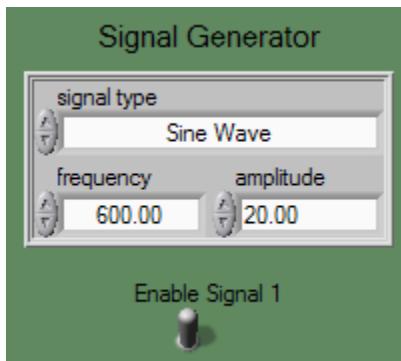
Another key idea is aliasing: when the sampling rate of an instrument is too low to capture all the frequency components in a signal, the higher-frequency components ‘fold back’ into the measured spectrum and appear at incorrect, lower frequencies. Square waves are especially susceptible because they contain infinite odd harmonics. If the sampling rate does not satisfy Nyquist for those higher harmonics (the Nyquist criterion requires sampling at least twice the highest frequency you want to capture), you will see unexpected peaks between the expected odd-harmonic peaks. Ask your TA if you have any questions about harmonics or aliasing. And with that behind us, let’s proceed to the Virtual Instrument overview:



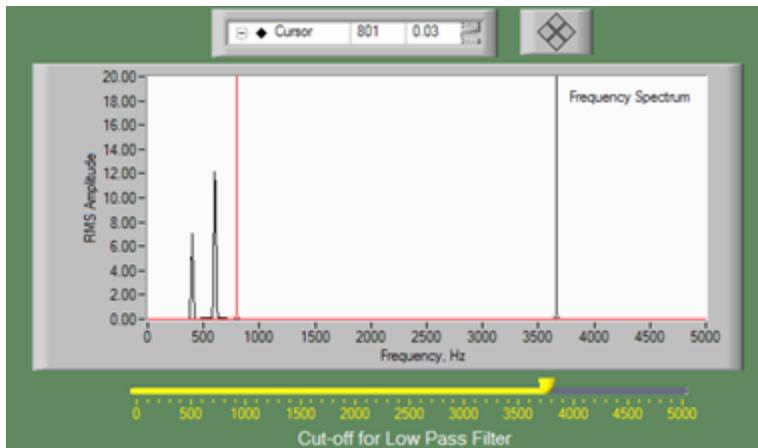
When the shortcut to this program is activated, this program will begin execution. Like the program described above, the buttons in the lower right-hand corner control the functionality of execution. In this program, two plots are presented corresponding to the time domain signal (top, red) and the frequency domain (bottom). In the latter case

only the amplitude spectrum is plotted. In the upper right-hand corner of the panel is a control to set the noise level (random white noise) and an indicator of the signal to noise level of the simulated signal. Beneath that are two toggle controls: one to control whether the noise is applied (added) to the signal, and a second to control (*enable overlay*) whether or not the digitally filtered signal (more below) is presented (black plot). This allows one to make observations of the effect of the digital filter on the noisy signal (noise level > 0), and is useful to observe the effects of the low pass filter on S/N, and the more subtle consequences of digital filtering on the phase shift or time delay and shape of the simulated waveform.

The program allows for the simulation of two signal components, and to control the nature of the waveform (sine, square, etc.), its amplitude and its frequency. The controls for these two “Signal Generators” are located in the upper left-hand corner of the front panel. You can control whether each is active with the corresponding toggle switches (*enable signal*). One of these sets of controls is shown below:



In the case of the plot of the frequency domain signal, there are some additional controls and features to note:

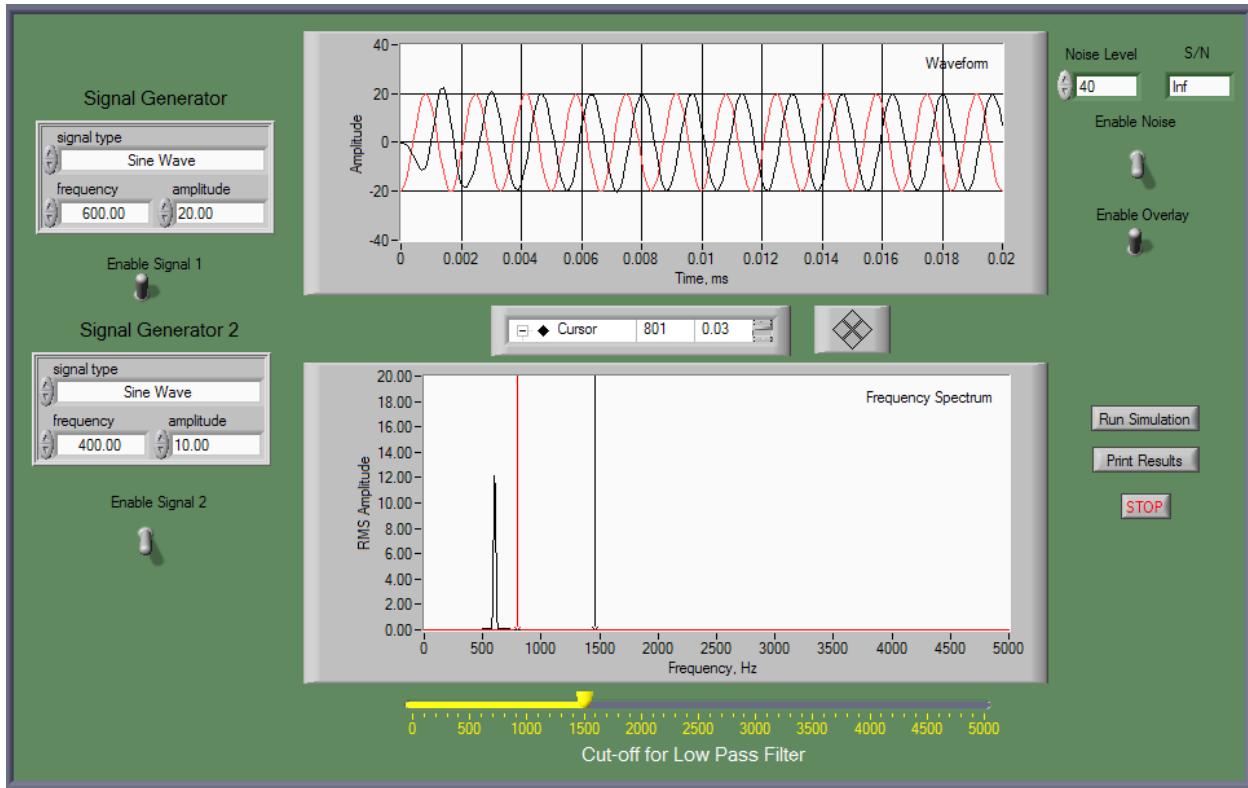


The red crosshairs correspond to a cursor which is locked to the plot, and when moved via mouse control will report the frequency and amplitude of a single point over which the cursor cross hairs are located. These are displayed in the box above the plot. The buttons to the immediate right of this indicator provide for an alternative means of moving the cursor position.

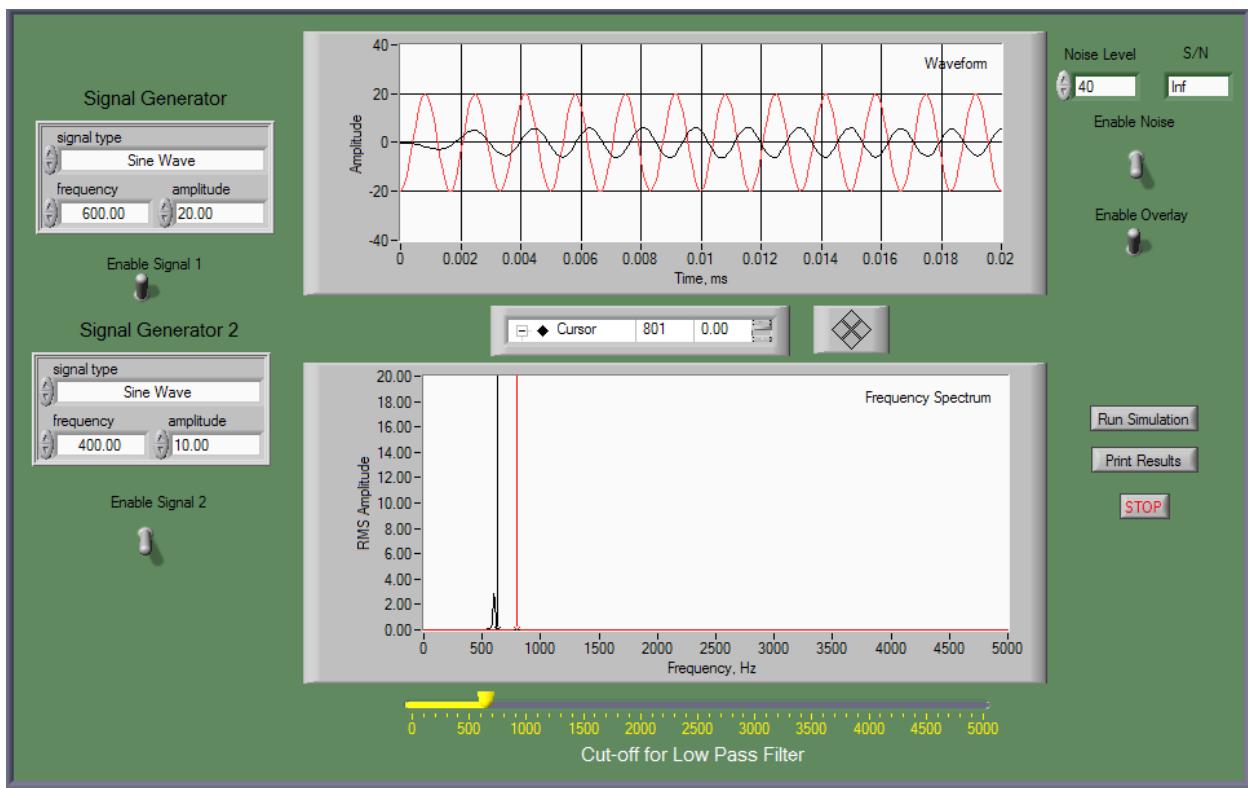
Below the plot is a control which allows you to control the cutoff frequency for the digital low pass filter. This filter is always on, and is applied to the signal generated by the signal generator controls with the noise added at the selected level. As such the effects of the filter can be observed in both the time and frequency domain plots. The cutoff frequency is set by using the left mouse button to move the pointer on this slide control. A second cursor (black) is controlled by the setting of this filter cutoff value, and is plotted as a reminder of the chosen cutoff frequency used.

Some example simulations follow which may help you to appreciate the nature of observations that can be made using this simulator.

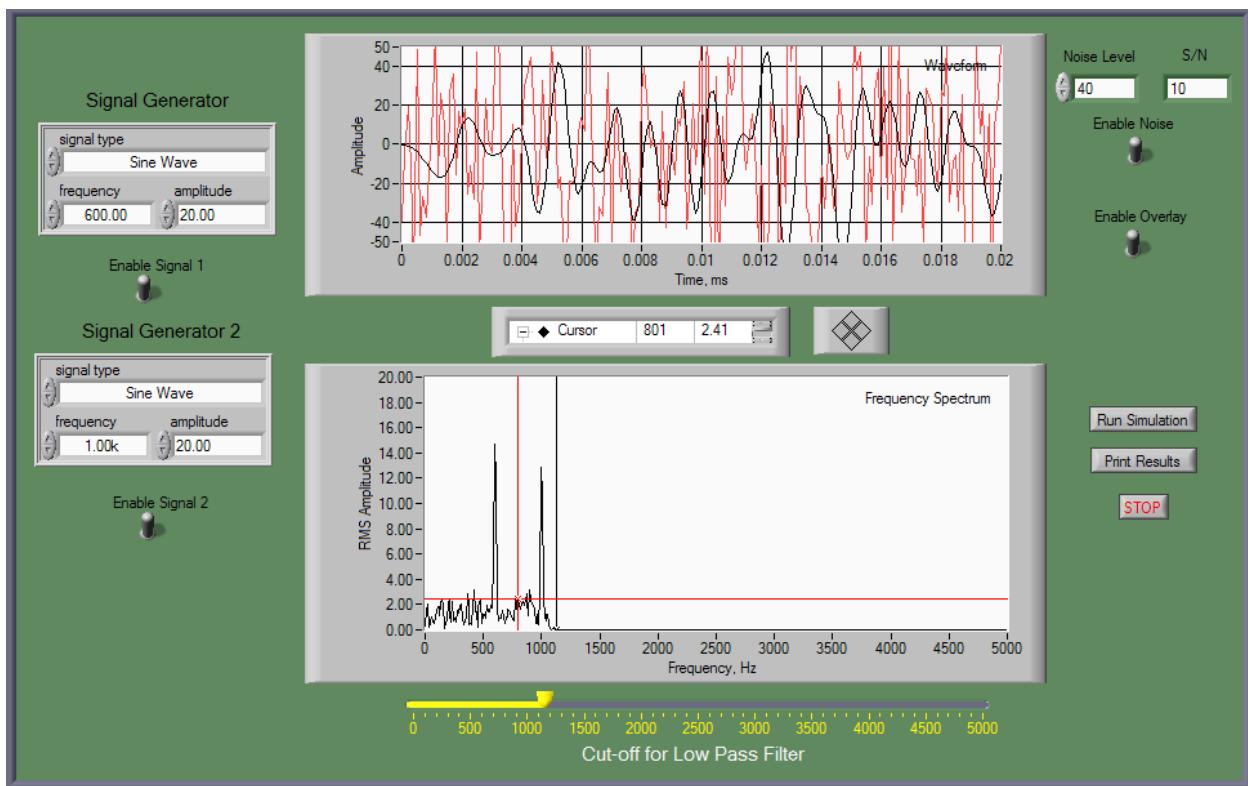
Example 1: Single sine wave (600 Hz), no noise, cutoff set to 1500 Hz. Note the phase lag in the filtered signal (black) relative to the unfiltered signal (red) in the time domain, yet the amplitude of the time domain signal is not affected. Note also the correspondence of the amplitude and frequency in the frequency domain to the settings of the signal generator. The frequency domain signal amplitude is RMS (root mean square), so that the amplitude appears as 0.707 of the amplitude setting in the signal generator.



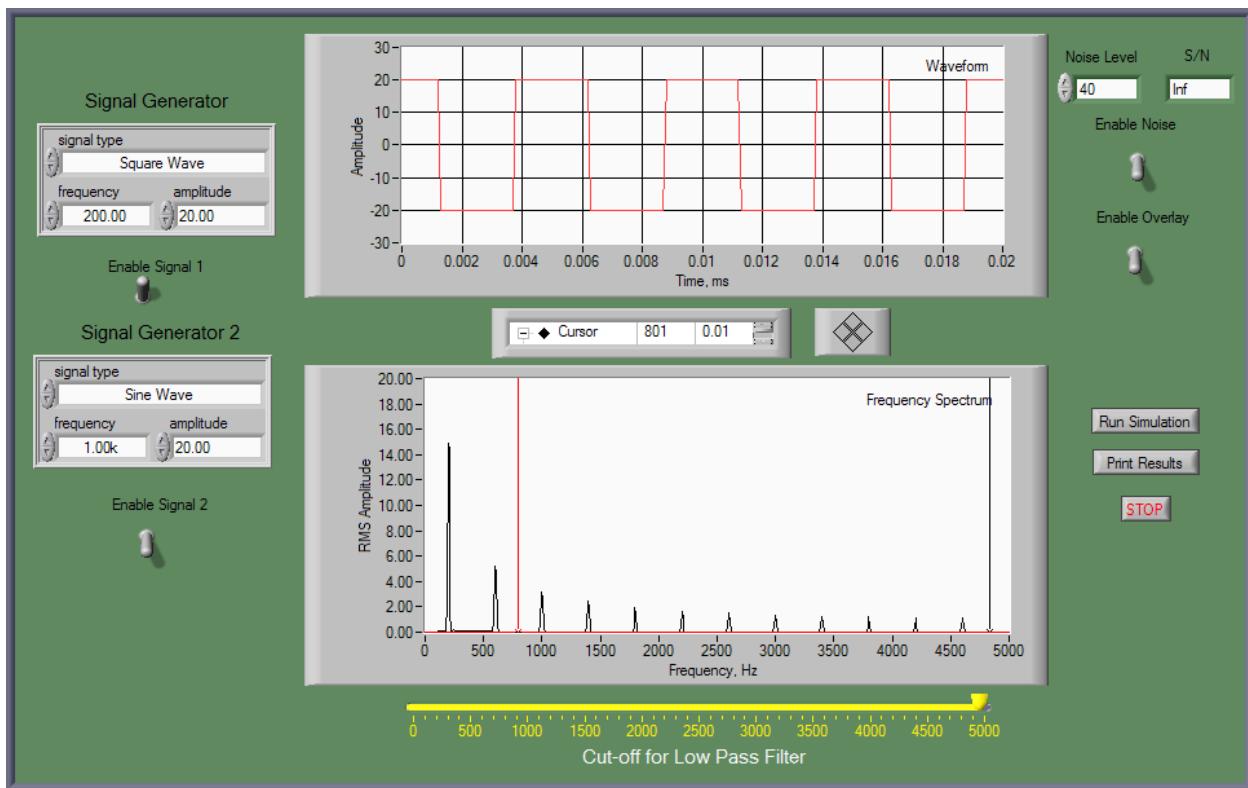
Example 2: As above, but the cut-off frequency for the filter has been lowered to 600 Hz. The effects are very pronounced in both the time and frequency domains.



Example 3: Two sine waves of equal amplitude with moderate noise, cut-off set to 1.2 kHz. Note effect of filter on both noise, and higher frequency sine component (1 kHz). Is the reduction in amplitude of the 1 kHz sine wave due to filter attenuation or the random nature of the noise at this frequency?



Example 4: Square wave, 200 Hz, no noise, filter cut-off set to 5 kHz. Note higher harmonics.

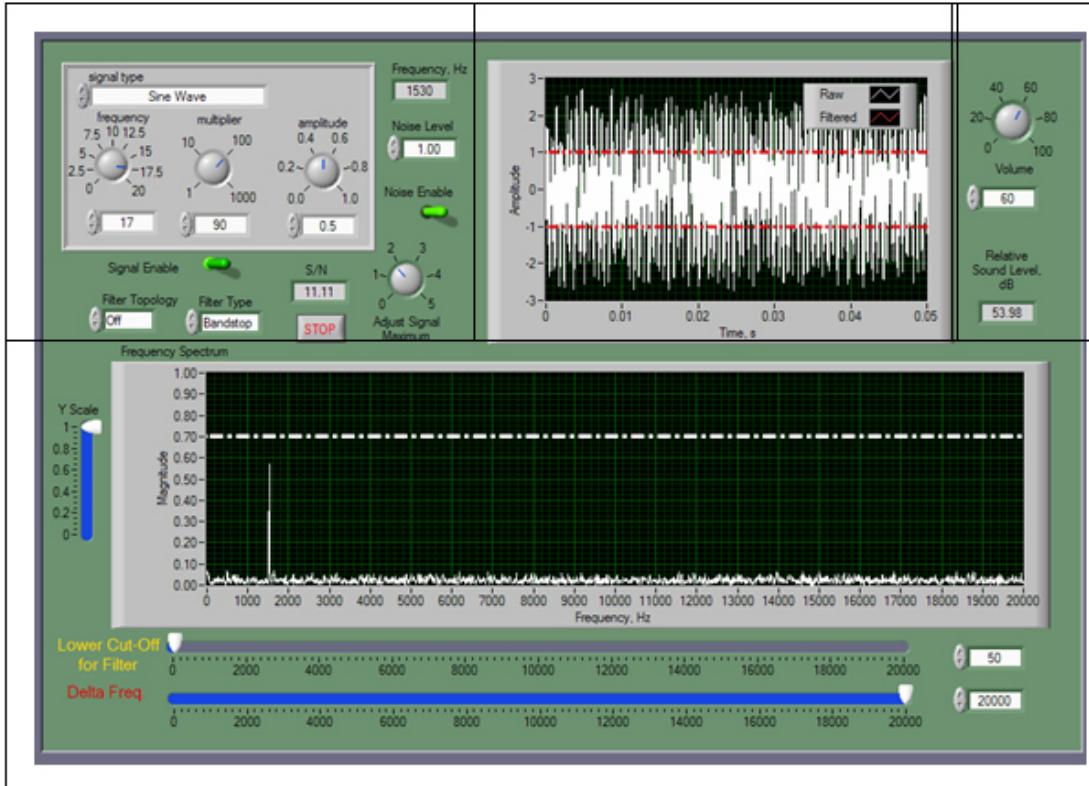


4.3. FFT Sound Check

This program adds the capability to hear the waveforms synthesized by the signal and noise generators, thus providing an additional dimension to the observations made while using it. In a sense it engages our ears as transducers, and the audio processing regions of our brains as the data acquisition and interpretation parts of the “apparatus”. In addition to this increased dimension of perception the program also has a more versatile implementation of the filtering of certain ranges of frequencies, allowing for enhanced understanding of their functionality as applied to digital signal processing. These filters employ mathematical algorithms to implement the attenuation of undesired frequency content of digitized signals, while preserving those frequencies which contain signal information along with noise components in those frequency ranges. Although a wide variety of algorithms are available, each with their own characteristics, the one chosen here is an inverse Chebyshev filter, chosen for its very abrupt attenuation of frequencies above and below the selectable or chosen cutoff frequencies.

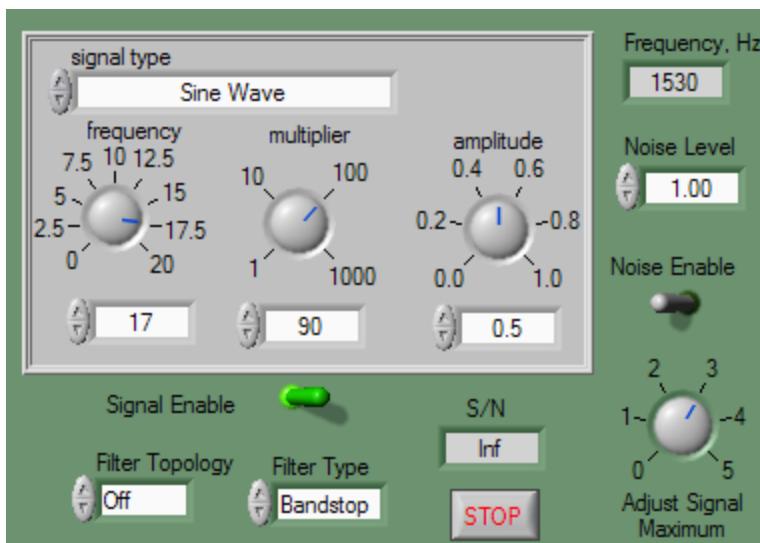
The front panel of this LabVIEW program is shown below.

Signal Generator Time Domain Signal Audio Volume



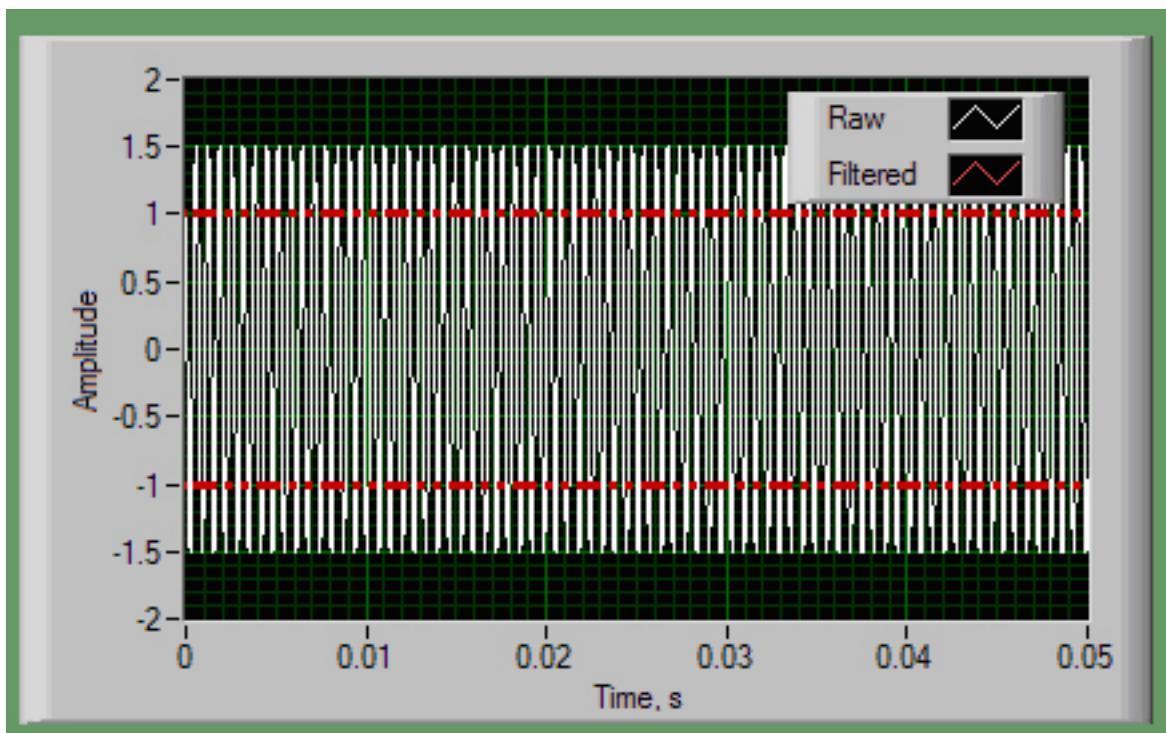
Frequency Spectrum and Cutoff Settings

It is partitioned into four regions, each of which will be discussed in turn. The signal generator part is located in the upper left-hand section, and contains controls to set the signal type, its frequency (two controls are included here for base frequency and its multiplier), its amplitude and includes a control to enable the signal source.



The selected frequency of the waveform is displayed in the upper left-hand corner of this section. Beneath that are controls which allow the setting of the noise level and to enable it. Beneath that is a control to set the overall level of the sum of the signal and noise. The signal to noise level is calculated and displayed for reference. Also included are controls for the filter (off vs band pass, low pass, etc.). The “STOP” button allows the user to exit the program (stop execution). The current version does not implement any printing or file storage, so when using the program, the user must record notes on observations made.

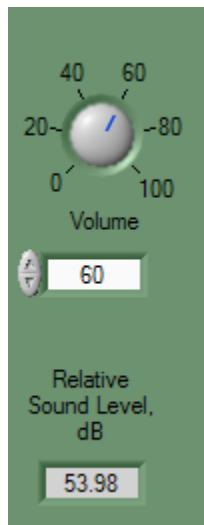
To the right of this section is a composite graph (time domain) of the clean or raw signal (white trace) overlaid on the filtered sum of the signal plus noise (red):



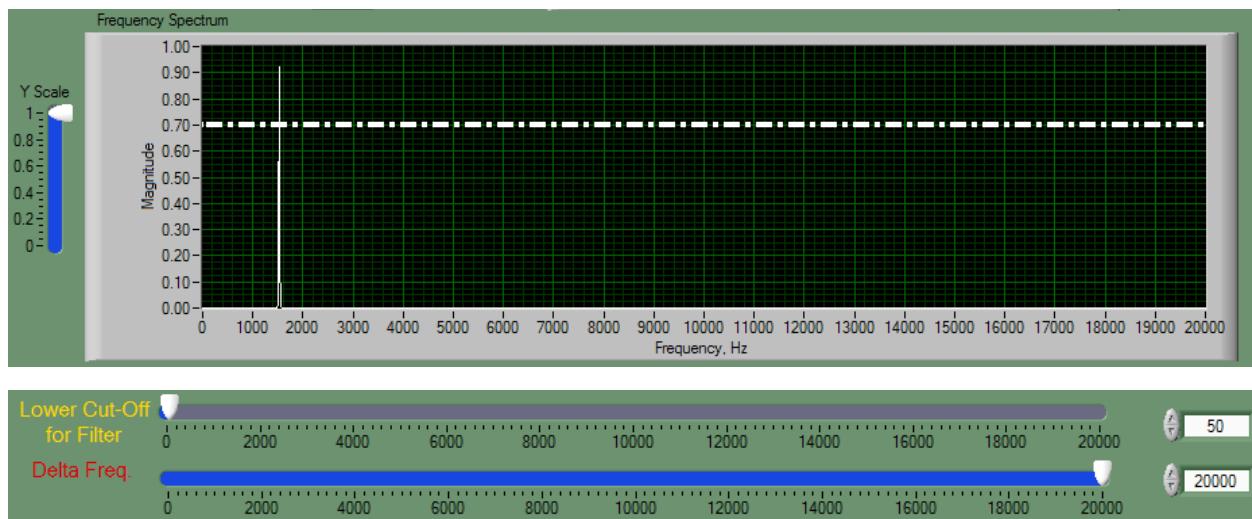
Overlaid on this graph are bold horizontal dashed lines which denote the maximum levels which can be accurately reproduced by a typical computer sound card. Settings of the overall level which cause excursions of the signal above or below these red lines will cause clipping of the waveform, resulting in distorted sound output with

anomalous high frequency content. It is very important to appreciate this aspect of the program to properly compare visual (graphical) representations of the signal with audio perceptions made when characterizing the composite signal (waveform plus any noise added). In this case the signal maximum is set too high and the audio signal heard will be clipped. The consequences of this distortion will be to add higher frequency harmonics, such that the audio output will sound like a square wave rather than a sine wave of the selected frequency!!!

To the right of this graph are controls and indicators which set and display the audio level sent to the sound card:

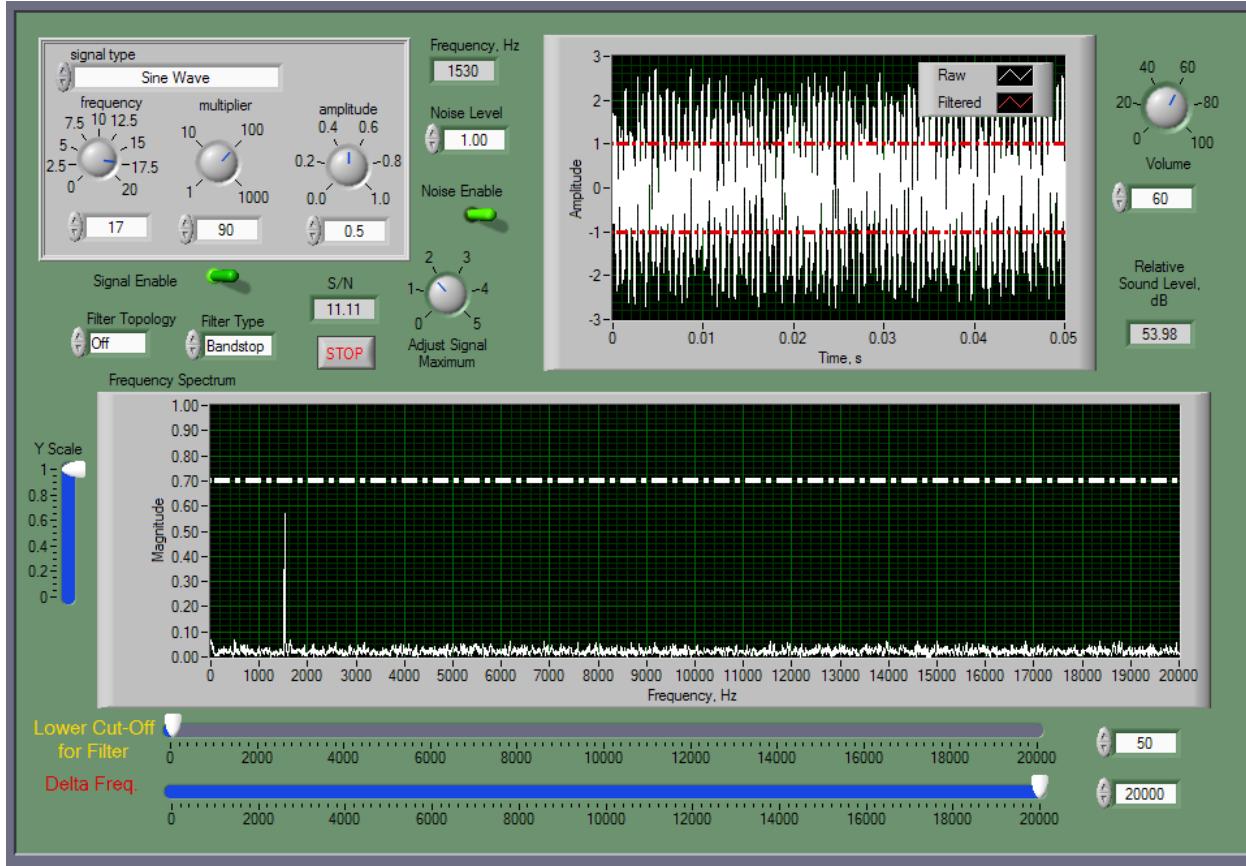


The bottom section contains a graph showing the frequency content (frequency domain) of the combined waveform plus noise as obtained using the FFT algorithm, along with slider controls which set the lower and upper cutoffs for the chosen filter (if selected):



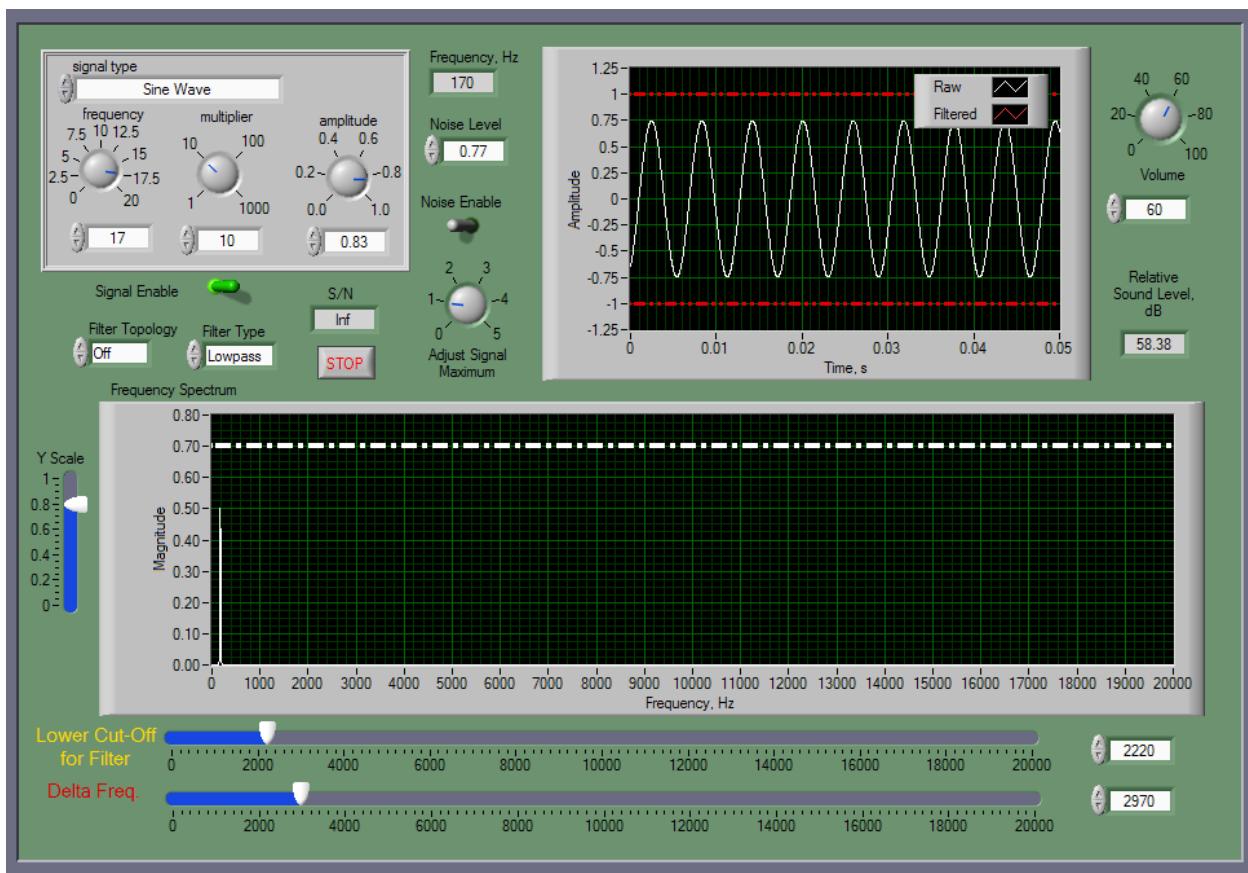
Note that the magnitude plotted here is RMS (root mean square) amplitude of the frequency components of the composite signal. The bold dashed white line in the frequency domain reminds us that the audio output in this case will be distorted (clipped). Note that the upper cutoff for the filter is set by selection of a "Delta Freq(uency)". This value is added to the lower cutoff setting to set the upper cutoff for those filter settings which employ this parameter (e.g. bandpass, notch, etc.). To the left of the graph of the frequency spectrum plot is a control which allows for variable scaling of the amplitude (y axis) presented on this graph. To the right of the cutoff sliders are indicators of the current settings of these values. The default setting is shown with the minimum lower cutoff set to 50 Hz, and the maximum delta frequency set to 20 kHz.

Shown below is an example of the appearance of the front panel for a 1.53 kHz sine waveform to which white (random Gaussian) noise has been added to yield a composite signal with S/N = 11.11. Whereas the time domain display seems very busy, the frequency domain display clearly indicates the frequency and amplitude of the sine waveform.

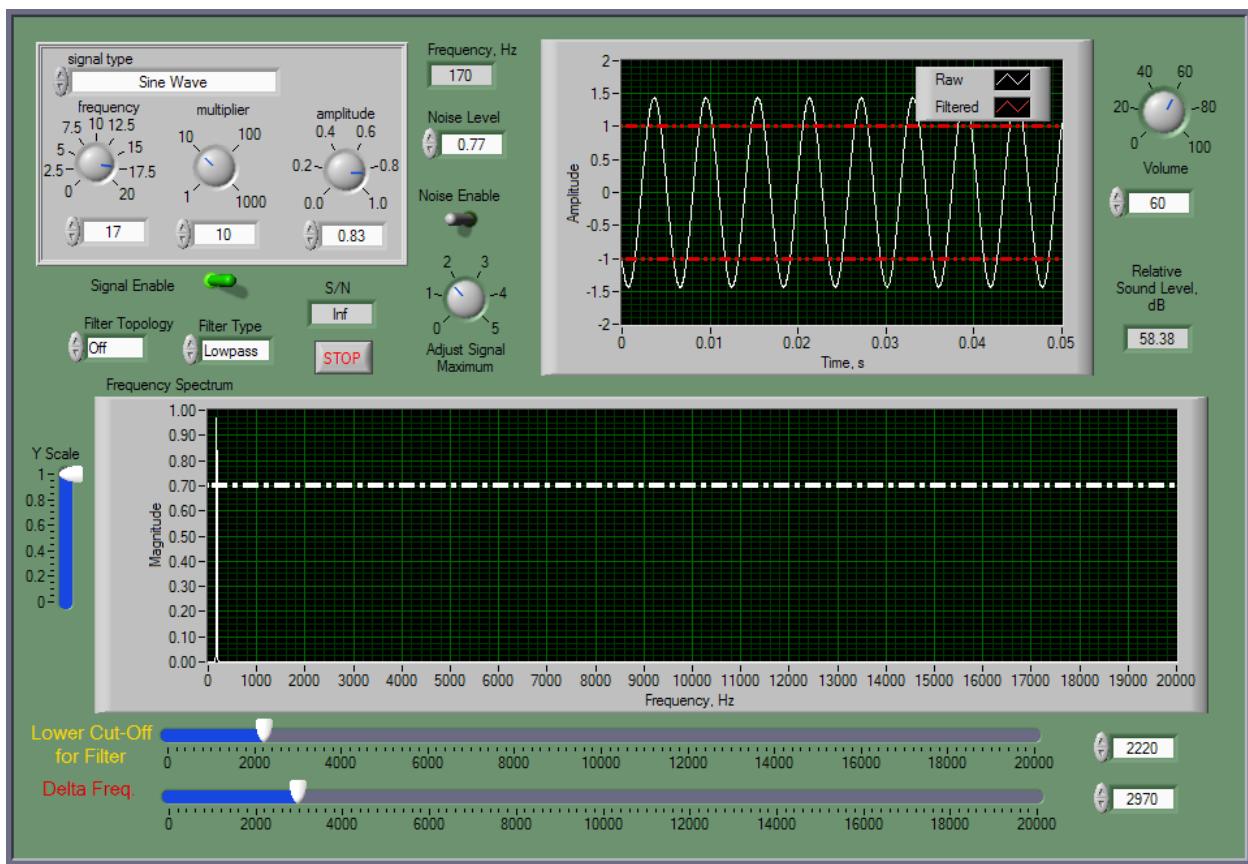


Note that the audio signal will be distorted in this case, even though all frequency components lie below the bold dashed white line in the frequency spectrum. This is indicated by the time domain signal exceeding the red dashed limits shown in the time domain representation. This is a result of the noise level being set too high, and thus the frequency components of the noise will be distorted, sounding harsher than it should. In this case the filter is off, and we observe random amplitudes of noise at all frequencies.

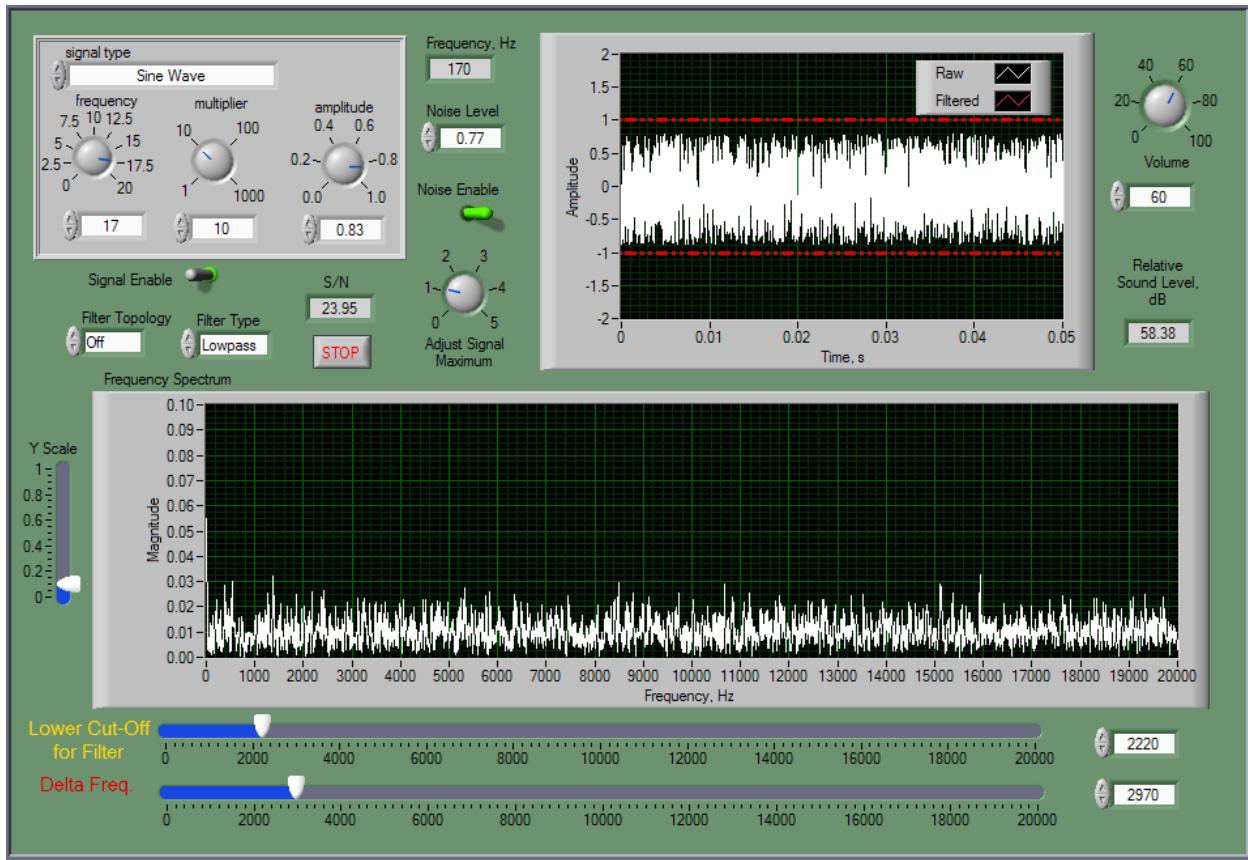
Examples:



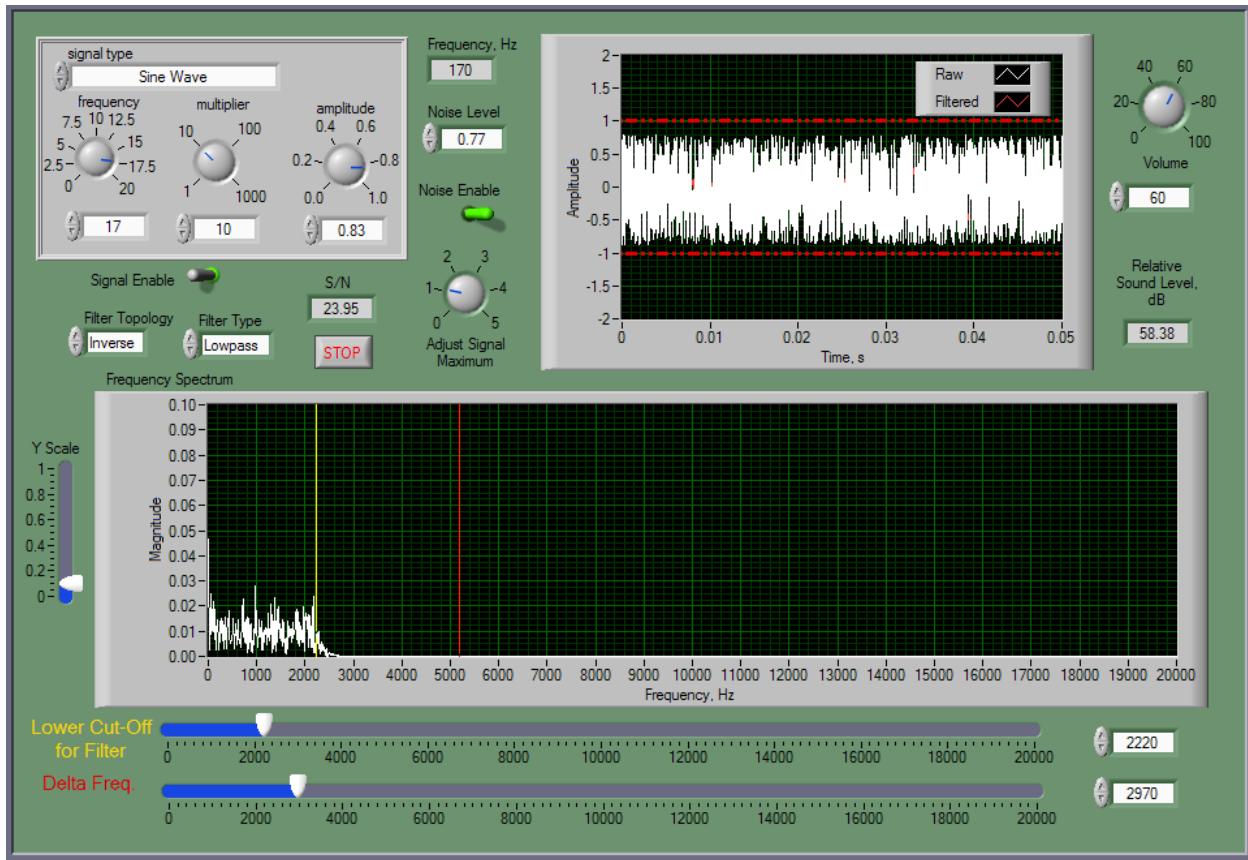
Proper setting of overall signal amplitude; audio signal will correctly reflect content.



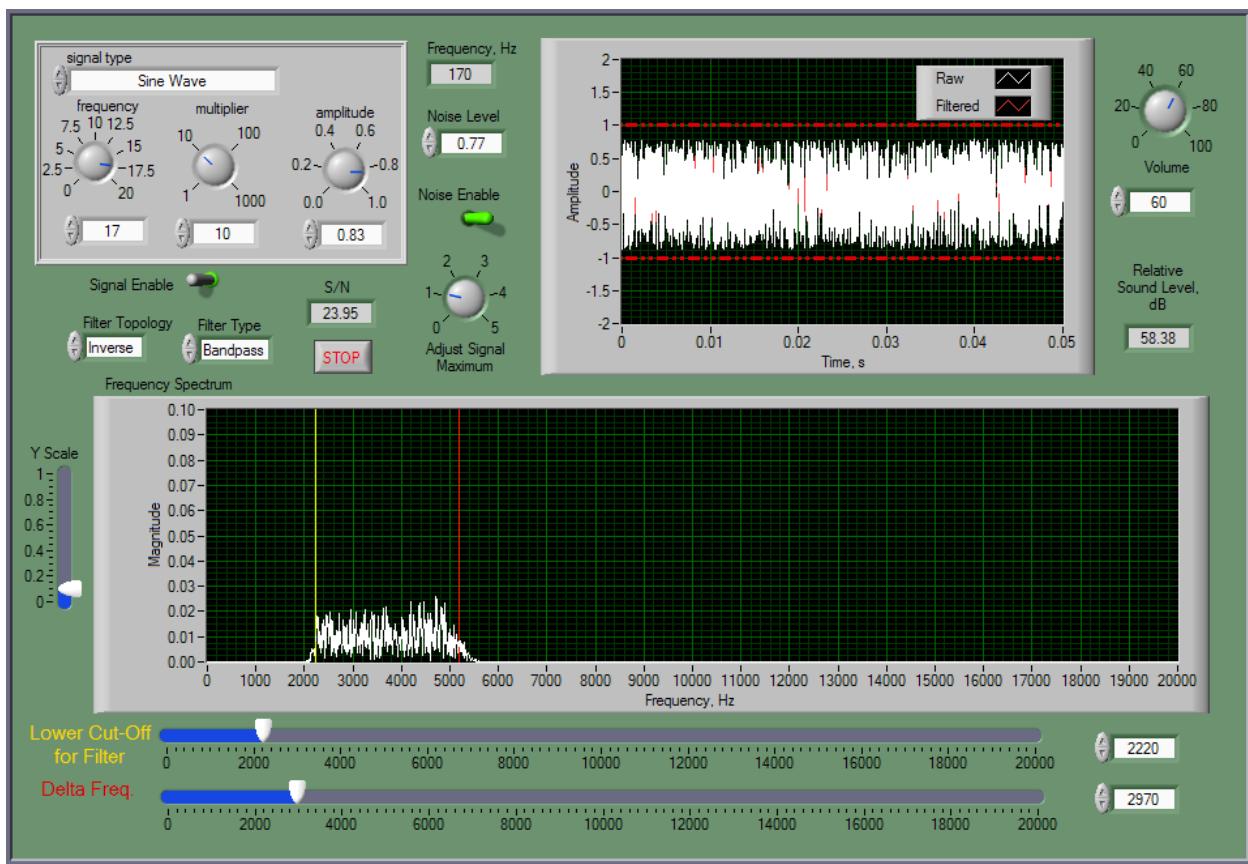
Improper setting of overall signal amplitude; audio signal will not correctly reflect content.



Proper setting of overall signal amplitude with only the noise generator enabled. Filter is disabled (off). The audio level is set correctly.



As above, with filter enabled and set to LOWPASS. Note the appearance of the yellow and red cursors on the frequency spectrum plot (bottom plot) when filter is enabled. The yellow cursor marks the lower cutoff for the filter and the red cursor marks the upper cutoff for the filter, which is meaningless for this filter type.



As above, with the bandpass filter setting. Only frequencies within the cutoff limits are allowed to pass unattenuated.

5. Experiments / Procedures

Download and Install LabView

1. Search “LabVIEW Community Edition” and follow the link to the National Instruments website.
2. Download the software for your computer. You will need to make a National Instruments account to download the software. Do this when prompted. The confirmation email may take about 5-10 minutes to send.
3. Once the confirmation is complete, download the version of the software that is compatible with your computer (Mac v PC), then follow the installation prompts and select installation options based on your preference.
4. Open the LabVIEW software you downloaded. On Mac it should be called LabVIEWCommunity, and on PC it should be called NI LabVIEW 2025.

Download the Virtual Instruments from the Canvas site

Look for this week’s entry on Canvas, click on the “Software” download button, unzip the file. In it should be files with the extension “.vi”. You will use the ones called “Signal Averaging S14”, “intro fft s14”, and “FFT Sound Check s14”. You can open those files using the LabView software you have already downloaded.

Table 1. Signal Averaging Experiments.

Experiment Number	Conditions	Purpose
S-1	No noise, single scan (“iteration”)	Describe the ideal “clean” spectrum.

Experiment Number	Conditions	Purpose
S-2	Noise added, single scan (“iteration”)	You may include a screen shot as <i>part</i> of your answer to this question, if you wish, and the same goes for the following questions. Determine signal to noise ratio where shoulder on left-hand peak begins to be obscured by noise. Use your discretion to determine when YOU think the shoulder has disappeared and justify your thinking.
S-3	Noise added, single scan (“iteration”)	Determine when right-hand peak begins to be obscured by noise.
S-4	Time between scans 1 s (1000 msec), noise level 2, 10 scans (scans are also sometimes called “iterations” in this experiment or in the Virtual Instrument)	What do you observe in terms of improvement in signal quality with successive scans?
S-5	Noise level 2, design an experiment to quantitatively evaluate how the signal to noise level (S/N) improves with number of scans averaged. An upper limit of $n = 1000$ should be sufficient.	Describe the results of averaging and compare to statistical prediction of S/N improvement. (Hint: graphical representation aids discussion).
S-6	As above, but with noise level set to 5.	Describe the results of averaging and compare to statistical prediction of S/N improvement.

Table 2. Introductory FFT Experiments. (Consider questions F3-F5 together: Please comment on the specific waveforms and their harmonics but also how they compare to each other.)

Experiment Number	Focus	Conditions	Purpose
F-1	Familiarization with signal generator controls.	Single and summed signals (i.e. both signal generators enabled), no noise, filter set to max cut-off, vary waveform type, frequency and amplitude	Explore frequency content of transformed data in the frequency domain. Does the FFT match your expectations based on the setting(s) you picked? Explain.
F-2	Effect of added noise, and function of filter	Single signal with noise added. Vary waveform type, frequency and amplitude (including amplitude = 0), as well as noise level. Vary cut-off frequency of filter.	Compare S/N in time vs frequency domains. Which is a better context in which to evaluate S/N? Note effect of filter on attenuation of frequency components (of both noise and simulated waveform) near cut-off frequency of filter, as well as above and below it.
F-3	Fourier series: square wave (odd harmonics).	Single square wave of various frequencies,	In the frequency-domain plot, identify which

Experiment Number	Focus	Conditions	Purpose
F-4	Fourier series: triangle wave (odd harmonics).	starting with base frequency $f_0 = 200$ Hz. No noise, filter cut-off set to maximum frequency.	harmonic peaks are present (odd vs even). Use the cursor to measure the RMS amplitude of at least the 1st, 3rd, 5th, and 7th harmonics (at $n \cdot f_0$). Compare the <i>relative</i> amplitudes to the expected square-wave scaling ($\sim 1/n$ for odd n). Note and explain any unexpected peaks (e.g., between harmonics or at frequencies not equal to $n \cdot f_0$).
F-5	Fourier series: sawtooth wave (all harmonics).	As above, but with a triangle waveform.	Repeat F-3, but test whether (i) only odd harmonics appear and (ii) the relative amplitudes fall off faster than square/sawtooth (triangle-wave scaling $\sim 1/n^2$ for odd n). Use at least the 1st, 3rd, 5th, and 7th harmonics.
		As above, but with a sawtooth waveform.	Repeat F-3, but determine whether both odd and even harmonics appear. Measure at least the 1st–7th harmonics (including even n) and compare the relative amplitudes to the expected sawtooth scaling ($\sim 1/n$). Explain how sawtooth differs from square even if the falloff scaling is similar.

Experiment Number	Focus	Conditions	Purpose
F-6	Investigation of frequency “aliasing”.	Single square wave of 300 Hz frequency. No noise, vary filter cut-off frequency.	Identify which peaks are aliased and explain why they appear.

Table 3. FFT Sound Check Experiments.

Experiment Number	Focus	Conditions	Purpose
SC-1	Familiarization with controls, displays and audio output.	Variable	Explore the functionality of the controls so that you are comfortable using this “instrument” for the

Experiment Number	Focus	Conditions	Purpose
SC-2	Frequency content of different signal types as perceived graphically and by the audio output	Set the frequency to 800 Hz; enable signal, no filtering and no noise. Adjust the signal maximum such that time domain amplitudes do not exceed the limits of the red dashed lines. Adjust the volume so that you can hear audio signals. Note the qualities of the sound output for the various signal types (e.g. square vs sine, etc.)	Calibrate your sensors (ears) in terms of perception of higher harmonic content of complex waveforms. The frequency spectrum quantitatively represents signal content. How do these tones sound, and what are the consequences of discontinuities in the waveform?
SC-3	Effects of Clipping	Set the (base) frequency to an 800 Hz sine wave; enable signal, no filtering and no noise. Adjust the signal maximum such that time domain amplitudes do not exceed the limits of the red dashed lines. Adjust the volume so that you can hear audio signal. Note the qualities of the sound output as you vary the signal maximum above and below the red line limits. Repeat for other base frequencies.	Examine the effects of clipping as perceived in the frequency content of the audio signal. Visualize what a clipped sine wave looks like. Explain if what you are visually observing is consistent with what you hear. What are your cautionary recommendations regarding the consequences of clipping of any waveform?
SC-4	Audio perception of white noise.	Signal disabled, noise enabled. Adjust signal maximum so that signal is not clipped. Set lower filter to 6 kHz and upper cutoff to 11 kHz.	Characterize white noise and investigate filtering. Compare functionality or topology of filter (low pass vs notch vs high pass, etc.) in terms of visual (frequency domain content) and audio perception of frequency content of filtered and unfiltered noise.
SC-5	Sensitivity of the human ear as a function of frequency	Signal disabled, noise enabled. Adjust signal maximum so that signal is not clipped. Keep this setting constant after	Semiquantitatively characterize the frequency response of your ears. Make sufficient observations to allow you

Experiment Number	Focus	Conditions	Purpose
		adjustment. While keeping the volume constant and with a filter setting of bandpass topology and delta F set to 50 Hz, sweep the lower cutoff throughout its range.	to create a plot of sensitivity of your ears as a function of frequency over the range of 20 – 20 kHz (this should be done in Excel). Repeat observations using an unfiltered sine wave rather than white noise. What are the lowest and highest frequencies you and your partners can hear? You may find it helpful to toggle the noise or signal on and off as you make your observations.
SC-6	Filtering of harmonics using various filter topologies.	Using the more complex waveforms (e.g. square, triangle and/or sawtooth) and various filter settings.	Comment on the effectiveness of filters in removing unwanted harmonics as perceived both visually (frequency spectrum) and aurally. In your discussion, consider the pitch and clearness of the audio (like is there feedback/fuzziness).

6. Report Guidelines

Upon completion of this lab, you will turn in your answers to the questions posed in the tables above along with a short summary. This “lab report” is different than the other lab reports (i.e., we don’t expect a formal write-up).

1. Discuss the results of each experiment specified in Tables 1-3, for each part of this exercise. Almost every row in the tables includes a specific topic/question to address.
2. Summarize what you learned about the following concepts:
 1. Effects of clipping on signal frequency content.
 2. How waveform type changes frequency content as perceived visually and aurally.
 3. Frequency characteristics of white (Gaussian) noise.
 4. What is meant by filter topology for the filters implemented in this program.
 5. Sensitivity of the human ear as a function of frequency.
 6. Effectiveness of the various filter topologies on removing unwanted noise components.

7. References

1. The Scientist and Engineer’s Guide to Digital Signal Processing Second Edition by Steven W. Smith.
<https://www.dspproject.com/pdfbook.htm>

2. NI FFT Tutorial: <http://www.ni.com/swf/presentation/us/fft/>
3. Sound processing and perception: <http://www.faqs.org/docs/sp/index.html>
4. The human ear as a transducer:
http://www.epd.gov.hk/epd/noise_education/web/ENG_EPD_HTML/m1/intro_2.html

Analytical Separation with Gas Chromatography-Thermal Conductivity Detection (GC-TCD)

1. Learning Outcomes

1. Configure gas chromatography (GC) inlet modes and explain how split/splitless settings affect peak shape and detection limits.
 2. Calculate and interpret chromatographic metrics (k' , N, H, R_s , alpha) from experimental data.
 3. Evaluate how flow rate and oven temperature impact retention, efficiency, and resolution in isothermal GC.
 4. Use van Deemter/Golay relationships to justify optimized operating conditions for a multi-component mixture.
 5. Report results with appropriate statistics (replicates, uncertainty, plots with axes/units/error bars).
-

2. Pre-Experiment Reading

- [Chapter 27: Gas Chromatography](#) — GC principles, columns, and detectors (including TCD).
-

3. Goals

1. Determine the impact of different injection settings on the shape of peaks.
 2. Examine the effect of temperature and mobile phase flow rate on retention for a five component mixture of aromatic compounds using isothermal gas chromatography.
 3. Compare your results to theoretical predictions and models of fundamental chromatographic behavior.
-

4. Background and Theory

Gas chromatography is a powerful method that is widely used for analysis of complex mixtures. The major sample requirements are that the sample be volatile and thermally stable at temperatures below about 300 °C. If these requirements are satisfied, GC is the method of choice. Relative to other chromatographic methods, gas chromatography generally has the advantage to short analysis time and high resolving power (when a high resolution capillary column is employed), making it the method of choice for analysis of complex mixtures. In this experiment you will compare experimental results to theoretical predictions regarding retention and efficiency in gas chromatography. Please refer to your instrumental analysis textbook for a general treatment of the theory and application of gas chromatography.

General Relationships

Chromatography is used to perform separations for many purposes such as isolation of synthesis products for a complex mixture or, as in this lab, separation of a complex mixture to identify and quantify components. The goal, therefore, of this separation is to resolve the components into discrete peaks so you can measure their concentrations in the sample, and to do so in a practically reasonable time frame.

Resolution for the peaks corresponding to solutes A and B is defined as the completeness of separation and is quantitatively measured experimentally by the following equation:

$$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_{bl,B} + W_{bl,A}} \quad (\text{eqn 1})$$

where $(t_R)_B$ and $(t_R)_A$ are the retention times measured at the peak maxima and $W_{bl,B}$ and $W_{bl,A}$ are the baseline widths of the peaks. Two peaks are completely separated when $R_s = 1.5$ or more. R_s is unitless; t_R and W must be measured in the same units whether time, distance, or volume of mobile phase.

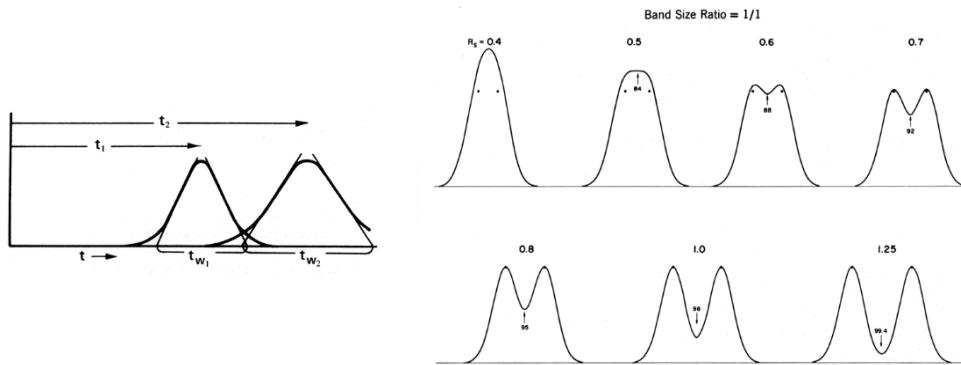


Figure 1. Resolution measures the completeness of separation of two components in a mixture. R_s is calculated using equation 1. At low R_s peaks are barely separated, as R_s increases separation increases.

Another term often used to describe the separating ability of a column is the number of theoretical plates (N). The number of theoretical plates is calculated by the following equation:

$$N = 5.54 \left(\frac{t_R}{w_{1/2}} \right)^2 = 16 \left(\frac{t_R}{W_{bl}} \right)^2 \quad (\text{eqn 2})$$

where t_R is the retention time measured at the peak maximum, $w_{1/2}$ is the width at half-height and W_{bl} is the peak width at baseline.

Additionally, chromatographers use another *experimental* measure of retention called capacity factor (k'). The capacity factor is a measure of retention relative to the system dead time (t_m), and is related to the partition equilibrium constant, K (eqn. 4). The dead time is the time it takes for solute that does not interact with the stationary phase (in this case air) to elute from the column. The capacity factor is calculated by the following equation:

$$k' = \frac{t_R - t_m}{t_m} \quad (\text{eqn 3})$$

The equilibrium constant which governs the thermodynamics of retention is given by a quantity which is directly related to k' according to:

$$K = \frac{k' V_s}{V_m} \quad (\text{eqn 4})$$

Where V_m is the volume of the mobile phase in the column and V_s is the volume of the stationary phase in the column. The expression in terms of time is valid at constant flow rate, and is a convenient and useful method of expressing relative retention. For reasons not addressed here, a value of k' between 2 and 5 generally provides a good compromise of speed and resolution. For complex samples, values of k' between 1 and 20 are generally considered acceptable.

Retention in gas chromatography is controlled by the vapor pressure of the solute in the stationary phase. Increasing the temperature will increase the vapor pressure and therefore decrease retention. For an isothermal separation, one can optimize retention to give the desired range of k' values simply by adjusting the temperature and flow rate.

Capacity factors are used to calculate the selectivity of a separation (α) of two compounds. The selectivity is another measure of how well peak maxima for solutes are separated in time on the chromatogram. It is different from resolution, because selectivity only takes the time separating peak maxima into account. Resolution on the other hand, takes the peak widths into account. If α is large, but the peaks are still very broad, then the two components may still not be completely separated. The selectivity between two peaks is calculated by the following equation:

$$\alpha = \frac{k'_B}{k'_A} = \frac{\frac{t_{R,B} - t_m}{t_m}}{\frac{t_{R,A} - t_m}{t_m}} = \frac{t_{R,B} - t_m}{t_{R,A} - t_m} \quad (\text{eqn 5})$$

Taking N , k' , and α and the resolution equation given above and some algebra we can derive another equation for resolution. This equation is given below:

$$R_s = \frac{\sqrt{N}}{4} (\alpha - 1) \frac{k'}{k' + 1} \quad (\text{eqn 6})$$

As stated, but not explained earlier, the separating ability of a column is related to the number of plates. Equation 6 shows that as the number of plates increases resolution will also increase, the same is true for selectivity and to a smaller extent capacity factor.

Effect of Mobile Phase Flow Rate on Plate Height and Efficiency

Band spreading in a chromatographic column results from several specific physical processes. These processes are described mathematically by the van Deemter equation:

$$H = C_m \bar{u} + \frac{B}{\bar{u}} + C_s \bar{u} \quad (\text{eqn 7})$$

where \bar{u} is the average linear velocity of the mobile phase (cm/sec, not ml/min!). The dependence of H on \bar{u} , shown graphically in Figure 4, yields an optimum velocity \bar{u}_{opt} when H is at a minimum. Although maximum efficiency (and best resolution) is obtained by operating the column at \bar{u}_{opt} , most separations are done somewhat higher velocities in the interest of minimizing the overall separation time, yet conserving the resolution require for a given analysis.

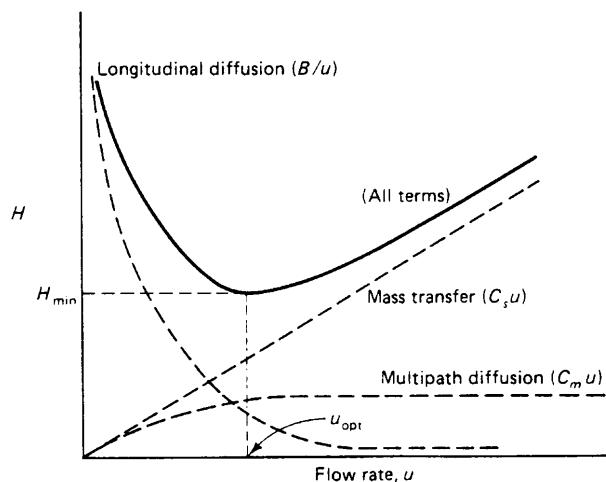


Figure 2. The van Deemter plot, showing the contribution of various terms in eqn 7 to the plate height for a given solute and temperature.

There are a number of variations of the van Deemter equation, depending of the specific column type. For packed columns, there is no simple rigorous treatment for the physical processes that result in solute dispersion, and a number of variations of the van Deemter equation have been proposed. Open-tubular or capillary columns such as those used in this apparatus are amenable to an exact, rigorous treatment, and the plate height is expressed by the Golay equation:

$$H = \frac{2D_m}{u^*} + \frac{1 + 6k' + 11(k')^2}{24(1+k')^2} \frac{r^2 \bar{u}}{D_m} + \frac{k'}{6(1+k')^2} \frac{(d_f)^2}{D_s} \quad (\text{eqn 8})$$

where r is the column radius, d_f is the film thickness, and D_m and D_s are the solute diffusion coefficients in the mobile and stationary phases, respectively. You should note that detailed expressions such as the Golay equation are useful primarily for intelligent column design and optimization of separation conditions. Experimentally, the plate height of a column is calculated from an individual chromatographic peak as described above. The plate height so calculated is sometimes referred to as apparent plate height, and may include contributions from extra-column sources such as the injector, connector tubing, and injector. For a well designed chromatographic system operated under uniform conditions (isothermal conditions and negligible pressure drop), the apparent plate height should be equal to the value predicted by the van Deemter or Golay equation.

The figure below provides graphical support for understanding of the Golay equation:

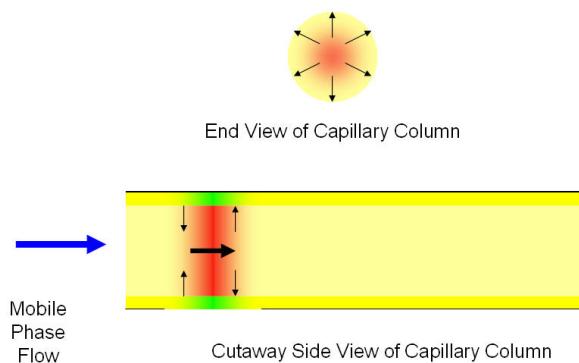


Figure 3. Diagram of analyte diffusion during gas chromatography separation.

This will be discussed when your instructor introduces you to the experiment, along with simulation of this equation to provide an appreciation for how various column parameters affect plat height. You will also use these simulations to compare the data you acquire to theory.

5. Chemicals, Supplies, and Safety

Chemicals

- 50 ml methanol
- 15 ml ~3 mg/mL mixture of ethylbenzene, p-xylene, and toluene, chlorobenzene and bromobenzene in methanol (“Sample A”)
- 15 ml ~10 mg/mL mixture of ethylbenzene, p-xylene, and toluene, chlorobenzene and bromobenzene in methanol (“Sample B”)

Supplies

- GC vials and lids
- Wash vials A and B in the carousel, filled with methanol, and in correct positions
- Pasteur pipettes and bulbs
- GC vial rack
- Waste container, Kimwipes, Sharpie

Safety

- **Flammable/toxic solvents and aromatics (methanol; toluene, ethylbenzene, p-xylene, chlorobenzene, bromobenzene).** Hazards: flammable vapors, skin/eye irritation, and inhalation exposure. Avoid by keeping vials capped, working in the designated area, wearing goggles/gloves, and keeping ignition sources away. If exposure or a spill occurs, alert the TA immediately, ventilate the area, clean small spills with appropriate absorbents, and wash skin/eyes with water; seek medical help for significant exposure.
 - **Hot injector/oven surfaces and pressurized/detector gases.** Hazards: burns from hot metal surfaces and risk from heated gas flows. Avoid by not touching heated zones, using only approved methods to change settings, and allowing cool-down before handling. If a burn occurs, cool the area under running water and notify the TA; if you smell gas or suspect a leak, stop the run if safe to do so and report it immediately.
-

6. Instrumentation

GC-TCD Instrument

An Agilent 7820A GC with Phenomenex ZB-5ms analytical capillary column and thermal conductivity detector (TCD). ChemStation software is used for data acquisition and analysis; some basics of the use of this software are described below. Your TA/Instructor will give you a brief introduction to the GC.

Computer Credentials

- The instructor, TA, or student login with university credentials.

Inlet and Injection modes

The system is equipped with an auto-injector that can be configured to operate in the following modes.

1. *Splitless Flow Mode.* The entire sample (0.5 or 1 μ L) is introduced to the column, thereby improving analyte detection. However, the introduction of a microliter of solvent overloads the column and can interfere with the detection of analytes that elute near the solvent peak when the column temperature remains constant during the separation (isothermal temperature control). This problem can be overcome by appropriate temperature programming. At injection, the column temperature is low. The analytes are retained in a

narrow band at the head of the column while the more volatile solvent passes through the column. After the solvent has eluted, the column temperature is increased slowly and the analytes elute more or less in order of their boiling points.

2. *Split Flow Mode*. Only a small fraction of the sample is introduced onto the column; most of it is split off to the atmosphere. This prevents overloading the column, but generally results in higher detection limits. Overloading is a condition where equilibrium cannot be reached at the stationary – mobile phase boundary because the stationary phase is saturated. Such would be the case if the solubility limit for a given solute in the stationary phase is exceeded. The fraction of each component that goes onto the column may also vary somewhat with molar mass (discrimination).

Regardless of the injector used, the injection of too much sample, either in terms of volume of sample or concentration of analyte can result in artifacts which are manifested in the shape of the peaks obtained. Ideally peaks should have a Gaussian shape. Peak shape artifacts can result for a number of reasons. Two easily controlled sources of these are (i) those resulting from the way in which the sample is introduced onto the head of the column (poor injection technique or inefficient volatilization within the injection port) and (ii) effects due to how the sample interacts with the stationary phase as it moves through the column. In the latter case, too high of a concentration of an analyte will cause non-ideal peak shape.

Injection of too large a volume of sample causes broad, flat topped peaks which compromises resolution. This is a consequence of the lack of efficiency of sample volatilization and introduction onto the head of the column. Even under conditions of good volatilization and introduction, if the concentration of analyte is too high, the solubility limit for that analyte in the stationary phase will be exceeded. Overloading the column will result in “fronted” peaks which appear like that shown in Figure 4B.

Fronting will obviously increase the apparent peak width and will also affect retention time. For proper (artifact free) evaluation of the effects of flow rate and column temperature on retention, efficiency and resolution, overloading of the stationary phase must be avoided to obtain useful results.

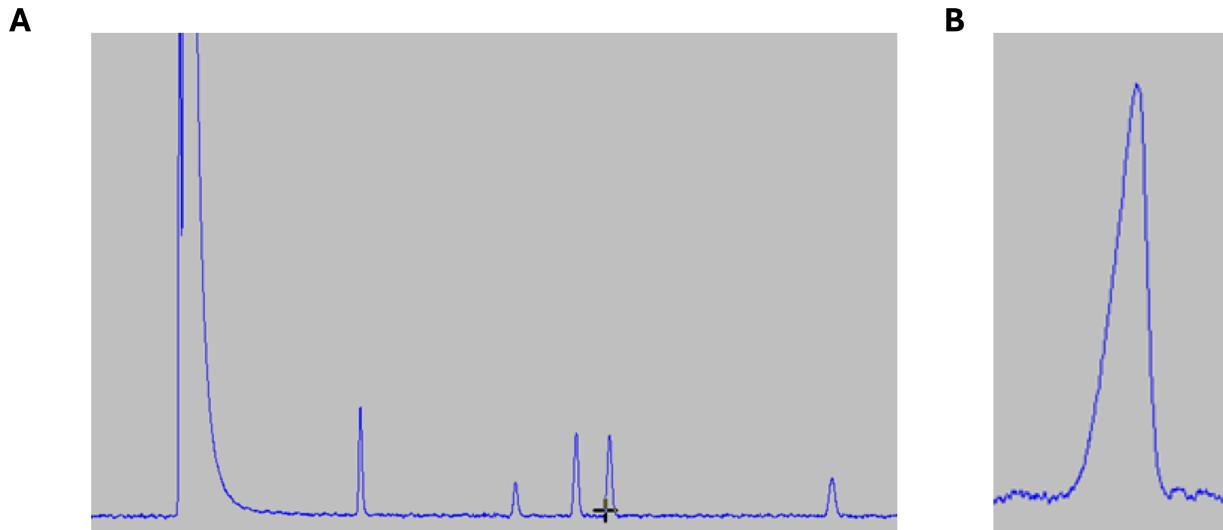


Figure 4. **A** Example of sharp symmetrical peaks and **B** peak fronting.

Column

Phenomenex ZB-5ms capillary column (isothermal $T_{\max} = 325^{\circ}\text{C}$); 10 m long x 0.18 mm internal diameter (ID) x 0.32 μm film thickness. The ZB-5 is an excellent general purpose column with good temperature stability. The non-polar stationary phase is (5%-phenyl)-methyl polysiloxane. Verify this information against the column identification label on the instrument. This non-polar column separates analytes according to their boiling points. As such, columns of this type are often referred to as “boiling point columns”.

Detector

A modulated single-filament thermal conductivity detector (TCD). The analytical and reference (H_2) gases are passed alternately over a tiny, heated filament held in a ceramic detector cell (this is the clicking noise you hear during operation). The temperature of the filament depends on the thermal conductivity of the gas flowing around it. A decrease in thermal conductivity, such as when organic molecules displace some of the carrier gas, causes the filament temperature to rise. This temperature change is sensed as a change in electrical resistance of the filament which varies with temperature change. The TCD has relatively low sensitivity compared to other detectors but has the advantages of being non-specific (responds to both organic and inorganic species) and non-destructive.

Instrument Settings

ChemStation Software

ChemStation® is the software program that is used to control the gas chromatograph and the data acquisition process, and also to perform basic data analysis on the acquired data. All settings for the GC operating parameters, data acquisition rate, and data analysis functions are stored in a user-defined method file. Performing a typical GC analysis involves loading and running the appropriate method file.

To launch software, open “Control Panel” icon from the desktop and then choose “Launch” from the listed GC 7820A instrument. You may get a series of errors, but you should ultimately select “Download to instrument” option so that the instrument opens in standby mode.

General Operating Conditions

Method File Name: 4243H2.M (Note: Consult your TA for the current default method, this may have been changed). This is the default method file that you should load for this analysis. You can change any of the settings as needed. If you wish to save the method after you make changes (e.g., new column temperature), save your modified method under a new file name, beginning all filenames with 4243 so that all method files associated with this course can be easily located. For the experiments to be carried out here, the parameters to be varied are the “initial flow” which can be changed under column parameters, and “oven temperature” which can be changed under “oven” settings in the method.

There are some default settings and your TA will walk you through the software. You will need to adjust the injection parameters, flow rate through the column and the oven temperature as variables in this experiment. Because a previous user may have altered the file, you should examine the file settings after loading the method. Be sure to record method details as you are adjusting settings throughout the experiment.

For the experiments, the split flow (20:1 with 20mL/min) is optimized for 0.5 μ L sample injection volumes. The effect of injection volume will be examined in part 2 at this split flow ratio. Additionally, you’ll explore the effect of needing washing procedure.

Expected Instrumental Responses

You will use two different samples in this set of experiments. The samples consists of five components (analytes) in a methanol solution. The analytes are: toluene, chlorobenzene, ethylbenzene, p-xylene and bromobenzene. Sample A is a mixture with these components present at ~3 mg/mL each. Sample B is a mixture with these components at a concentration of ~10 mg/mL.

A good response for Sample A is shown below (NOTE: this was from a previous version of the software and a different column so it will look different and retention times may vary). The first large peak is the solvent (methanol), followed by smaller analyte peaks eluting in the order given above. Note good separation (baseline resolution) of the components in the sample with good symmetric, Gaussian peak shape is accomplished rapidly (Figure 5A). You should expect symmetric peak shapes for all experiments involving 0.5 μ L injection volumes in this experiment. If you notice tailing peaks please notify the TA. A bad response is shown in Figure 5B - while a very rapid separation is evident, quantitation is likely to be compromised due to poor resolution of peaks.

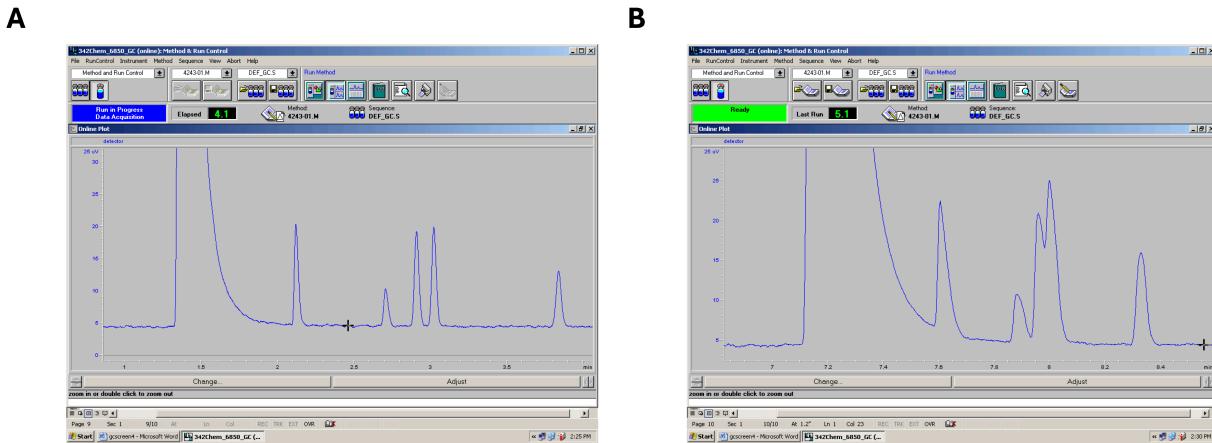


Figure 5. A. Example of good peak shape and resolution. B. Example of poor separation.

7. Experiments to be Performed

There are several sets of experiments to be carried out as detailed below. For the conditions suggested, most runs will be completed in 5-10 minutes or so with maximum run times around 15 minutes. Even so, there are many runs required to acquire all the data needed for this experiment. You must make efficient use of your time.

IMPORTANT: when you set up a sequence, you need to disable the auto-print setting, which is available in the sequence settings menu. If you do not disable this setting, the computer will not automatically run all samples back-to-back: you will have to manually trigger each run, which will prevent you from running samples overnight.

Part 1: Evaluate the reproducibility of the autoinjector and the importance of washes using Sample A.

- Using an oven temperature of 60° C, and a flow rate of 1.0 ml/min, perform at least three analyses of 0.5 uL of the sample (i.e. an injection volume of 0.5uL). Bracket your group of injections with blanks. In other words, blank-sample-sample-sample-blank. You should obtain a chromatogram such as that shown in Figure 4A, with sharp, nearly symmetric peaks for each solute in addition to the air and solvent peaks (which will be tailed).
- Adjust the parameters of the injection procedure to eliminate the pre- and post- needle washes and redo the triplicate injection of the sample. Be sure to start and the series of runs with a blank. So again, blank-sample-sample-sample-blank.

Part 2: Assess effects of injection volume and analyte concentration on peak shape using Samples A and B.

- Using sample A, an oven temperature of 60° C, and a flow rate of 1.0 ml/min, inject samples of 0.5, 0.75 and 1.0 uL of sample A. Be sure to include pre- and post-wash steps within your injection method.
- Repeat the step above using Sample B.

Part 3: Examine effects of flow Rate on retention and plate height using Sample A

- Using sample A, an oven temperature of 60° C, and the flow rates specified below, inject samples of 0.5 uL for this part of the experiment. As you adjust the flow rate (ml/min) also note the calculated average linear velocity, u (cm/s), which appears on the instrument set up screen. You will need this information to construct the van Deemter plots for each solute. Flow rates (ml/min) to be examined: 0.5, 0.7, 1.0, 1.20, 1.40, 1.60, and 1.75 mL/min.

Part 4: Determining effects of oven temperature on resolution using Sample A

- Using a flow rate of 1.0 ml/min and the oven temperatures specified below, inject samples of 0.5 uL to examine this phenomenon. Oven temperatures (isothermal): 50, 55, 60, 65, 70, 75, and 80 °C.

Shutdown Procedure

Load the method called “4243standbyH2.M” when you have finished your experiments for the day. If you are running an overnight sequence, have the final line in your sequence be empty except for the method cell, which should be configured to invoke this standby method.

Waste Disposal

All the waste solutions are to be disposed of in the waste bottle(s) dedicated to this experiment. Clean any glassware or other supplies or materials you used and return it to where you found it. Discard of any pasteur pipettes in the sharps waste container.

Data Export

1. Open offline mode.
2. Select run from left hand side.
3. Using Windows Key + Prnt Scrn Key, screenshot data and save.
4. To record all peak data (retention times, areas, heights, widths, etc), you need to export the values to a text file. Follow these steps:
 - Open the data file from which you wish to export the data. Make sure the peaks are integrated. Click on File, Export File, CSV File.
 - A window should pop up called Write Data to CSV. Make sure the selected data source is Integration Results. Then click the checkbox in the bottom left-hand corner for Write to Clipboard. Then press OK.
 - Make sure all the checkboxes under the Select Contents menu are selected. Then press OK.
 - Right-click on the desktop and create a new text document. Paste your results into that document. Repeat this process so that the document contains all of the peak data for all of your samples.
 - Then save the text document to a USB drive. Use the USB stick to transfer the document to your computer and open it in Excel using the delimiter options to parse the file nicely.

8. Report Guidelines

Your report should consist of a short introduction and methods section, but then primarily be focused on the results and discussion. General “results and discussion” guidelines: Report all results, calculations and plots, along with explanatory prose as guided by the following. Your report should also include an “author contributions” section describing the role each member played in this experiment and the contributions to writing.

Part 1

Statistically evaluate the reproducibility of the autosampler injections with and without the needle washes. Report the mean, standard error, and confidence interval (at 95%) for each of the following: peak height, peak area, retention time and peak width for the third most strongly retained species in the sample. Comment on the results, particularly commenting on the uncertainty for each of the peak parameters (e.g., peak height). Can the results with and without the needle washes be pooled? Explain.

Consider peaks 2-5 in all samples (exclude blanks and excluding the solvent peak). Calculate the total area of those four peaks for each sample. Now, again for each sample, determine the percentage of its total that is contributed by each of peaks 2-5 (For example, in sample 1, peak 2 was 25.6% of the total area of sample 1’s peaks 2-5, in sample 2 peak 3 was 24.8% of the total area of sample 1’s peaks 2-5, etc.). Now, assume that the data from runs with and without washes can be pooled. What is the 95% confidence interval for the percentage contribution of each of peaks 2-5 across all samples?

Now assume that the least strongly retained peak (not the solvent, but analyte peak 1) is an internal standard. For each sample, normalize the areas of peaks 2-5 to the area of peak 1 in that sample. With these normalized areas, repeat the calculations you just did in the previous paragraph to determine the 95% confidence interval for the percentage contribution of each of peaks 2-5 across all samples. Comment on any differences in the 95% confidence

intervals obtain without normalization versus those intervals obtained with normalization. If there are any differences, why might they exist? What sorts of variation might the normalization have corrected for?

Part 2

Discuss your observations with respect to peak shape for 0.5, 0.75 and 1.0 μL injections of the samples. Explain why an ideal peak should be Gaussian shaped? What do your results show? Interpret your observations of change in peak shape with increased injection volume and/or analyte concentration.

Part 3

Using Excel, carry out calculations of k' , N and H for each component of the sample at each flow rate employed. Also compute the velocity of the solvent at each flow rate. Plot H for each analyte in each sample vs that sample's flow velocity (for the solvent, in m/s). Comment on the results relative to what is predicted by the van Deemter equation.

The mechanism or basis of separation for a poly-dimethylsiloxane stationary phase such as that used here is that retention is primarily attributable to the differences in the heat of vaporization of the sample components. Examine (plot) your data for the *lowest flow rate* and fit k' to normal boiling point for the five components. What is the form and equation for the models you tried? Is this consistent with the mechanism stated above?

Assuming that your data support this mechanism, it seems possible that at a given stationary phase temperature, *all of the results* from part 3 can be examined together (*all components and flow rates*). Examine this possibility, and report your results and conclusions.

Part 4

For each temperature used in part 4, examine the effect of temperature on the resolution of peaks 2 vs 3 and 3 vs 4 as a function of column temperature. In the process of doing this, you will need to calculate alpha for these component pairs. Recalling that alpha is a measure of the ratio of partition coefficients (k'), note the magnitude of a required to obtain an “acceptable” separation. Relative to the chromatograms you obtained for this part, are the calculated values of R_s consistent with the rule of thumb that a value of R_s of approximately 1.5 is required to obtain baseline resolution?

Part 5

Make a general recommendation for *optimal conditions to obtain a quantitative analysis* of this sample using the HP-5 column. Consider that the optimization should be based on obtaining baseline resolution of all peaks in the shortest analysis time.

Table of Chromatographic Equations and Relationships.

$u = L/t_M$	u = column length / retention time of mobile phase
$V_M = t_M F$	V_M = volume of mobile phase eluted with the sample
$k' = (t_R - t_M)/t_M$	k' = retention factor = ratio of retention times of solute and mobile phase
$K = \frac{k' V_M}{V_S}$	K = equilibrium constant = ratio of concentrations of solute in solution and in the mobile phase
$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M}$	α = separation factor = ratio of retention factors of two solutes
$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B}$	R_s = resolution = ratio of peak widths at half-height
$N = 16 \left(\frac{t_R}{W} \right)^2$	N = number of theoretical plates = measure of column efficiency
$H = L/N$	H = height equivalent to a theoretical plate = measure of column efficiency
$(t_R)_B = \frac{16R_s^2 H}{u} \left(\frac{\alpha}{\alpha - 1} \right)^2 \frac{(1 + k'_B)^3}{(k'_B)^2}$	

9. References

- Chapters 26 and 27 in Skoog et al.

Quantitative and Qualitative Analysis of Kerosene with Gas Chromatography-Mass Spectrometry (GC-MS)

1. Learning Outcomes

1. Explain electron ionization mass-selective detector (EI-MSD) operation and relate chromatographic retention to physicochemical properties of n-alkanes.
 2. Optimize gas chromatography (GC) temperature programming to achieve target separations and justify parameter choices.
 3. Identify components using retention time trends and mass spectral patterns (parent/daughter ions, intensity ratios).
 4. Quantify components using response factors and calculate mass percentages from TIC data.
 5. Synthesize GC-MS results into a defensible interpretation of kerosene composition.
-

2. Pre-Experiment Reading

- [Chapter 20: Molecular Mass Spectrometry](#) — mass spec fundamentals (ion sources, analyzers, spectra).
-

3. Goals

1. Optimize a GC method for separation of an alkane mixture
 2. Predict the elution of n-alkanes
 3. Evaluate the mass spectra to identify and quantify the unknown compounds in a kerosene sample
-

4. Background and Theory

An electron impact ionization mass spectrometer using a linear quadrupole mass analyzer is a common specialized detector used in conjunction with gas chromatography. The quadrupole is shown in the following figure:

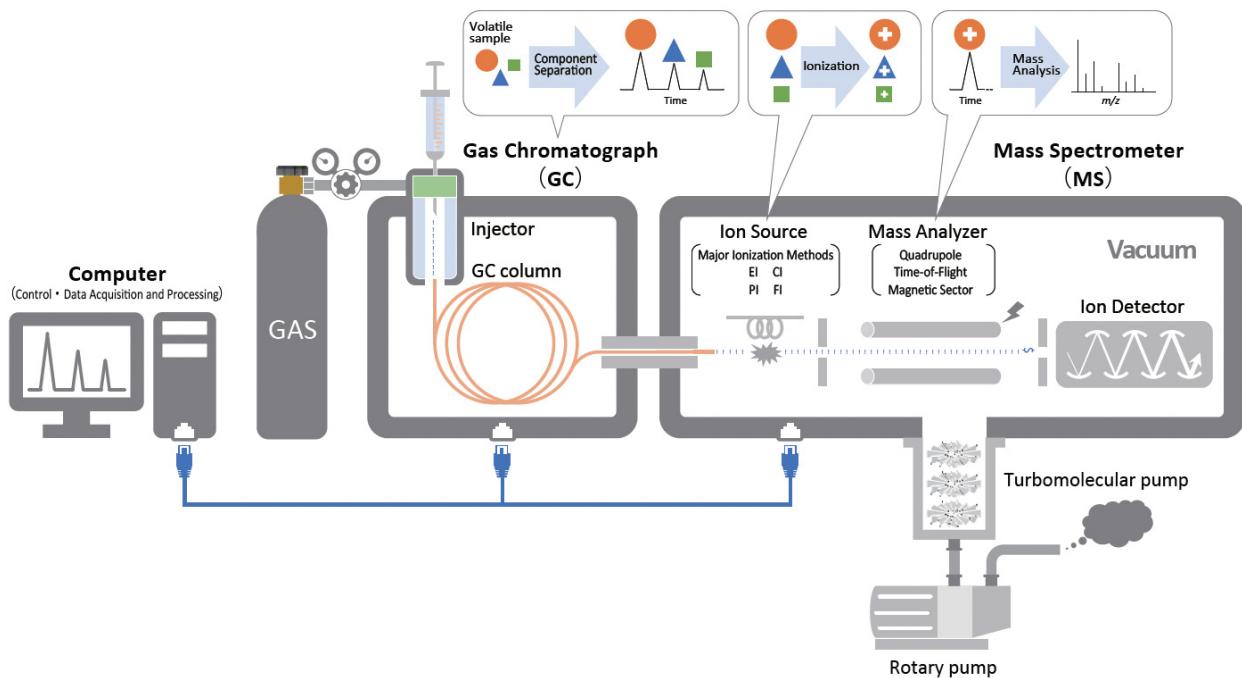


Figure 1: A gas chromatography-mass spectrometry system. Image from www.jeol.com/products/science.

In a GC/MS analysis, the GC generally utilizes a smaller bore open-tubular column and a splitless injector, though ours is a split injector. The column outlet is connected directly to the mass spectrometer ion source. The sample is often injected into the splitless injector as a dilute solution in a volatile solvent, and the GC column oven is operated in the programmed temperature mode.

During the initial, low temperature portion of the run, the injection solvent vaporizes and elutes from the column while the higher boiling sample components are retained at the head of the column. In order to avoid damaging source and detector components, the mass spectrometer is maintained in a standby mode and no data is collected while the relatively large amount of solvent elutes from the column. (Samples should always be diluted in a solvent. Injecting concentrated sample will cause the MS detector to shut down and may cause serious damage.) After essentially all of the solvent has eluted the column temperature is increased steadily, the ion source and detector are activated and the MS detector signal is monitored. As the sample components elute from the column outlet, they are ionized in the source by electron impact and are accelerated through the quadrupole mass filter to the ion detector.

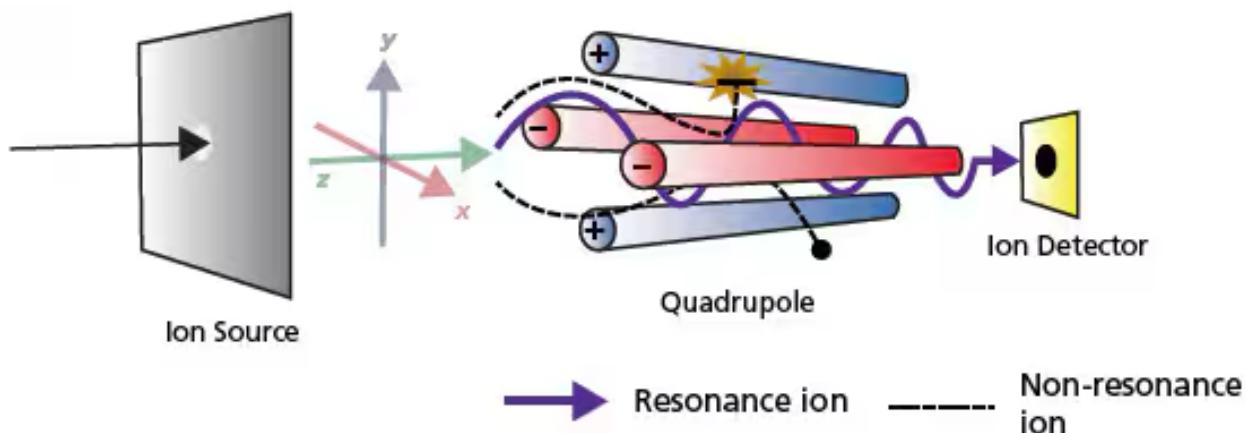


Figure 2: A quadrupole mass filter. Image from www.ssi.shimadzu.com.

When used as a chromatographic detector, the mass spectrometer is often referred to as a mass-selective detector (MSD). The MSD can be operated in several modes and several types of data can be generated. Mass spectral scans are repeated continuously during data collection at a user-determined rate, typically about 10 Hz (ten scans/second). The simplest form of the output in this mode is a total ion chromatogram (TIC), which represents the total ion current (sum of all peak signals), as a function of time. A TIC looks like any other chromatogram produced by a simple one-dimensional GC detector (e.g., flame ionization or thermal conductivity), in which each component has a characteristic retention time and peak areas are related to the quantity injected on the column. However, the data for every MS scan during the run is stored in a file that includes the time each scan was done. It is a simple matter then to examine the mass spectrum of the component(s) eluting at a specific retention time.

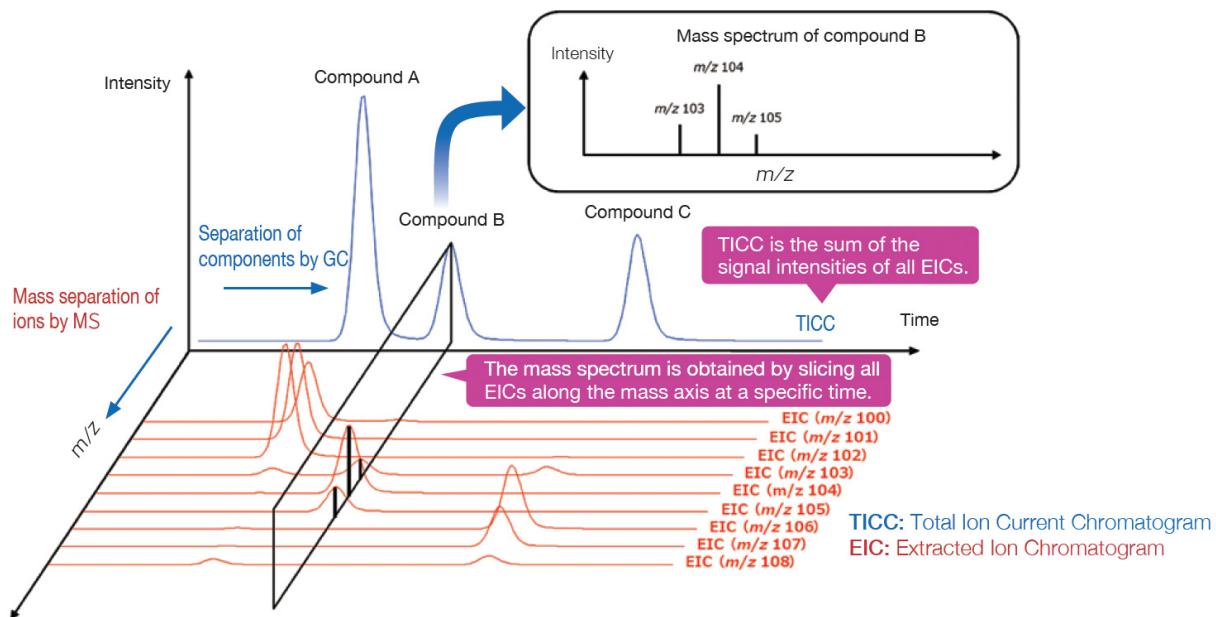


Figure 3: A total ion chromatogram (sometimes called TICC) and an extracted ion chromatogram (EIC), which is related to SIM mode. Image from www.jeol.com/products/science.

The mass spectrum for each peak provides a wealth of additional information which can be used to either identify or confirm the presence of a substance in several ways:

- To determine the identity of an eluting component.
- If the parent peak is present, molecular weight is known.
- The fragmentation pattern can be used to provide structural information. This approach can be especially powerful for advanced users.
- A computerized library search can be performed, based on the mass spectrum, to provide one or more candidate structures. Fragmentation patterns for electron-impact (EI) mass spectra obtained under carefully controlled conditions, usually at 70 eV, are highly reproducible. For this reason, the EI technique lends itself well to computerized library searching.

The following information can be used to confirm the presence of a known component.

- The presence of the parent ion peak.
- The fragmentation pattern. In particular, the intensity ratios of specific daughter ion peaks can be used as supporting evidence. This is especially useful to confirm peak purity (i.e., no co-eluting substances).

In addition to the TIC mode, the MSD can be used for selected-ion monitoring (SIM). In this approach, which is used primarily to determine specific components in a sample, full spectral scans are not performed, but the MSD is set to monitor only specific ions, such as the parent ion and one or more daughter ions. The intensities of these peaks are used for quantitation, and the intensity ratios for confirmation of peak purity. Because more time is spent collecting data on the peaks of interest, this approach can provide significant improvements in signal-to-noise ratio and lower detection limits.

SIM representation of the data acquired in TIC mode is easily accomplished by post run data processing in which the series of mass spectral scans for the entire TIC is searched for a specified range or ranges of m/z, and the intensity of peaks in these ranges are then plotted vs elution time.

5. Chemicals, Supplies, and Safety

Chemicals

- Standard solution of n-alkanes (C₁₁–C₁₄) in isoctane (equal concentrations).
- Kerosene sample (prepared as 1 mg/mL in isoctane).
- Isooctane solvent (run solvent blanks to check purity as needed).

Supplies

- Wash vials A and B in the carousel, filled with isoctane, and in correct positions.
- GC vials and lids
- Pasteur pipettes and bulbs
- Waste container, Kimwipes, Sharpie

Safety

- **Flammable hydrocarbon solvents/samples (isooctane, kerosene, n-alkanes).** Hazards: flammable vapors and inhalation exposure. Avoid by keeping samples capped, minimizing open handling, using the designated ventilated area, and eliminating ignition sources. If a spill or exposure occurs, notify the TA, ventilate, use appropriate absorbents, and wash skin/eyes with water; seek medical help for significant exposure.
- **Hot GC inlet/oven and MS source/vacuum system.** Hazards: burns from heated surfaces and risks from opening instrument compartments during operation. Avoid by not touching heated zones, not opening instrument panels, and allowing full cool-down before any handling by staff. If a burn occurs, cool under running water and inform the TA; if abnormal odors/smoke or instrument alarms occur, stop the run if safe and report immediately.

6. Instrumentation

GC-MS Instrument

An Agilent 7890B gas chromatograph (GC) with HP-5 analytical capillary column and 5977B mass spectrometry detector (MSD). ChemStation Software. Some basics of the use of this software are described below.

Computer Credentials

- Username: N/A
- Password: N/A

Inlet and Injection modes

The system is equipped with an auto-injector that can be configured to operate in the following modes.

Splitless Flow Mode. The entire sample ($0.5 \mu\text{l}$) is introduced to the column, thereby improving analyte detection. However, the introduction of a microliter of solvent overloads the column and can interfere with the detection of analytes that elute near the solvent peak when the column temperature remains constant during the separation (isothermal temperature control). This problem can be overcome by appropriate temperature programming. At injection, the column temperature is low. The analytes are retained in a narrow band at the head of the column while the more volatile solvent passes through the column. After the solvent has eluted, the column temperature is increased slowly and the analytes elute more or less in order of their boiling points.

Split Flow Mode. Only a small fraction of the sample is introduced on the column; most of it is split off to the atmosphere. This prevents overloading the column, but generally results in higher detection limits. Overloading is a condition where equilibrium cannot be reached at the stationary – mobile phase boundary because the stationary phase is saturated. Such would be the case if the solubility limit for a given solute in the stationary phase is exceeded. The fraction of each component that goes onto the column may also vary somewhat with molar mass (discrimination).

Column

HP-5 capillary column (isothermal $T_{\max} = 325^{\circ}\text{C}$); 10 m long x 0.18 mm internal diameter (ID) x $0.32 \mu\text{m}$ film thickness. The HP-5 is an excellent general purpose column with good temperature stability. The non-polar stationary phase is (5%-phenyl)-methyl polysiloxane. Verify this information against the column identification label on the instrument. This non-polar column separates analytes according to their boiling points. As such, columns of this type are often referred to as “boiling point columns.”

Detector

The mass spectrometer (MS) consists of an electron impact ion source, a quadrupole mass filter, and an electron multiplier for detection of the ions. The process for detection of the analytes off the column includes transforming the analytes to ions that are sorted based on the mass/charge (m/z) ratio. As part of the ionization process, molecules can fragment into smaller ions, resulting in a characteristic pattern of ions for each type of molecule. This mass spectrum enables identification of unknown molecules.

Instrument Settings

ChemStation® is the software program that is used to control the gas chromatograph and the data acquisition process, and also to perform basic data analysis on the acquired data. All settings for the gas chromatography-mass spectrometry (GC-MS) operating parameters, data acquisition rate, and data analysis functions are stored in a user-defined method file. Performing a typical GC-MS analysis involves loading and running the appropriate method file.

To launch software, open “HCAMS 318” icon from the desktop.

7. Experiments to be Performed

Your objective in this experiment is to identify each n-alkane present at 5 % or greater (mass basis) in kerosene. Once you have determined the appropriate (optimal) oven programming conditions using the GC (Part 1, below), carry out Part 2, which is replicate analyses of the kerosene sample (at 1 mg/ml in iso-octane solution).

Part 1: GC Programming of a GC Oven for Optimization of the Experimental Run

The first part of this experiment is to optimize a GC separation method using a standard solution of equal concentrations of n-alkane standards ($C_{11} - C_{14}$) in iso-octane solvent at a concentration of 1 mg/ml. For the column this instrument is equipped with, the ideal elution times for the standards are: $C_{11} \sim 8.5$ min, $C_{12} \sim 10.4$ min, $C_{13} \sim 12.1$ min and $C_{14} \sim 13.8$ min. These are the elution times you are striving to reproduce in your initial runs. Your reproduction does not need to be exact, but the closer you are, the easier it will be to analyze your kerosene samples.

To reproduce the retention times specified above, it is suggested that you make a new method that starts with the oven program at 60°C , and commences the ramp after 3 minutes, ending the ramp (and the run) once you are 10°C above the boiling point of the highest boiling point analyte in your standard solution. Begin your experiments with a high ramp rate ($15^{\circ}\text{C}/\text{min}$), and work your way down to one which approximates the retention times mentioned

above. Try at least four different ramp rates and use some basic math to determine which rate, from among your 4+ rates, is the best match for the provided retention times.

Once you find your optimal ramp rate, run several replicate injections of the standard at that rate so you can perform a statistical analysis of the retention times and peak areas. For all experiments, you should use injection volumes of 0.5 μ l or less. Since the mass spectrometer is very sensitive, it is always a wise decision to also run the solvent to check its purity. This minimizes the frustration of interpreting solvent impurity peaks in the total ion chromatogram (TIC) of a sample.

Important notes: - On this version of ChemStation, it is critical that you specify a Data Path in your sequence; otherwise the data are not saved. Please make a folder within the “4243 SpXX” folder that you can find on the desktop and use it as the file path. - Messages about “Computer running out of memory” can be ignored. - To protect the MSD detector, we do not collect mass spec data until after the solvent peak has eluted \sim 1.75 min. Note that almost all of the solvent has eluted after 2.5 min post injection. So, don’t override the solvent delay! This prevents the detector from turning on before the solvent elutes, which protects the detector. We want the solvent delay!

Part 2: Qualitative and Quantitative GC-Mass Spectrometric Analysis of Kerosene

In this part of the experiment you will analyze a sample of kerosene, at concentrations of 1 mg/ml in isooctane. Be sure you run blanks as part of your sequence, and do multiple injections of the kerosene sample so that statistics can be performed.

Waste Disposal

All the waste solutions are to be disposed of in the waste bottle(s) dedicated to this experiment. Clean any glassware or other supplies or materials you used and return it to where you found it. Discard of any pasteur pipettes in the sharps waste container.

Data Export

1. Open “CAMS318 Data Analysis”.
2. Find your data on the left-hand side.
3. Once the desired chromatogram is open, select the “Chromatogram” tab on top.
4. Click “Integrate”.
5. Re-open the “Chromatogram” tab on top and select “Integrate and Label Peak Areas”.
6. Re-open the “Chromatogram” tab on top and select “Integration Results...”
 - This will pull up the area, retention time, and everything into a table.
7. To view MS data, right click to draw a box around the desired peak; the mass spec will pop up underneath the chromatogram.
8. To export data, open the Snipping Tool and screen shot desired data.
9. To export Abundance values per m/z value
 - Select “Spectrum”, then “Tabulate”
 - Select “Print”, then “Microsoft Print to PDF”
 - File contains the method, time interval obtained from chromatogram, and abundance values per m/z.
10. Save under the desired name and copy to a flash drive.

8. Report Guidelines

Your report should consist of a short introduction and methods section, but then primarily be focused on the results and discussion. General “results and discussion” guidelines: Report all results, calculations and plots, along with explanatory prose as guided by the following. Your report should also include an “author contributions” section describing the role each member played in this experiment and the contributions to writing.

Part 1

- Discuss the methodology and process for optimization of the ramp rate when using the alkane standards. Using your optimized runs, provide a total ion chromatogram and produce a table of the retention times and peak areas, with appropriate statistics based on replicate measurements.
- For a typical programmed temperature run of a homologous series, there is a nearly linear relationship between retention time and boiling point (which, in the case of n-alkanes, is highly proportional to molar mass). For the data you collected with your optimized ramp rate, generate a plot and linear equation with which you can examine the relationship between retention time and molar mass. Predict the retention times of neighboring n-alkanes (C_{10} and C_{15}) that might be present in the kerosene sample. Be sure to explain based on GC theory why your results make sense or do not make sense.
- Provide a mass spectrum for each of the four solutes in the standard sample. From the mass spectrum of each component, identify the parent ion peak and two or more daughter ion peaks and use them in the calculation of intensity ratios. Two good daughter ion candidates are the butyl and pentyl fragments which are fairly intense peaks and can provide highly reproducible intensity ratios.

Part 2

- Provide a TIC of the kerosene sample including a table of retention times and peak areas. Use the retention time data (observed and predicted) for the standards ($C_{11} – C_{14}$) you obtained in Part 1 to provide initial identification of the corresponding components ($C_{11} – C_{14}$) in the kerosene sample as a column in your table.
- Examine the mass spectrum for each peak ($C_{11} – C_{14}$) in the kerosene sample and provide confirming evidence for its identification based on the presence of the parent ion peak and daughter ion peaks, as well as the corresponding intensity ratios. The data (observed and predicted) for each component in the standard and kerosene sample, should be placed in a table in a format to facilitate comparison. Among other information for each n-alkane include the formula, the calculated molecular mass, and the observed mass of the parent ion.
- In order to identify the presence of other n-alkanes in the kerosene (including, for example, C_9 , C_{10} , C_{15} , C_{16} , etc.), apply the regression model you created in Part 1 to predict the retention times of additional n-alkanes. For any candidate peaks, compare their retention times against the model and examine the mass spectra for the presence of the appropriate parent ion and daughter ion peaks, as the corresponding intensity ratios. Although you will not have standard data for these components, the information obtained for the standards can be used to make rational arguments about what their identities likely are. As you go, consider the mass spectrum for each n-alkane candidate and indicate in the table whether the fragmentation pattern is consistent with what you expect for a normal alkane, and also note if there are any irregularities.
- Report the full distribution of n-alkanes in the kerosene sample as a percent of the sum of all peak areas in the original kerosene sample. You can ignore any peaks less than 5% the abundance of the most abundant component.
- Finally, consider that kerosene represents a cut from a distillation tower in the range of boiling points centered about that for C_{13} , and that operating conditions for that tower are usually carefully controlled to yield a narrow range of alkanes in the collected kerosene fraction. You might also find it useful to look up the boiling points for $C_9 – C_{15}$ n-alkanes for reference. In your report, be sure to comment on the relative concentrations you observed in the kerosene analysis for the n-alkanes in light of the distillation process which is used to yield the kerosene fraction from crude oil.

9. References

1. Chapters 20, 26, & 27 in Skoog et al.

Separation of Bioactive Heterocyclic Compounds with High Performance Liquid Chromatography (HPLC)

1. Learning Outcomes

1. Develop and evaluate an isocratic HPLC method by adjusting mobile phase composition and flow rate to achieve target resolution.
 2. Interpret chromatograms using retention time, peak shape, and resolution metrics to justify method choices.
 3. Build calibration curves and quantify unknowns with appropriate statistics (replicates, uncertainty, confidence intervals).
-

2. Pre-Experiment Reading

- Chapter 28: High-Performance Liquid Chromatography — HPLC theory and instrumentation.
-

3. Goals

1. Create and evaluate HPLC methods for optimal separation of an aqueous mixture
 2. Quantify unknown concentration with appropriate statistics based on replicate measurements
-

4. Background and Theory

Analytical separation

The goal of any analytical separation method is to provide adequate resolution and quantitation of all analytes of interest within a reasonable time. For determination of small organic molecules in aqueous mixtures, reversed phase liquid chromatography (RPLC) is widely used because of its high resolving power and wide range of applicability. No single set of conditions is suitable for all types of samples, though reverse phase mobile phases are comprised of polar solvents (e.g., water, methanol, acetonitrile) and the stationary phase is non-polar. A particular analysis by RPLC generally requires the identification and optimization of a number of parameters, including column configuration, stationary phase, mobile phase, detection method, sample injection solvent, and calibration method.

Column configuration

For much of the 1990s, the standard analytical column was a 4.6-mm i.d. x 250-mm long stainless steel column, packed with 5- μm porous silica particles with a covalently bonded stationary phase. Modern trends include the use of shorter columns packed with 3- μm or smaller particles to minimize the production of waste while providing shorter analysis times. As the particles get smaller, chromatography theory (e.g. Van Deemter or Golay) shows that band broadening decreases and the number of theoretical plates increases, thus increasing resolving power. While smaller particles yield numerous chromatographic advantages, use of smaller particle sizes was limited until recently because the liquid chromatography (LC) pump technology did not have adequate ability to provide the higher pressures required to push the liquids through the smaller particles. As pump technology has improved, UHPLC (ultra-high performance liquid chromatography), or LCs that can utilize columns with smaller particle diameters, has become the preferred choice.

Stationary phase

A C18 stationary phase, or octadecylsilica, is the most widely used in RPLC, offering satisfactory retention for a wide range of organic solutes. Similar phases offering weaker retention include C8, C4 and C1. Other phases containing polar substituents or ionizable groups extend the application of HPLC to the separation of a wide variety of analytes, though these are not considered to be RPLC. A C18 stationary phase is achieved by bonding the octadecyl-chain to a porous silica particles.

Mobile phase

The success of a reversed phase HPLC separation requires selection of an appropriate combination of stationary phase and mobile phase composition to achieve selective retention. The mobile phase typically consists of water with added organic modifier. Because water is highly polar, most organic molecules of low to moderate polarity are strongly retained on the nonpolar stationary phase if pure water is used as the mobile phase. An organic modifier such as methanol or acetonitrile must be added to elute the solutes from the column. The added organic modifier lowers the polarity of the aqueous mobile phase, making it a better (stronger) solvent for non-ionized organic solutes.

For relatively simple mixtures containing fewer than 6-12 solutes, the strength of the mobile phase is normally adjusted to yield a range of solute retention factors between 1 and 10, or 0.5 to 20 for more difficult separations (see reference chapters for definition of “retention factor”). In many cases, changes in the modifier can affect retention factors of each solute differently, and it can be difficult to find a modifier concentration that yields the optimum combination of speed and resolution. For such cases, optimization software can be very useful. To be useful, the optimization software requires some basic information about the characteristics of the chromatographic system, including the effect of modifier concentration on retention.

Detection

A variety of detectors are used with in conjunction with HPLC. One of the most common detectors is a UV detector, where detection is based on measuring the absorbance of eluting solutes. Most UV detectors have signal outputs for a digital integrator, an analog strip chart recorder, or both. The integrator is most widely used today, with the output scaled to 1 volt for 1 absorbance unit, or other scale. The analog recorder output typically has adjustable scaling and time constant, or rise time. The time constant is adjusted to produce a chromatogram with a baseline that is relatively free from noise, which producing solute peaks that accurately reflect the change in concentration in the detector flow cell with time. A time constant that is too great (long rise time) may result in distorted peaks, causing loss of resolution and unsatisfactory quantitation.

Sample injection

For a classical “standard analytical” column (250 x 4.6 mm ID), a 20- μ L or smaller injection loop is usually used. Larger sample sizes may result in loss of efficiency and resolution due to extra-column band broadening. Smaller diameter columns require the use of smaller injection volumes. For quantitative HPLC using manual syringe injection, the full-loop loading technique is preferred, in which total volume introduced from the sample syringe is at least 5 times the volume of the sample loop. Partial-loop loading is sometimes desirable to avoid undesirable effects on the chromatography due to the injection solvent. It is an alternative in which a volume not exceeding 50% of the capacity of the sample loop is introduced from the syringe. When doing manual injections, partial-loop loading can be less precise, compared to the precision when using modern auto-injectors, which have peak-area precisions around relative standard deviation (RSD) < 0.5%. The amount of solute injected should also be limited to avoid nonlinear detector response. In general, absorbance values around 1 or greater may yield calibration curves with negative deviations from Beer’s law. The sample injection solvent ideally should be the same as the mobile phase. In any case, it is best to avoid using a solvent that is stronger than the mobile phase.

Quantitative Analysis - Calibration Curves

A calibration curve usually employs external standards, in which a series of solutions containing the solutes at known concentrations is prepared and injected using the same conditions as those for the sample.

5. Chemicals, Supplies, and Safety

Chemicals

- **Note to TAs: please start the equilibration process on the HPLC before lab begins.**
- 1 L Nanopure water to be used as mobile phase (should be installed and on top of HPLC system)
- 1 L HPLC grade methanol to be used as mobile phase (should be installed and on top of HPLC system)
- 1 L HPLC grade 60/40 nanopore/methanol for seal wash (should be installed and on top of HPLC system)
- Five 100 ml Individual 0.3 mg/ml stock standard solutions in 95/5 (by volume) water/methanol for resorcinol, nicotinamide, theophylline, caffeine, and uracil
- One 100 ml individual 0.1 mg/ml stock standard solution in 95/5 (by volume) water/methanol for theobromine
- 100 ml six-component stock standard solution in 95/5 (v/v) water/methanol for resorcinol, nicotinamide, theophylline, caffeine, and uracil (all at 0.3 mg/ml), and theobromine at 0.1 mg/ml
- 100 ml six-component unknown solution in 95/5 (by volume) water/methanol for resorcinol, nicotinamide, theophylline, caffeine, uracil, and theobromine (unknown concentrations)

Supplies

- LC vials and caps
- Pasteur pipettes and bulbs
- LC vial rack
- Waste container, Kimwipes, Sharpie

Safety

- **Flammable/irritant solvents (methanol-water mobile phases; standards).** Hazards: flammable vapors and skin/eye irritation. Avoid by keeping containers closed, working in the designated area, and wearing goggles/gloves. If a spill or exposure occurs, alert the TA, absorb and dispose of waste properly, and rinse skin/eyes with water; seek medical help for significant exposure.
- **High-pressure fluid lines and fittings.** Hazards: leaks or line failures can spray solvent under pressure. Avoid by ensuring fittings are secure, not bending lines, and never opening the system while pressurized. If a leak occurs, stop the pump, relieve pressure per TA guidance, and clean up only after the system is safe.

6. Instrumentation

HPLC Instrumentation

1. Dionex HPLC system.
2. Dionex HPLC Pump.
3. Rheodyne 7125 injector with 5 μL loop.
4. HPLC column, 150 x 4.6 mm internal diameter (ID), Phenomenex Gemini C18, 3- μm particles (manufacturer and details may vary; you should record details on column used). This column should be stored in 65/35 acetonitrile (ACN)/water when not in use for more than a few weeks.
5. Linear UV-Visible detector, model UVIS 200 (variable wavelength, adjustable range and rise time, 1 V/absorbance unit (AU) integrator output). Flow cell is 316 stainless steel, 6-mm path length, and 9 μL volume.
6. HPLC column heater, Eppendorf CH-30.
7. Data system: SRI Instruments PeakSimple model 333 single channel chromatography data system with 20-bit high resolution A/D board. USB 2.0 for Windows PC.

Computer Credentials

- Username: chem342
- Password: N/A

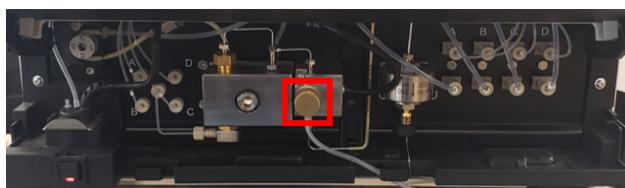
Instrument Settings

Purging the system

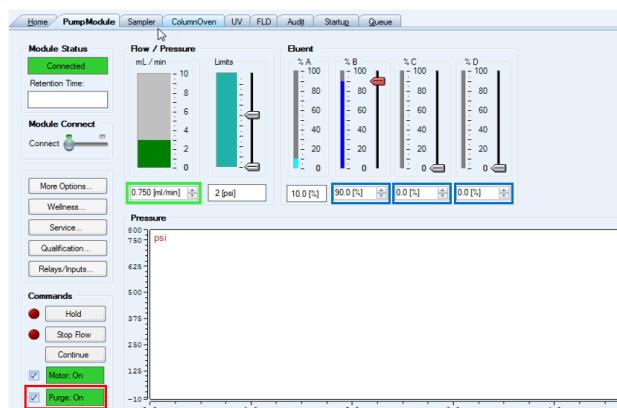
Initially, there may be air bubbles in the lines which draw the solvents from the containers to the column. These bubbles can cause a multitude of problems when running experiments. So, in general, it is wise to remove them prior to the experiment. This is done through a process called purging.

WARNING Do not execute this section without TA or Instructor supervision as the high pressure can ruin the column if done incorrectly.

The first step in the purging process is to slightly loosen the knob which is within the red box seen in the picture below.



This ensures that the column is protected from the high flowrate of the purging process. After, go to the terminal and set the Eluent percentages all to 25% (outlined by the blue boxes) and finally, click the “purge button” (outlined in red) to initiate the purging process.



A final warning message should pop up as a final reminder to ensure that the knob described above is loosened. If it is, hit continue and wait till the purging process is over (approx. five minutes). After the purge is finished, tighten the knob again and proceed. *reminder to only do this with supervision*

Equilibration

Equilibration is a necessary part of any experiment procedure; it is especially important for any work with liquid chromatography. To properly equilibrate the column, the first step is to ensure that any residual analytes are completely off of the column. This is done by first setting the desired flow rate (Green box in picture above). In the case of this experiment the flow rate is **0.5 ml/min**. The second step is to set the eluent percentages to that seen in the table below :

Step	% A	% B	% C and D	Equilibration time (minutes)
1	0	100	0	10
2	50	50	0	10
3	100	0	0	10
4	X	Y	0	10

The step 4 of the table above can be changed dependent on the method you are running. Steps 1-3 of the table above only need to be run during the initial start-up of the instrument. When changing the method, only equilibrate using step 4 with a composition of eluents that matches the desired method. Once equilibration is complete, the instrument is ready to run samples. Log equilibration pressures in the notebook next to the instrument.

Method development

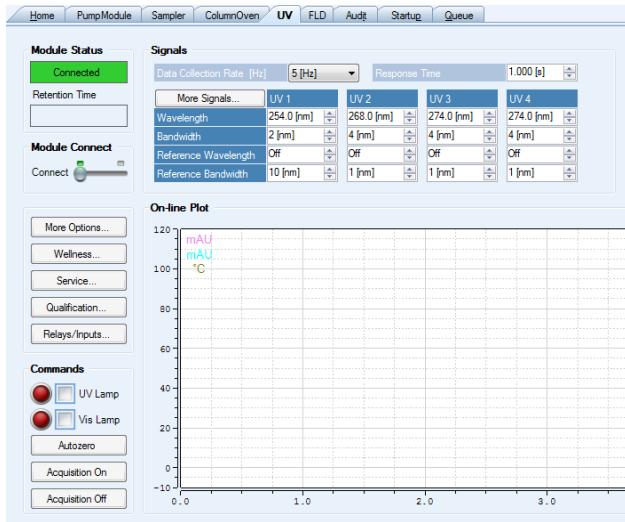
While there are plenty of parameters that can be changed, **this experiment will only focus on the effects of changing the compositions of eluents A and B or changing the flow rate.** Under the “Create” tab of the Chromeleon Console, click the “Instrument method” button. This will pop up a new window titled General settings for system, from here follow the table below.

Window	Guidelines
General Settings for System	Set a 10-minute run time and uncheck the FLD box as there will be no fluorescence measurements taken for this experiment. Leave Diagnostics Channels all selected.
General Settings for Pump	Do not change anything here.
Flow Gradient for Pump	First click the “Remove Equilibration Stage” as this was performed in the previous step. Then set the parameters for your run. Do not use runs with solvents C or D and the flow rate should not exceed 0.6 ml/min. Generally, we will want an isocratic run for simplicity, however if you have time feel free to run a method with a gradient.
General Settings for Sampler	Do not change anything here.
Inject Mode for Sampler	Do not change anything here.
General Setting for ColumnOven	Click the box to enable temperature control and ensure that it is set to 30 °C.
Columns for ColumnOven	Click to Activate Column_A.
MSV Time Program for ColumnOven	Do not change anything here.
Channel Settings for UV	UV-Vis will be the method of detection for these analytes, however, there should not be anything to change here. Just make sure that the channels are set to 254, 268 and 274 nm, bandwidths = 4, reference bandwidths disabled as suggested by the software.

Finally hit finish, go to the upper left-hand corner and save the method to your groups respective folder following the path listed below : Instrument Methods/Chem 4243 Spring XX Methods/Your groups folder (where XX is the current year). Ensure to save the method as something that can be easily referenced (i.e. Group_TA A%-B% X flow). After it is saved this window can be closed.

Turning on the UV-Vis

Go to the UV tab on the main terminal. While here, click the UV Lamp. This can take awhile to turn on, so be patient and only click once. Once on, do the same with the Vis lamp.



Setting up a Sequence

Again, go to the “Create” tab on the console and click “Sequence”. This will pop up a new window. In this tab set your number of vials and starting position (**ensure that you are starting at the correct position and color**). Set up each sample with a “Type” of “Unknown”. Leave injection volumes at 10ul. In the next tab, select the desired method to be run (you can only use one method per sequence) but do not change the processing method. Click finish which will open another tab prompting you to save the sequence, use the following path: Sequences/Chem 4243 Spring XX Sequences/Your groups folder (where XX is the current year). Again, save this sequence as something that can be easily referenced. Once saved, a new window will pop up. In the table, name your samples and again ensure that the starting position is correct. After the final check, save the changes, then press start. You may then need to go back to the window called UltiMate3000_1, then go to Queue, make sure your sequence is queued, make sure the “after running the queue” is set to “run Smart Shutdown” then hit start. You can watch the progress of your run in the UV tab.

Collecting the Data

Once the run is finished, go to the sequence and double click on any of the chromatograms. This will pop up a new window containing the data. Under the “Data Processing” tab of this new window, copy and paste the table into an excel file. For each sample, ensure that all data from every channel of the UV-Vis are gathered. This can be done by double clicking on the different channels in the channels tab. *Make sure to label each data set for the correct channel* A snapshot of each of the chromatograms will also be required. Once all data is collected, save this Excel file and put it on a thumb drive for future analysis.

Shutdown

Once finished with the experiments, the lamps will turn off automatically and the system will cease to pump any volume of liquid. After ensuring that these controls have turned off, you can simply exit from the Chromeleon software (assuming you have collected all the data) and turn off all the compartments of the HPLC, starting at the bottom and working your way up.

Failure to complete proper flushing procedures may result in poor performance or damage to the system. Bonded silica packing materials are susceptible to loss of stationary phase by hydrolysis if left in contact with aqueous mixtures for long periods of time, especially if the pH is less than 3 or greater than 7. In addition, buffer chemicals left in the system may lead to solid deposits which cause pump seals to leak, leading to irreproducible retention times.

7. Experiments to be Performed

Part 1: Method Development and Optimization

- Using the steps outlined above and the mixed standard provided, develop a method that accurately resolves each peak in the given standard. Explore how changing the settings of the method under the “Flow Gradient for Pump” tab impacts the separation. It is recommended that the eluent B stays within 30-70% and the flow rate stays within 0.4-0.6 ml/min. Do not use eluent C or D (i.e., those will remain at 0%). Attempt 3-4 different methods with changing eluent B% while maintaining a constant flow rate of 0.5 ml/min.
- After determining the optimal conditions (i.e., good resolution of all analytes in a reasonable amount of time), observe what effects changing the flow rate has on the chromatogram. Good practice entails having a nanopure water blank before and after every sample injection; however, in the interest of time for this section, just run a nanopure water blank at the end of this section instead of between every single injection.

Part 2: Quantitative Analysis of an Unknown Sample

- Run a set of individual standards for each analyte using the method you developed during Part 1 to determine the exact elution time for all individual analytes.
- While running the individual standards, use the provided *mixed stock solution* that contains all the five to prepare solutions that, once analyzed with your method, will simultaneously generate a calibration curve for each analyte. This calibration curve will be used in the determination of the concentrations of each analyte in a provided unknown sample. The unknown will have the following concentration ranges. Highest possible concentration of analytes in unknown: 0.3 mg/mL Uracil, Nicotinamide, Theophylline, Resorcinol, and Caffeine; 0.1 mg/mL Theobromine. Lowest possible concentration of analytes in unknown: 0.05 mg/mL Uracil, Nicotinamide, Theophylline, Resorcinol, and Caffeine; 0.01 mg/mL Theobromine. Do not use individual standards to make your calibration curve. Use the mixed stock. Ensure that enough replicate measurements are collected to run statistics.
- Measure the unknown solution with the LCMS and determine the concentration of each analyte in the unknown including your measurements’ standard deviations and confidence intervals at 95% confidence.

Waste Disposal

All the waste solutions are to be disposed of in the waste bottle(s) dedicated to this experiment. Clean any glassware or other supplies or materials you used and return it to where you found it. Discard of any pasteur pipettes in the sharps waste container.

Data Export

- Open your finished run.
- Copy and paste data on the bottom of the screen into an Excel spreadsheet.

8. Report Guidelines

Your report should consist of a short introduction and methods section, but then primarily be focused on the results and discussion. General “results and discussion” guidelines: Report all results, calculations and plots, along with explanatory prose as guided by the following. Your report should also include an “author contributions” section describing the role each member played in this experiment and the contributions to writing.

Part 1

Describe the method choices that you employed and the impact of the mobile phase composition on the resolution of the compounds. Discuss based on equilibrium/thermodynamics why the separation of the molecules would change based on the mobile phase composition. Similar to mobile phase composition, describe how flow rate changes

affected the separation. Be specific in your description of the ‘quality’ of the separation and include resolution numbers (see appendix). Additionally, include retention times of each analyte for the optimized conditions.

Part 2

Determine which analyte corresponds to the peak within the chromatogram and describe how you decided. Does this match what would be predicted based on the analyte hydrophobicity (note: $\log K_{ow}$ is a property of the molecule as a measure of molecule hydrophobicity)? Calibrate the instrument for each analyte, including proper statistics for each fit based on replicate measurements. For at least 1 analyte, produce a calibration curve with both peak height and peak area (i.e., 2 calibrations for the one analyte) and discuss which calibration produces the least uncertainty. Determine the concentration of each analyte in the unknown including the confidence interval at 95%. Include a qualitative discussion of the retention times of replicate injections and a quantitative measurement of the precision of the retention times for uracil (or another compound if uracil behaved unexpectedly).

Table of Chromatographic Equations and Relationships.

$$u = L/t_M \quad \text{Retention time ratio}$$

$$V_M = t_M F \quad \text{Volume of elution solvent}$$

$$k' = (t_R - t_M)/t_M \quad \text{Relative retention time}$$

$$K = \frac{k' V_M}{V_s} \quad \text{Distribution coefficient}$$

$$\alpha = \frac{(t_R)_B - t_M}{(t_R)_A - t_M} \quad \text{Separation factor}$$

$$R_s = \frac{2[(t_R)_B - (t_R)_A]}{W_A + W_B} \quad \text{Resolution factor}$$

$$N = 16 \left(\frac{t_R}{W} \right)^2 \quad \text{Number of theoretical plates}$$

$$H = L/N \quad \text{Plate height}$$

$$(t_R)_B = \frac{16R_s^2 H}{u} \left(\frac{\alpha}{\alpha - 1} \right)^2 \frac{(1 + k'_B)^3}{(k'_B)^2} \quad \text{Retention time of B}$$

9. References

- Chapters 26 and 28 in Skoog et al.

Spectrophotometric Determination of Health-related Compounds in Mountain Dew with UV-Visible Spectroscopy (UV-Vis)

1. Learning Outcomes

1. Compare single-beam and double-beam UV-Vis instruments and assess how configuration and cuvette material affect spectra.
 2. Prepare standards, acquire absorbance spectra, and construct calibration curves with appropriate regression and statistics from replicate measurements.
 3. Determine concentrations of benzoic acid and caffeine in unknowns from UV-Vis data with quantified uncertainty.
-

2. Pre-Experiment Reading

- Chapter 13: Introduction to UV/Vis Absorption Spectrometry — basics of UV-Vis measurement.
-

3. Goals

1. Operate a UV-visible spectrometer
 2. Explore the comparison between a double-beam versus single-beam spectrophotometer
 3. Interpret spectra depending on the analytes present
 4. Calculate the concentration of caffeine and benzoic acid in Mountain Dew
-

4. Background and Theory

Quantifying with UV-visible spectroscopy

Remember that UV-visible spectroscopy applies Beer's law for quantifying the absorption:

$$A = \varepsilon bC$$

Where

$$\varepsilon$$

is the molar absorptivity, a wavelength dependent value, b is the path length, and C is concentration. Beer's law also applies to a medium containing more than one kind of absorbing substance. Provided there is no interaction among the various species, the total absorbance for a multicomponent system is given by:

$$A_{total} = A_1 + A_2 + \dots + A_n$$

or

$$A_{total} = \varepsilon_1 b C_1 + \varepsilon_2 b C_2 + \dots + \varepsilon_n b C_n$$

Where the subscripts refer to absorbing components 1, 2,...n. The above equation indicates that the total absorbance of a solution at a given wavelength is equal to the sum of the absorbances of the individual components present. This relationship makes possible the quantitative determination of the individual constituents of a mixture, even if their spectra overlap. If enough spectrometric information is available, all of the components of mixtures can be quantified without separation. For a two-component mixture (compound X and Y) with overlapping absorbances, one could solve for the concentration of each species, [X] and [Y], by measuring the absorbances at two different wavelengths, λ_1 and λ_2 . The problem is mathematically equivalent to having two simultaneous equations with two unknowns.

$$A_1 = \epsilon_{X,1} b C_X + \epsilon_{Y,1} b C_Y$$

(total absorbance at λ_1)

$$A_2 = \epsilon_{X,2} b C_X + \epsilon_{Y,2} b C_Y$$

(total absorbance at λ_2)

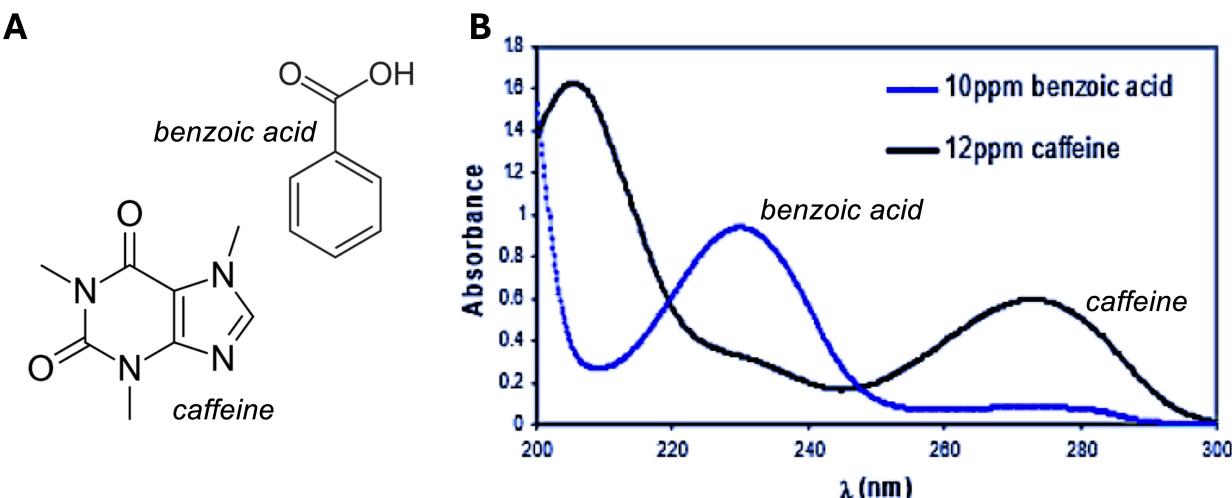
The four molar absorptivities:

$$\epsilon_{X,1}, \epsilon_{Y,1}, \epsilon_{X,2}, \epsilon_{Y,2}$$

can be evaluated from individual standard solutions of X and Y, or better, from the slopes of their Beer's law plots.

Molecules of interest in soda

Caffeine is added as a stimulant and sodium benzoate is a preservative. Benzoic acid was the first chemical preservative allowed in foods by the USA Food and Drug Administration (FDA) and it is widely used in acidic foods (pH 2.5-4). It is normally added as the salt, sodium benzoate, because it is ~200x more soluble in water. (CONSIDER: at pH 2.5, what will be the predominant form, benzoic acid or benzoate?). Although benzoic acid is a human-made food additive, benzoic acid can occur naturally in several fruits (e.g., Scandinavian cloudberry has several times the FDA legal limit!).



Caffeine, 182.2 g/mol; benzoic acid, 122.1 g/mol, pKa = 4.20; UV absorbance of benzoic acid and caffeine in 0.1 M HCl

In this analysis, we shall limit ourselves to non-diet soft drinks because the sugar substitute, aspartame, also absorbs UV radiation that slightly interferes in the analysis. We also avoid darkly colored drinks because the colorants also absorb in the region of interest. In this experiment, we shall analyze Mountain Dew. There will be some UV absorbance from the colorants in these drinks, which will give rise to a small systematic error.

5. Chemicals, Supplies, and Safety

Chemicals

- **Note to TAs:** Remind students to first execute their solution preparation plan, then come to TA/instructor for an instrument overview.
- 4 L 0.1 M HCl
- 1 L 500 mg/L stock solution of caffeine in 0.1 M HCl
- 1 L 200 mg/L stock solution of sodium benzoate in 0.1 M HCl
- 250 ml 30 mg/L calibration solution of sodium benzoate in deionized (DI) water
- 50 ml degassed and filtered Mountain Dew

Supplies

- Pasteur pipettes and bulbs
- Quartz and polystyrene cuvettes
- Waste container, Kimwipes, Sharpie

Safety

- **Corrosive acid solutions (0.1 M HCl) and standards.** Hazards: skin/eye irritation and burns from splashes. Avoid by wearing goggles/gloves, using pipettes carefully, and keeping containers closed. If exposure occurs, rinse skin/eyes with water for at least 15 minutes and notify the TA.
 - **UV light sources and fragile cuvettes.** Hazards: UV exposure and glass/plastic breakage. Avoid by keeping instrument lids closed during scans and handling cuvettes gently. If a cuvette breaks, do not pick up shards by hand; alert the TA and use appropriate cleanup tools.
-

6. Instrumentation

UV-Vis Instruments

1. Cary 60 UV-visible spectrophotometer
2. Cary 5000 UV-visible/near-infrared (NIR) spectrometer

Computer Credentials

Cary 60

- Username: chem4644
- Password: guest

Cary 5000

- Username: admin
- Password: 3000hanover

Instrument Settings

UV-visible spectrometry is an often-employed tool for a wide variety of applications. In this experiment, you will both explore the UV-vis spectrophotometer instrumentation and use the instrumentation to measure two major species in soft drinks. UV-visible spectroscopy is the measurement of the absorption of electromagnetic irradiation in the UV to visible energies. When molecules absorb this energy of light, they are excited from an electronic ground state to an electronic excited state (i.e., this is an electronic transition as opposed to say an excitation to vibrational levels like in infrared (IR) spectroscopy). Because of its wide application, it is important that you understand the capabilities and limitations of this equipment.

There are various configurations to UV spectrometers. In this lab, you will use both a single-beam and a double-beam instrument. Both types of spectrometers use a monochromator to scan through the relevant wavelengths of

light. A single-beam spectrometer directs the path of light through the sample compartment in one path, which is through the sample holder.

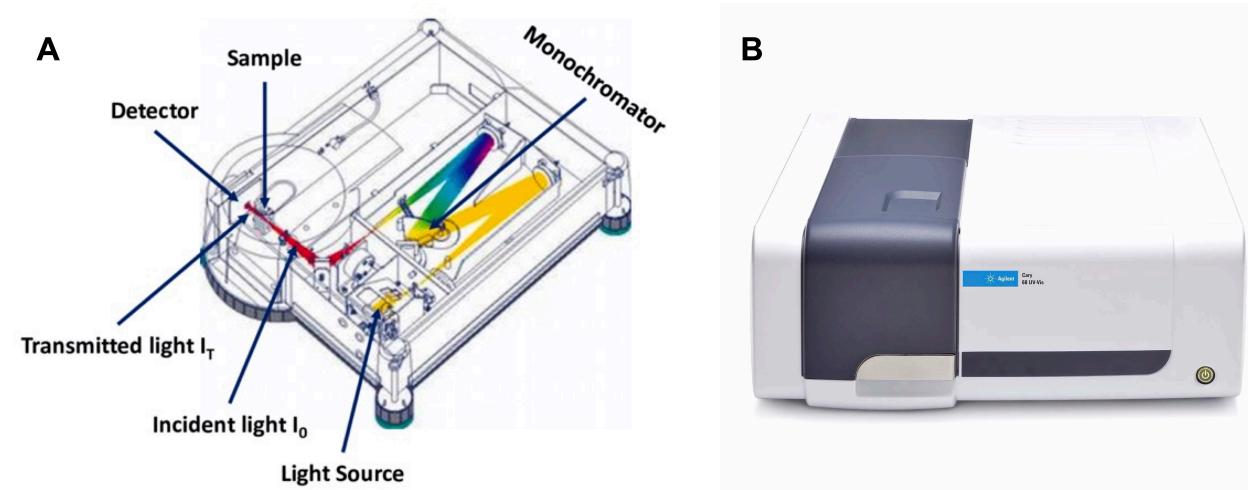


Figure 1. Cary 60, a single-beam UV-vis spectrometer.

In contrast, a double-beam spectrometer splits the light beam to go through 2 separate sample holders. One beam is directed through the solution with the molecule of interest and the other beam is directed through the solvent (the reference). Ideally, the solvent does not absorb UV light (i.e., it is transparent to UV light) but this is not always the case. Additionally, taking measurements through a reference cell also allows for correction for temporal fluctuations in the light source. Besides the configuration of the spectrometer, other sampling considerations are the materials and dimensions of the sample holders. Cuvettes are manufactured with a wide range of materials (borosilicate glass, quartz, polystyrene, etc) and they can have different sizes.

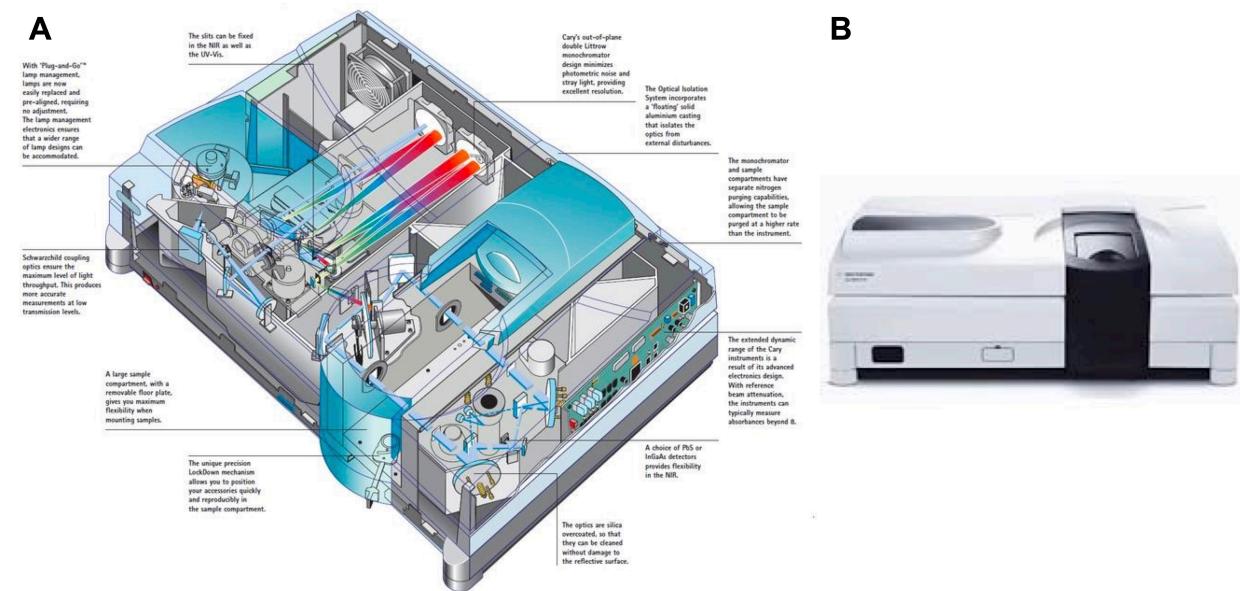


Figure 2. Cary 5000, a double-beam UV-vis spectrophotometer

7. Experiments to be Performed

There are two major parts to this experiment. First, you explore the differences between the single-beam and double-beam UV-visible spectrometers (Part 1). The second portion of this experiment will involve evaluating sample cuvette material performance and quantifying unknown concentrations of benzoic acid and caffeine (Part 2). Note the following for the instruments:

- For Cary 60:
 - Turn on the instrument using the switch/button on the front. You do not need to turn on the power bar that controls the Peltier attachment.
 - You will use the “Scan” application located in the Cary WinUV folder on the computer’s desktop.
 - You need only record the reference spectrum of the solvent you used once prior to running your samples.
 - Specify the following in the Setup menu:
 - Set the wavelength range to scan from 600 to 200.
 - Set the instrument to operate in single beam mode.
 - Toggle ‘scan controls’ to advanced, then set data interval 0.5 nm, averaging time 0.1 s.
- For the Cary 5000:
 - Turn on the instrument using the switch/button on the front. You do not need to turn on the power bar that controls the Peltier attachment.
 - You will use the “Scan” application located in the Cary WinUV folder on the computer’s desktop.
 - The solvent can be placed in the reference cell to automatically incorporate reference correction into your measurements.
 - Specify the following in the Setup > Cary Options menu:
 - Set the wavelength range to scan from 600 to 200.
 - Set the instrument to operate in double beam mode.
 - Toggle ‘scan controls’ to advanced, then set data interval 0.5 nm, averaging time 0.1 s.

Part 1: Comparison of Single-Beam and Double-Beam Spectrophotometry

- *Prepare a set of 10 caffeine standards in the range of 1 to 100 mg/L in a 0.1 M HCl solution. Your TA has made a 500 mg/L stock solution of caffeine in 0.1 M HCl for your dilutions.*
- *On the Cary 60 spectrophotometer and Cary 5000 UV-vis spectrophotometer (if available), measure the absorbance spectra of each standard in enough analytical replicates to be able to perform statistics. Don’t just run a spectrum of the exact same solution, pour a new aliquot for each replicate. You should use the quartz cuvettes here.*
- *You may store the caffeine standards you prepared in Part 1 for use in Part 2 below, if needed.*

Part 2. Measurement and Quantification of Health-related Compounds in Mountain Dew

- *Make a set of calibration standards of sodium benzoate in 0.1 M HCl (5-6 standards in concentration ranges 1-50 mg/L). To help, your TA has made a 200 mg/L stock solution for these dilutions.*
- *Make one calibration solution (30 mg/L) of sodium benzoate in deionized (DI) water. Your TA may have made this solution for you.*
- *Measure cuvette material effects using the Cary 5000 or the Cary 60 by choosing a standard in the middle of the concentration range, then measuring the absorbance spectrum of that standard when it a polystyrene cuvette versus a quartz cuvette is used.*
- *On the Cary 5000 or the Cary 60, measure the absorbance spectra of each standard in enough analytical replicates to be able to perform statistics. Don’t just run a spectrum of the exact same solution, pour a new aliquot for each replicate. Based on your result from the cuvette test above, carefully select which cuvettes to use.*

- *On the Cary 5000 or the Cary 60, measure the absorbance spectrum of the Mountain Dew.* To measure the absorbance of the soda, first confirm that the Mountain Dew has been allowed to have the CO₂ removed and has been filtered for particulate. If this hasn't been done yet, you should warm ~20 mL of soda in a beaker on a hot plate to expel CO₂ and filter. Pipet 2.00 mL of cooled/filtered soda into a 50 mL volumetric flask and dilute to the mark with 0.1 M HCl. Measure enough replicates to be able to perform statistical analysis.

Waste Disposal

All the waste solutions are to be disposed of in the waste bottle(s) dedicated to this experiment. Clean any glassware or other supplies or materials you used and return it to where you found it. Discard of any pasteur pipettes in the sharps waste container.

Data Export

Cary 60

1. Open "Scan" software, adjust parameters, and collect data.
2. When collecting data, save as a .BSW or .DSW.
3. Re-open .BSW or .DSW data and "Save As" a .CSV.

Cary 5000

1. Open "Scan" software.
 2. When collecting data, save as a .BSW or .DSW.
 3. Re-open .BSW or .DSW data and "Save As" a .CSV.
-

8. Report Guidelines

Your report should consist of a short introduction and methods section, but then primarily be focused on the results and discussion. General "results and discussion" guidelines: Report all results, calculations and plots, along with explanatory prose as guided by the following. Your report should also include an "author contributions" section describing the role each member played in this experiment and the contributions to writing.

Part 1

- If you were able to collect measurements on both spectrophotometers, explore and discuss the differences between the two spectrometers.
- For the Cary 60 (and Cary 5000, if you made measurements with that instrument), qualitatively describe the appearance of the spectra and approximate S/N values. Run statistics on your calibration standards from replicate measurements and report the dynamic ranges and limits of detection.

Part 2

- Compare the sodium benzoate calibration standards made in water versus 0.1 M HCl. Discuss the relative importance of the calibration measurements as it relates to unknown quantification.
 - Discuss the influence of cuvette material on the appearance of the spectra.
 - Determine the molar absorptivities for your standards. Considering the absorbance at two different wavelengths, determine the concentrations of benzoic acid and caffeine in the original soda, reported with a 95% confidence interval. Comment on the appearance of the UV-visible spectra of the soda and any spectral characteristics that may contribute to measurement error. Why does this error exist?
-

9. References

1. Chapters 7, 13 & 14 in Skoog et al.
2. Review of Quant concepts here: http://dpuadweb.depauw.edu/harvey_web/eTextProject/version_2.1.html

Assessing Diagnostic and Analytical Reagents Used in Biomedical Science with Voltammetry

1. Learning Outcomes

1. Operate the potentiostat to collect linear sweep and cyclic voltammograms for the ferri/ferrocyanide system.
 2. Evaluate how scan rate, electrode material, and concentration affect peak currents and potentials.
 3. Use voltammetric data to estimate key parameters (e.g., formal potential, diffusion relationships) with appropriate plots.
-

2. Pre-Experiment Reading

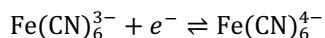
- Chapter 25: Voltammetry — electrochemical voltammetric methods.
-

3. Goals

1. Perform linear sweep voltammetry and cyclic voltammetry on a ferricyanide solution
 2. Calculate various parameters of the electrochemical system
-

4. Background and Theory

There are several parts to this experiment, but in general, this is an opportunity to learn about voltammetry and the Gamry Potentiostat and related software. There are many ways to employ electrochemistry to learn about chemical and biochemical systems. Electrochemistry, voltammetry in particular, is used extensively. Voltammetry comprises a group of electro-analytical methods in which information about the analyte is obtained by measuring current as a function of applied potential. Depending on the pattern by which the voltage is applied (i.e., the waveform), the voltammetry experiment is known by different names. In this week's experiment, you will explore linear sweep voltammetry, and more specifically cyclic voltammetry, on a one-electron ferricyanide model system at a Pt or glassy carbon working electrode:



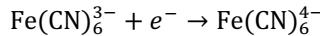
For the above, Standard Reduction Potential = 0.361 V vs SHE. Cyclic voltammetry (CV) is a versatile electroanalytical technique for the study of electroactive species. CV monitors redox behavior of chemical species within a wide potential range. The current at the working electrode is monitored as a triangular excitation potential is applied to the electrode. The resulting voltammogram can be analyzed to obtain fundamental information regarding the redox reaction. Cyclic voltammograms are the electrochemical equivalent to the spectra in optical spectroscopy.

The potential at the working electrode (WE) is controlled vs a reference electrode (RE), a Ag/AgCl(s)/(satd. KCl) electrode. The controlling potential that is applied across the working electrode and the auxiliary electrode is the excitation signal. The excitation signal is varied linearly with time; first scan positively (-100 mV vs. RE to 500 mV vs. RE). Then the potential is scanned in reverse, causing a negative scan back to the original potential to complete the cycle. Single or multiple cycles can be used on the same surface. A cyclic voltammogram is the plot of the response current at the working electrode to the applied excitation potential.

As the potential is scanned positively (forward scan, here) and is sufficiently positive to oxidize $\text{Fe}(\text{CN})_6^{4-}$, the anodic current is due to the electrode process:



The electrode acts as an oxidant and the oxidation current increases to a peak. The concentration of $\text{Fe}(\text{CN})_6^{4-}$ at the electrode surface depletes and the current then decays. As the scan direction is switched to negative, for the reverse scan the potential is still sufficiently positive to oxidize $\text{Fe}(\text{CN})_6^{4-}$, so anodic current continues even though the potential is now scanning in the negative direction. When the electrode becomes a sufficiently strong reductant, $\text{Fe}(\text{CN})_6^{3-}$, which has been forming adjacent to the electrode surface, will be reduced by the electrode process:



resulting in a cathodic current which peaks and then decays as $\text{Fe}(\text{CN})_6^{3-}$ in the solution adjacent to the electrode is consumed. In the forward scan $\text{Fe}(\text{CN})_6^{3-}$ is electrochemically generated from $\text{Fe}(\text{CN})_6^{4-}$ (anodic process) and in the reverse scan this $\text{Fe}(\text{CN})_6^{3-}$ is reduced back to $\text{Fe}(\text{CN})_6^{4-}$ (cathodic process). The quantities of note in a CV plot are the anodic peak current (i_{pa}), cathodic peak current (i_{pc}), anodic peak potential (E_{pa}), and cathodic peak potential (E_{pc}). Measuring i_p does involve the extrapolation of the baseline current (the dotted lines in the diagram below):

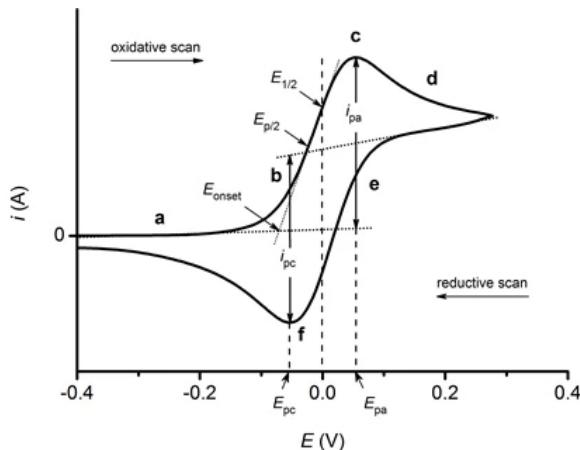


Figure 1. Example Voltammogram.

The peak potential separation, ($E_p = E_{pa} - E_{pc}$), is a key diagnostic for reversibility. For a reversible one-electron couple at 25($^\circ$ C), (E_p) is expected to be near 59 mV; in this lab, values in the (50)-80 mV range are typically considered reasonably close. Peak currents should also be similar, with ($|i_{pa}|/|i_{pc}|$) near 1 (use 0.8-1.2 as a practical threshold). For quasi-reversible or irreversible behavior, electron transfer is slower, so (E_p) increases (often with scan rate) and the anodic/cathodic peak symmetry is reduced.

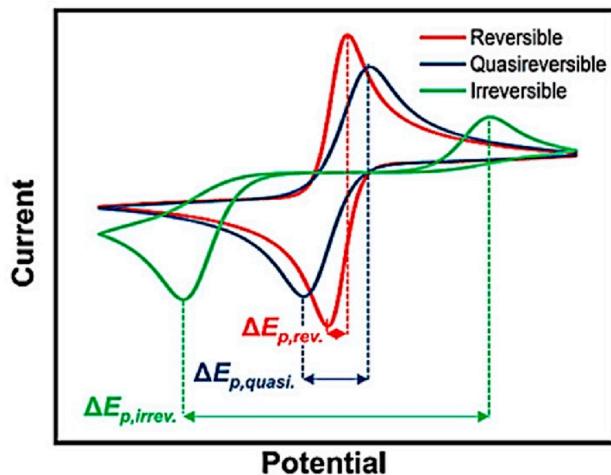


Figure 2. Reversibility in voltammetry.

Redox couples in which half reactions rapidly exchange electrons at the working electrode are said to be electrochemically reversible. The formal reduction potential $E^{\circ'}$ (different from E° , strictly speaking) for such a reversible couple is the mean of E_{pa} and E_{pc} , and the i_{pa} and i_{pc} are very close in magnitude.

$$E^{\circ'} = \frac{E_{pa} + E_{pc}}{2}$$

The number of electrons involved in the redox reaction for a reversible couple is related to the difference of peak potentials by:

$$E_{pa} - E_{pc} = \frac{59 mV}{n}$$

For slow electron transfers at the electrode surface, i.e. irreversible processes, the difference of peak potentials widens. The peak current in reversible systems is given by the Randles-Sevcik equation,

$$i_p = 2.69 \times 10^8 n^{3/2} A D^{1/2} v^{1/2} C$$

where i_p = peak current (in A); n = # electrons involved; A = electrode area (in m^2); D = diffusion coefficient, m^2/s ; C = concentration, mol/L and v = scan rate, V/s.

For a given redox couple at fixed concentration and scan rate, this relation shows that peak current scales with electrode area ($(i_p A)$) and with the square root of diffusion coefficient ($(i_p D^{1/2})$). Therefore, when comparing different working electrodes, raw peak currents should be area-normalized (for example, by comparing (i_p/A) , current density). The diffusion coefficient is a property of the species in solution and should be similar regardless of whether Pt or glassy carbon is used; large differences in calculated (D) often indicate non-ideal behavior (e.g., slower kinetics, surface fouling, uncompensated resistance, or baseline/peak-picking error).

Thus, i_p increases with square root of v and is directly proportional to concentration of the species. The values of i_{pa} and i_{pc} are very similar for a one-step reversible couple leading to their ratio to be unity. Ratio of peak currents may differ from unity if the reactions coupled to other electrode processes.

5. Chemicals, Supplies, and Safety

Chemicals

- **Note to TAs: Use polishing pad and 0.05-μm alumina slurry to polish the working electrodes**
- 1 L nanopore water and nanopure spray bottle with which to clean glassware to minimize contamination
- Solid potassium ferricyanide crystals
- 100 ml 3.0 M KCl for storing the Ag/AgCl reference electrode
- 1 L 1.0 M KNO₃ supporting electrolyte solution

Supplies

- Ag/AgCl Reference Electrode (attach to white lead on potentiostat)
- Pt working electrode, diameter 1.6 mm (attach to blue/green lead on potentiostat when directed)
- Glassy carbon working electrode, diameter 3.0 mm (attach to blue/green lead on potentiostat when directed)
- Pt auxiliary electrode (attach to red/orange lead on potentiostat)
- Electrode polishing equipment (alumina and pad)
- Waste container, Kimwipes, Sharpie
- Small stir bar
- Scoopula

Safety

- **Ferricyanide solutions and salts.** Hazards: toxic if ingested and should not be mixed with strong acids (cyanide gas risk). Avoid by wearing gloves, keeping solutions away from acids, and disposing of waste properly. If exposure occurs, rinse skin/eyes with water and notify the TA immediately.
 - **Electrical equipment and electrodes in liquids.** Hazards: shock risk or equipment damage from wet connectors or improper lead handling. Avoid by keeping leads dry, following startup/shutdown steps, and not touching electrode leads during runs. If a spill occurs near the instrument, stop the run if safe, power down under TA guidance, and clean up once the area is safe.
-

6. Instrumentation

Voltammetry Instrument

- Gamry Interface 1000 Potentiostat
- Gamry Framework Software
- Echem Analyst

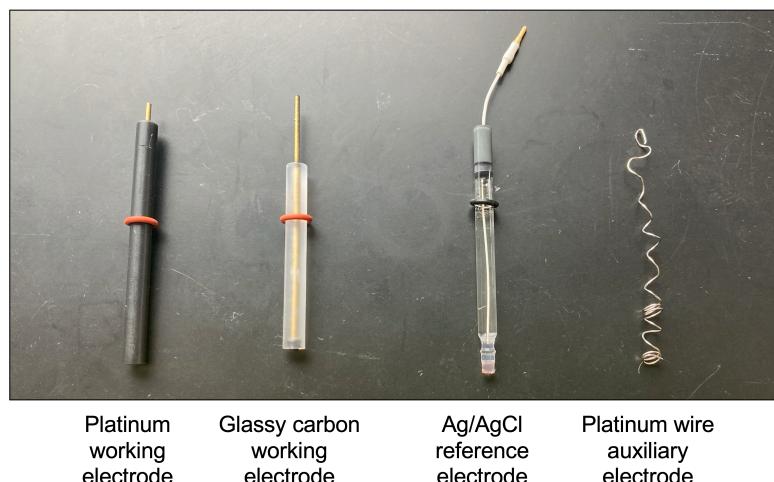


Figure 3. Electrodes used in this experiment.



Figure 4. Voltammetry set up in this experiment.

Instrument Settings

Computer Credentials

- Username: N/A
- Password: N/A

Startup Procedure

- Turn on the potentiostat with the black button on the front.
- Turn on the computer and launch the Gamry Framework software, which will operate the electrochemical system.

Preparation and Use of the Pt Disk or GC Electrode

[Note: your TA or your instructor will complete this step]

In the procedures that follow you will use a solid working electrode that consists of a platinum disk embedded in a Kel-F sheath. Because contaminating deposits will form on solid electrode surfaces with use, the electrode should be cleaned and polished prior to conducting the experiments by polishing with a fine abrasive. The polishing procedure entails using a polishing pad and a slurry of 0.05- μm alumina for this purpose. The pad is stored in a dust-free container and must be kept clean to avoid damaging the electrode. Wet the pad with distilled water and add a drop or two of the alumina slurry. The electrode is held perpendicular to the pad and moved in small circles while applying gentle pressure for about 10-15 seconds. It is then rinsed with distilled water.

After cleaning, the electrode can be used for a series of experiments. If degradation in the response is suspected, obtain a voltammogram using the same test solution that was used for the freshly polished electrode. If a significant decrease in current is observed, the electrode should be polished again. Avoid excessive polishing.

Shutdown Procedure

When you are finished using the system:

- Close the Gamry Framework software application.
 - Disconnect the electrode leads.
 - Rinse the electrodes with distilled water and place them in their storage containers. Store the reference electrode in a vial of 3 M KCl.
 - Turn off the potentiostat.
-

7. Experiments to be Performed

- Prepare 50ml of 2 mM potassium ferricyanide with 1.0 M KNO₃ supporting electrolyte. That means that you need to weigh out the right amount of potassium ferricyanide, then dissolve it in 1.0 M KNO₃. Aqueous solutions of potassium ferricyanide should be prepared fresh daily, as they tend to hydrolyze with time. Electrochemistry is also sensitive to contaminants in solution so be sure all glassware you use is particularly cleaned out and that you are using doubly-distilled water (nanopure).

Part 1: Exploring Scan Rate

- Fill the voltammetry vessel with your solution and place it on the stir plate, then add the stir bar. Cover it with the lid.
- Place electrodes, starting with the Pt working electrode, then the reference electrode, then the auxiliary electrode, in the sample solution and connect them to the potentiostat as described in the Supplies section, above. Prevent the stir bar from hitting the electrodes.
- Prior to measurements, stir the solution, though your measurements will be taken in a ‘still,’ or unstirred solution.
- In Gamry Framework, choose the ‘Experiment’ menu and within the physical electrochemistry tab, you will be using the cyclic voltammetry option. Specify what you want the name of the output file to be. The electrode area is: see Supplies section for electrode-specific diameter; max current: 0.3; equil. time: 5; expected max: 10; electrode setup: ON. Use the following cyclic voltammetry parameters: Initial E (V): 0; Scan Limit 1 E (V): 0.600; Final E (V): 0; Scan rate (mV/s): 100 – at least initially; Cycle: 1; Step size: 0.2 mV. electrode type = solid.
- Use the same solution to obtain cyclic voltammograms at the following scan rates (v): 20, 50, 75, 100, 125, 150, 175, and 200 mV/s. Make sure to collect triplicate scans for each scan rate. Between each scan, stir the solution in the cell to restore initial conditions and allow the system to acquire quiescence before applying the excitation potential scan.

Part 2. Effects of Electrode Composition

- Switch the working electrode from Pt to the glassy carbon electrode. Using the same solution as in Part 1, collect triplicate voltammograms at 100 mV/s, making sure to stir the solution between each scan.

Part 3. Effects of Concentration

- Switch back to the Pt working electrode again. Obtain voltammograms for solutions of different concentrations of K₃Fe(CN)₆ in 1.0 M KNO₃. You should make three new solutions, each with a different concentration, derived from the 2 mM solution you’ve run in the previous parts (i.e. use the 2 mM solution as a stock). Collect triplicate voltammograms at 100 mV/s, making sure to stir the solution between each scan.

Waste Disposal

All the waste solutions are to be disposed of in the waste bottle dedicated to this experiment. Using nanopure water, rinse all electrodes off into a beaker, then transfer the waste to the waste bottle. Clean any glassware or other supplies or materials you used and return it to where you found it. Discard of any pasteur pipettes in the sharps waste container.

Data Export and Analysis

You need to analyze your data in the program Echem Analyst. Make sure to save all your scans with unique data identifiers. To open scans, choose File > Open and choose the data you'd like to process. From there, a new menu called "cyclic voltammetry" should come up and from there, you can fit your data for the appropriate parameters.

Finding Min/Max

1. Click "Cyclic Voltammetry".
2. Click "Min/Max".
3. Write down or put into Excel min and max.

Pull off as .CSV

1. Click "DataViewer".
 2. Click "Copy to Clipboard".
 3. Select "As Text (Data Only)".
 4. Paste into Excel.
-

8. Report Guidelines

Your report should consist of a short introduction and methods section, but then primarily be focused on the results and discussion. General "results and discussion" guidelines: Report all results, calculations and plots, along with explanatory prose as guided by the following. Your report should also include an "author contributions" section describing the role each member played in this experiment and the contributions to writing.

Part 1

First, include and describe the observed voltammograms, explaining the various features of the voltammogram. Plot i_{pc} vs $v^{1/2}$ and i_{pa} vs $v^{1/2}$. Comment on whether these trends make sense based on theory. Plot E_p values vs v and comment on the reversibility of the redox reactions. Verify the formal standard electrode potential of the Fe(II)/Fe(III) systems and number of electrons involved in the half reaction, n , from appropriate plots. Determine the diffusion coefficient ratio of the two species. Comment on these results.

Part 2

Compare the parameters of the voltammograms collected with different WE, which includes discussion about why differences are observed. Run appropriate statistics on replicate measurements to determine if the data collected are statistically the same or different. Extend this comparison to diffusion coefficients calculated using the data from each electrode. NOTE: Be sure to account for the electrode area differences in the comparisons in this section.

Part 3

Compare the voltammograms that were collected of different concentration solutions. Plot the i_{pc} and i_{pa} versus concentration, considering the error. Does the data correspond to the predicted behavior? Compare these data to those obtained for CV at a stationary electrode.

9. References

1. 22 and 25 in Skoog et al.

Quantifying Bioactive Compounds with Quantitative Fluorescence Spectrophotometry

1. Learning Outcomes

1. Configure spectrofluorimeter settings (slits, photomultiplier tube (PMT) voltage, smoothing, averaging) and describe their effects on signal and resolution.
 2. Acquire excitation/emission spectra and quantify quinine using a calibration curve with appropriate statistics from replicate measurements.
 3. Evaluate quenching effects (e.g., potassium iodide (KI)) and discuss implications for quantitative fluorescence measurements.
-

2. Pre-Experiment Reading

- [Chapter 15: Molecular Luminescence](#) — includes fluorescence phenomena.
-

3. Goals

1. Adjust parameters of the spectrofluorimeter and characterize and quantify how changing the parameters affects the fluorescence spectra
 2. Characterize and quantify the fluorescence of quinine, including constructing a calibration curve and examining the role of quenching to its effect on quantitative results.
 3. Determine the concentration of quinine in commercial tonic waters.
-

4. Background and Theory

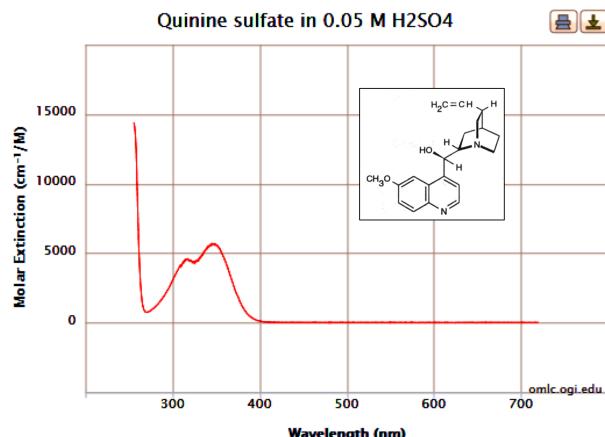
Quinine

The use of quinine in carbonated mineral water began in the mid 1800s for combating malaria. This practice is still continued today, though not for its anti-malarial action, but as a beverage for the slightly bitter taste it imparts to the palate. Quinine is an alkaloid occurring naturally in the bark of trees or shrubs of the various species of two Rubiaceous genera, *Cinchona* and *Remijia*, indigenous to the higher eastern slopes of the tropical Andes in South America¹. The medicinal properties of *Cinchona* bark were first recognized in the seventeenth century and until 1942, the sole source of quinine, a drug which was for years the only existing specific anti-malarial remedy. In recent years however, another alkaloid quinidine, the stereoisomer of quinine, has been used in the treatment of heart conditions². In this experiment, the technique of fluorescence spectroscopy will be utilized to determine the percentage quinine content in commercial samples of tonic water/bitter lemon³.

¹ Fluorescence and Phosphorescence Spectroscopy: Physiochemical

² Qualitative Analytical Chemistry, Schenk, G., Hahn,R., Hartkopf,

³ Chemistry of the Alkaloids, Pelletier, S.W., Nostrand Reinhold



UV-vis absorbance spectrum and chemical structure of quinine. Spectrum is from the sulfate salt of quinine.

Fluorescence fundamentals

The absorption of UV or visible light resulting in the excitation of an electron from a ground to an excited state is the first step in molecular fluorescence. As such, the absorption spectrum in absorbance spectroscopy and the excitation spectrum in fluorescence spectroscopy normally have the same wavelength dependence. This is a very fast process (ca. 10^{-17} s). If the excited state is sufficiently stable to last for about 10^{-9} seconds, the excited molecule will undergo a number of collisions, and some of its energy will be dissipated as heat. The energy of the electron after collisions will be somewhat lower than that achieved on excitation, and when the molecule relaxes to the ground electronic state by emitting light (fluorescing), the light given off will be of lower energy, or longer wavelength. Most stable, fluorescing molecules have singlet ground states, that is, their electrons are all spin-paired. The rules of quantum mechanics say that the only allowed absorption transitions for such molecules are to singlet excited states. In large, planar, conjugated molecules, the lifetime of the excited singlet state may be sufficiently long to allow almost all the excited molecules to lose energy in collisions, and decay to the bottoms of their excited state energy wells. As a result, the emitted (fluorescent) light may be shifted as much as 50 to 100 nm toward the red from the exciting wavelength. This is often referred to as the Stokes effect. To make matters more interesting, some compounds in the excited state may undergo singlet to triplet state transitions which leave a molecule in a metastable excited state, one from which relaxation occurs more slowly. Because the relaxation from these triplet excited states to the singlet ground states is forbidden, requiring the electron to flip its spin, this delayed luminescence signal persists, and is called phosphorescence. These processes are summarized in the diagram below.

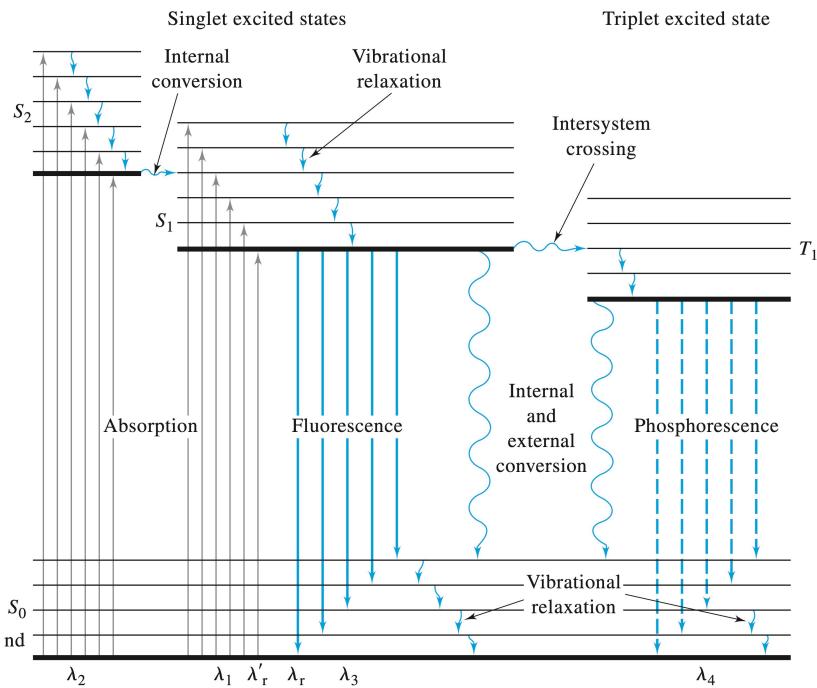


Figure 1. State Diagram for a Molecular Species

Another way of thinking about the relationships between the energy states involved in molecular luminescence is to think of the absorption process as a means of “preparing” excited states from which we may observe luminescence (fluorescence and phosphorescence). The relationship between the absorption, fluorescence and phosphorescence spectra for a hypothetical molecule in solution are shown below. You can rationalize why they appear the way they do in reference to the state diagram shown above.

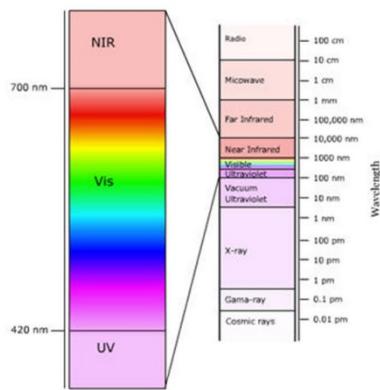


Figure 2. The UV-vis-NIR region of the electromagnetic spectrum (left) and comparison of absorption, fluorescent emission and phosphorescent emission spectra (right)

Because absorption of a photon must precede emission, emission spectra depend on the wavelength of excitation used for both fluorescence and phosphorescence. Emission intensity and the appearance of the spectrum are both affected. An example is given in which the 3-D fluorescence spectra for this sample shows how the emission intensity (z axis) depends on both the choice of excitation and emission wavelength (x and y axes):

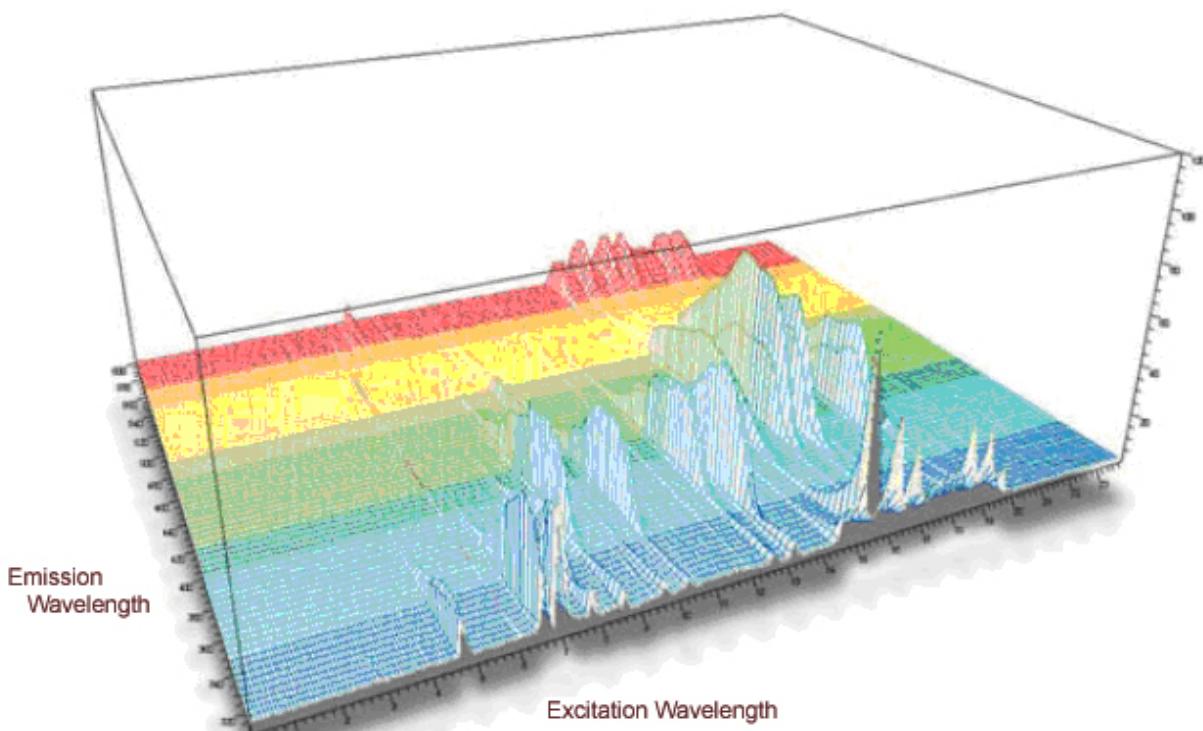


Figure 3. 3D fluorescence spectra

The following is a figure showing the excitation and emission spectrum of a real molecule, anthracene. The emission spectrum (solid line) was obtained by recording the intensity of the fluorescent emission while exciting the sample using 250 nm light, and the excitation spectrum (dashed line) was obtained by monitoring the fluorescence intensity at 397 nm while scanning the excitation wavelength.

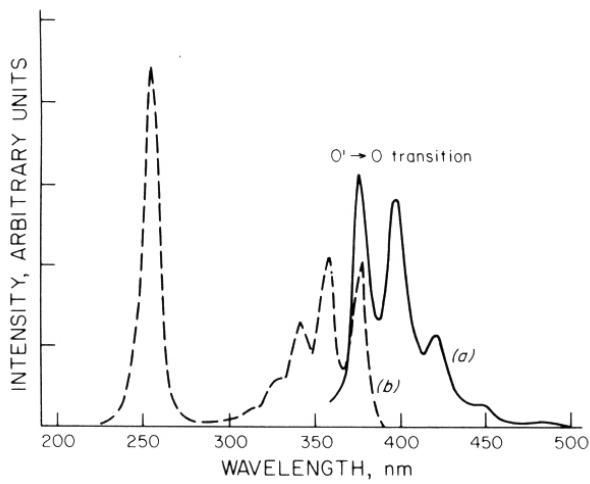


Figure 4. Excitation and Emission Spectra for Anthracene.

You may be wondering how we can make such measurements of excitation and emission spectra. You may also be wondering why one would choose to use luminescence vs absorption spectroscopy if the first step in the latter cases is the same as that involved in absorbance measurements. These are good questions, and we will return to later. The answers lie in a consideration of the quantities measured using the appropriate instruments and how these measurements relate to the concentration of the analyte molecules.

Absorbance vs fluorescence measurements

In absorbance measurements the intensity or power (flux of photons) of light absorbed by the sample is determined in the presence (P) and absence (P_0) of the absorbing sample. As “monochromatic” light is *transmitted* through a sample cuvette (shown in dark blue), the incident power is reduced by absorption of light (see Figure 5A).

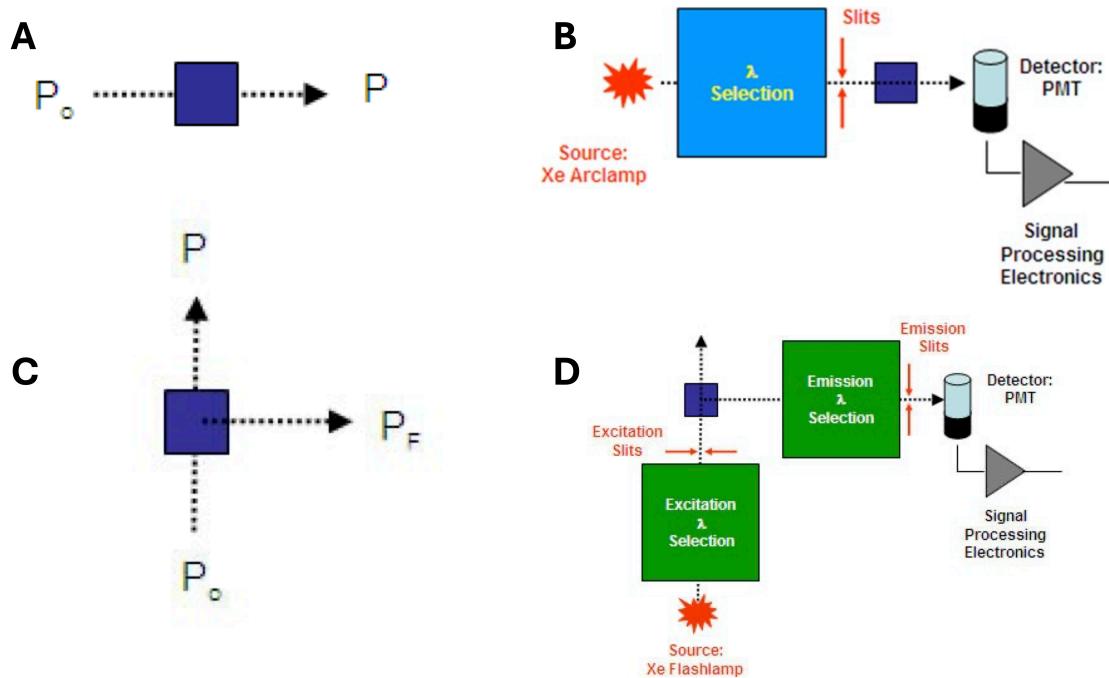


Figure 5. **A** Definition of Incident (P_0) and Transmitted Power (P) for a Transmission Experiment. **B** Block Diagram of an Absorption Spectrophotometer. **C** 90° Geometry for Fluorescence Measurements with Definition of Incident (P_0), Transmitted (P) and Fluorescent (P_F) Power. **D** Block Diagram of a Fluorescence Spectrophotometer.

The instrumentation (single beam spectrophotometer) used for such measurements is shown below in block diagram form (Figure 5B). Here a narrow band of light is selected from a broadband light source (Xe arc lamp) using a monochromator. The bandwidth of the “monochromatic” light is determined by the setting of the output slits of the monochromator: the wider the slit setting, the wider the range of wavelengths passed on to the sample.

It is convenient to determine the absorbance, A , by making two measurements. The first is a measurement of P_0 using only solvent (blank measurement), while the second involves replacing the solvent with a solution of the absorbing compound and measuring P . Absorbance is defined by the log ratio of these power levels as measured by the detector and associated electronics, and is related to concentration according to the Beer-Lambert Law:

$$A(\lambda) = \log\left(\frac{P_0(\lambda)}{P(\lambda)}\right) = \varepsilon(\lambda) b c$$

A similar situation is considered when measuring fluorescence, except that we must discriminate between the light used for excitation and that arising from fluorescent emission (P_F) from the sample: To do so a 90° geometry is typically employed (Figure 5C). A four-sided quartz cell is chosen for most measurements, as often the wavelengths of the light used are in the UV region.

Fluorescence instruments have two monochromators: an excitation monochromator and an emission monochromator. The first monochromator directs light of a certain selected wavelength band to the sample, while the second monochromator collects and analyzes the light emitted from the sample (at right angles to the incident beam). A block diagram of such an instrument is shown (Figure 5D).

Imagine a compound which absorbs energy at 350 nm, and exhibits fluorescent emission at 400 nm. An excitation spectrum can be recorded by setting the emission monochromator at 400 nm, and scanning the excitation

monochromator from, say, 300 nm to 500 nm. The excitation spectrum will be a plot of intensity registered by the detector at 400 nm (emission) vs. wavelength of the excitation monochromator, and will look a lot like an absorbance spectrum you might observe with a UV-visible spectrophotometer. To measure the emission spectrum, you would set the excitation monochromator at 350 nm, and then scan the emission monochromator from, say 250 nm to 500 nm. The emission spectrum will be a plot of intensity (usually in arbitrary intensity units) as a function of wavelength of the excitation monochromator. With these spectra in hand, then the measurement of fluorescence in an analytical context can be optimized for sensitivity by choosing the excitation wavelength which provides the *maximum* absorbance, and the emission wavelength which provides the *maximum* emission. Intensity measurements would then be taken with the respective monochromators set to these wavelengths. Only when both of these maxima are very near one another must a compromise be made to avoid excitation light to be scattered (Rayleigh scattering) through 90° and reach the detector.

This situation occurs for the example shown in Fig. 3, anthracene. Here the choice of 375 nm for both the excitation and emission monochromators would give rise to scattered light being recorded by the detector in addition to the fluorescent emission from anthracene. Hence the choice of 250 nm for excitation and 375 or 397 nm for the emission monochromator settings.

The quantitative relationship between fluorescent intensity and concentration is given by $P_F = P_0 K (1 - 10^{-A})$, where P_F is the power of the fluorescent light measured by the detector, P_0 is the power of the incident radiation, A is the absorbance of the solution at the excitation wavelength, and K is a constant for the optical geometry of instrument. You may think of K as an efficiency factor.

At low concentrations, P_F is nearly a linear function of A (since $(1 - 10^{-A})$ is approximately $2.303A$ for small A), and so

$$P_F = 2.303 P_0 K A = 2.303 P_0 K (\epsilon b C).$$

This approximation works at concentrations so low that $A < 0.01$. The terms in brackets are those of Beer's Law, and have the same significance as they did in UV-Vis absorbance measurements. A plot of fluorescent power (intensity) vs. concentration should be linear at low concentrations where A at the excitation wavelength is < 0.01 . This kind of plot can function as an analytical calibration curve: a series of solutions of known concentration can be studied, and their fluorescent intensities plotted as a function of concentration. A sample containing an unknown amount of solute can then be measured, and its intensity located on the working curve. The corresponding concentration can then be located on the concentration axis by dropping a vertical line. At higher values of A , the relationship for fluorescence intensity becomes nonlinear (concave downward calibration plots are obtained), but these working "curves" can still be used, although they are less convenient. See the figure below.

Quantitative considerations and the relationship to absorption of light

$$F = K'(P_0 - P)$$

$$\frac{P}{P_0} = 10^{-\epsilon bc}$$

$$F = K'P_0(1 - 10^{-\epsilon bc})$$

$$F = K'P_0 \left[2.303\epsilon bc - \frac{(2.303\epsilon bc)^2}{2!} + \frac{(2.303\epsilon bc)^3}{3!} \right]$$

$$F = K'P_0 2.303\epsilon bc$$

$$F = Kc$$

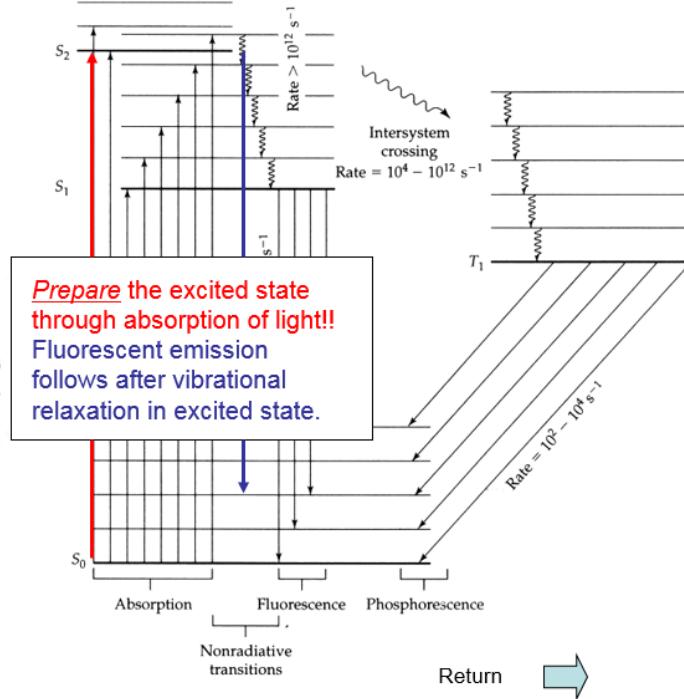


Figure 6. Quantitative considerations related to the absorption of light.

Quenching is a term applied to internal absorption of fluorescent light, and may take two forms. First, excited solute molecules may lose “all” their excitation energy to other solutes, solvent molecules or impurities during collisions, and return to their ground states without emitting light. Dissolved oxygen and heavy halides such as iodide can quench in this manner. Alternatively, another solute molecule, unexcited by the excitation source, may absorb energy from an excited neighbor in a mechanism called “self-quenching”. The latter mechanism occurs at high solute concentrations, and may also cause working curves to acquire concave downward curvature at high concentration.

5. Chemicals, Supplies, and Safety

Chemicals

- Commercial tonic water samples (diet and non-diet)
- Solid anthracene sample in polymethyl methacrylate (PMMA) (sample #1)
- 1000 ppm quinine sulfate in 0.1 M sulfuric acid
- 0.1 M sulfuric acid for blanks and all quinine sulfate dilutions
- Commercial tonic water samples (diet and non-diet, as assigned)
- Solid potassium iodide (KI) for quenching experiments

Supplies

- Pasteur pipettes and bulbs
- A four-sided quartz cuvette (1 cm)
- Waste container, Kimwipes, Sharpie

Safety

- **UV radiation from the xenon lamp.** Hazards: eye/skin damage from high-energy UV light. Avoid by keeping the sample compartment closed during measurements and never looking into the light path. If exposure is suspected, stop the run, close the lid, and notify the TA immediately.
- **Acidic solutions and salts (1.0 M sulfuric acid, quinine sulfate, KI).** Hazards: corrosive/irritant splashes and skin/eye exposure. Avoid by wearing goggles/gloves, using pipettes carefully, and keeping containers capped. If exposure occurs, rinse skin/eyes with water for at least 15 minutes and inform the TA.

6. Instrumentation

Fluorescence Instrument

The primary instrument to be used for this lab is the Cary Eclipse Spectrofluorimeter.

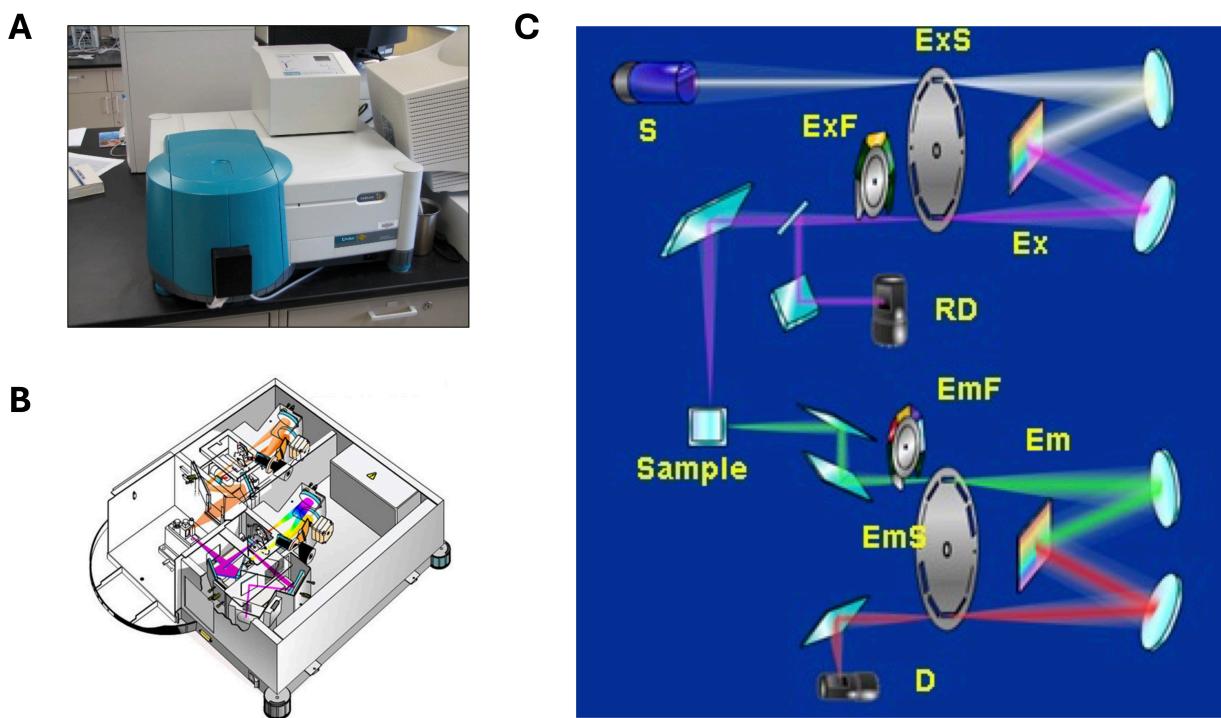


Figure 7. The Cary Eclipse Spectrofluorimeter, photo and block diagrams.

Source (S): The Cary uses a xenon discharge lamp operated in a pulsed mode as its source of UV-vis radiation for sample excitation. This is a high intensity source. The pulsed mode of excitation is employed to avoid photo-degradation of light-sensitive samples, and to provide longevity to the source. The pulsed mode also offers the advantage of more stable (constant) output over time, compared to continuous operation. In either case the intensity of light reaching the sample is monitored by a reference detector (RD, Fig. 7) which is a PMT. All measurements of emission intensity are *normalized* to the response of this detector. The details of the design of this source are shown below, where we can see the use of special optics to efficiently capture and focus the light output from each pulse.

Excitation Monochromator: The excitation monochromator selects the radiation band to be used to excite the sample, and consists of a set of slits mounted on a selector wheel (ExS), a reflection grating (Ex) which can be rotated for wavelength selection and a set of filters mounted on a selector wheel (ExF). All of these are selectable via software control. The position of the grating determines the center of the excitation band, while the choice of slit position determines the width of the excitation band. Note that the position of the filter wheel selects the same slit width for both the entrance and exit slits of the monochromator. The filters remove higher order diffraction bands from the

grating output. The selection of the appropriate filter is accommodated by software and coordinated with the selection of the center band of the grating.

Sample Cell: Figure 7 shows the entrance of the excitation radiation from the left into the liquid sample contained in the cuvette, with fluorescent emission exiting at 90° relative to the excitation beam. Note the excitation beam is “monochromatic, having been selected by the excitation monochromator, while the emission covers the entire UV-vis-NIR wavelength region. Typically, a four-sided quartz cell is employed. You can consider why we’d use quartz and why all four sides be must be transparent. You can also consider the consequences of small particulates suspended in the liquid sample.

Emission Monochromator: The design of the emission monochromator, depicted in Fig. 7, is identical to that of the excitation monochromator.

Detector: The detector for fluorescent emission is also shown, positioned on the output focal plane of the monochromator. The detector of choice for fluorescence measurements is a photomultiplier tube (PMT). Why is a photodiode or avalanche photodiode a poorer choice for this application?

Software

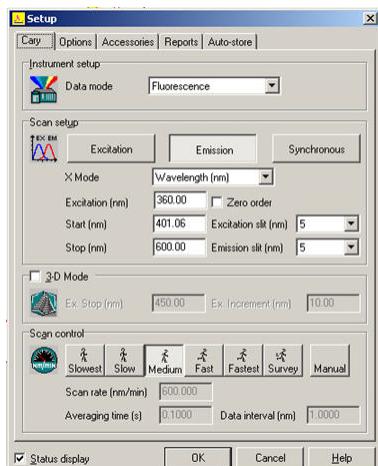
There are two programs which should be used to optimize the efficiency of these experiments. One is for obtaining spectra and is called “Scan”, and the other is for making quantitative measurements and is called “Simple Reads”. They are both accessible in the Cary Eclipse folder that is located on the desktop.

The “Scan” Application

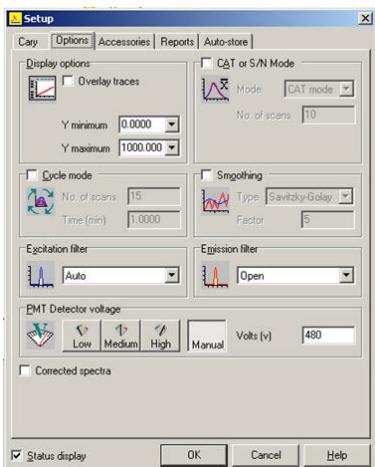
The first program is the “Scan” application. Unless you already know which excitation and emission wavelengths you want to use, you should start with this program. Note the “Status Display” window in this application, which provides a summary of current setup and activity. This program allows the user to find the optimum emission and excitation wavelengths to be used later in the experiment. It also offers control over almost all of the features of the spectrofluorimeter and certainly the ones necessary to optimize sample readings.

While you may print various graphs and reports directly from this software, you may also find it useful to store CSV text files for later import into programs such as Excel. *Note that the entire current report and graph data is saved when you do this, so you may want to investigate storing, retrieving and clearing graphs and reports now, so that you can plan on how to store data for the various experiments you will carry out.*

Slit width, signal-to-noise ratio (S/N), PMT voltage, excitation and emission wavelength selection, and scan rate are all important for increasing S/N and getting signal in general. Control over these parameters is available in the setup menu (shown below), which is accessible from a button on the application or a drop-down menu on the tool bar. Selecting the “Setup” button allows one to completely control all features of the scanning mode. The “Cary” tab is most often used, and allows for selection of mode and scan parameters. Slit sizes can be selected here, along with scanning parameters. Note that there are default settings and manual settings which may be selected.

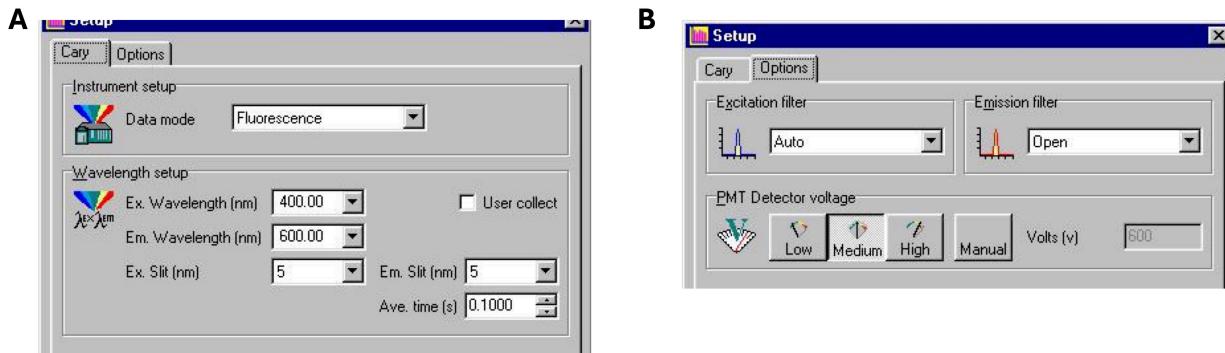


The “Options” tab (see below) allows one to control the display of plots, whether or not signal averaging is used, smoothing functions to remove noise, filter selection and PMT voltage.



The “Simple Reads” Application

After the optimum excitation and emission wavelengths and other parameters have been determined, the “Simple Reads” program allows fast acquisition of samples without graphical output at selected wavelengths. “Simple Reads” also has a Settings button, which allows control over slit width and PMT voltage (controls are located similarly to the Scan program) and gives a single reading of intensity. This allows for fast optimization of slit widths and also for taking readings for calibration curves because only one excitation frequency is scanned and one emission frequency gathered relative to scanning a range of both excitation and emission frequencies. Shown below are the screens under “Simple Reads” for controlling the experiment type and scanning ranges under the Cary tab (left), and the slit widths and PMT voltage under the options tab (right).



Instrument Settings

Computer Credentials

- Username: CHEM4633
- Password: guest

Startup

These instructions assume the use of the basic sample holder which accommodates a single 1-cm cell. A thermostatted cell holder is also available. Note the location of the label on the sample cell. For best precision when making measurements, always place the cell in the holder with the same orientation.

- Open the sample compartment to verify that the proper sampling accessory is installed before powering on the instrument.

- Turn on the power with the switch on the lower right front corner of the instrument.
- Turn on the computer and double click on the Cary Eclipse icon on the desktop.
- If you are a new user, click on the Cary Eclipse Tour icon for a brief introduction to the instrument.

Warning. The xenon lamp emits high-energy UV radiation that can cause serious damage to your eyes if viewed directly. The lamp emits only when making measurements. Close the compartment lid when making measurements.

Scan (Excitation or Emission Spectra)

- Setup
 - Double click on the Scan shortcut in the Cary Eclipse window. The scan window will open and after a minute the stoplight icon should turn green.
 - A small Status Display window shows the current settings. If not visible, select View/Status Display from the main menu.
 - Click on the Setup... button. A window will open showing you the various setup options. Check the following on the page with the Cary tab:
 - Data Mode: fluorescence
 - Scan setup: choose Emission or Excitation.
 - Enter the appropriate wavelengths for your experiment.
 - Slits: The default values of 5 mm are a good starting place for the excitation and emission slits.
 - Scan Control: medium is a good starting value
 - Click on the Options tab.
 - If you check the Overlay Traces box you will get the spectra overlaid in one window. If the box is unchecked, each spectrum you take will appear in its own window.
 - When you are done, click Okay. The setup window will disappear.
- Obtain the spectrum
 - Click on the big Start button. Enter a sample name when prompted. The instrument will acquire a spectrum. When it is done, click on the vertical arrow icon and the data will be scaled to fill the window. You can move the cursor along the spectrum and read its coordinates in the lower right corner.
 - If you choose Graph ->Remove all traces (or click the trash can icon) the spectra will be removed from the window.
- Finish
 - Select File/Exit to close the application.

Simple Reads

- Setup
 - Click on the Simple Reads shortcut in the Cary Eclipse window. The Simple Reads window will open and after a minute the stoplight icon should turn green.
 - Click on the Setup... button. A window will open showing you the various setup options.
 - Set the excitation and emission wavelengths.
 - Note that there are also places to set the excitation and emission slit widths and to change the averaging time. Try the default values of 5 mm slits and 0.10 sec.
 - When you are satisfied with the setup, click OK. The setup window will close.
- Take readings
 - Click the Read button. The instrument will take a reading and display it on the screen. Each time you click Read, a new value is recorded.
 - When you have completed your readings, you can print a report or save the file in Rich Text Format (RTF) and then import your data into Excel.

- Finish
 - Select File/Exit to close the application.

Shutdown

- At the end of the lab period, close the Cary Eclipse software (File/Close).
 - Shut down the PC
 - Switch main power button on the instrument to OFF.
-

7. Experiments to be Performed

In the following experiments you will learn how to use and calibrate the spectrofluorimeter, perform a quantitative analysis, and learn about sensitivity and detection limits.

Part 1: Familiarization with the instrument and the software using anthracene and the “Scan” application

Experiment 1-1. Study of the effect of Emission Slit Setting on Resolution, Spectral Appearance, and Signal to Noise Ratio (S/N).

- Using the anthracene sample, set mode to “fluorescence” with the scan setup to “emission”. Set the excitation and emission slits to 1.5 nm, the excitation wavelength to 360 nm and the start and stop wavelengths to 350 and 500 nm, respectively. Go to the “Options” tab and set the PMT voltage to “Medium” (600 V). You have configured the instrument to record emission spectra in the 350-500 nm range using 360 nm excitation. Record the emission spectrum for each of the following *emission* slit settings: 1.5, 2.5, 5, 10 and 20 nm, leaving the *excitation* slit set to 1.5 nm for all cases. Save the data so that you can plot these data on a common set of axes for comparison.

Experiment 1-2. Study of the effect of PMT Power Supply Voltage Setting on Spectral Output and S/N.

- Again using the anthracene sample, with the settings last used above (set mode to “fluorescence” with the scan setup to “emission”, both slits at 1.5 nm, the excitation wavelength to 360 nm and the start and stop wavelengths to 350 and 500 nm, respectively), and the PMT voltages varied as follows: 400, 500, 600, 700, 800, 900, and 1000 V, record the emission spectra for each of these PMT voltage settings. Save the data so that you can plot these data on a common set of axes for comparison.

Experiment 1-3. Study of the effect of Spectral Smoothing Algorithms on Resolution and S/N.

- Again using the anthracene sample, set the PMT voltage to 600 V. With the other settings set as above for Parts 1-1 and 1-2 (set mode to “fluorescence” with the scan setup to “emission”, both slits at 1.5 nm, the excitation wavelength to 360 nm and the start and stop wavelengths to 350 and 500 nm, respectively), record the emission spectra using each of the following smoothing choices on the “Options” tab: Savitsky-Golay, factor = 5; Moving Average, factor = 5; Boxcar, factor = 5. Save the data so that you can plot these data on a common set of axes for comparison.

Experiment 1-4. Study of the effect of Signal Sampling and Averaging on S/N.

- The Cary and its software provide for several modes of signal averaging. We will investigate 2 of them. In the first one we will examine the choice of averaging time under the “Scan Control” settings on the “Cary” tab. In the second case we will carry out signal averaging (CAT mode) which can be selected under the “Options” tab. For the first set of measurements, turn off any smoothing, and set the parameters as above for part 1-2 (PMT voltage = 600 V, set mode to “fluorescence” with the scan setup to “emission”, both slits at 1.5 nm, the excitation wavelength to 360 nm and the start and stop wavelengths to 350 and 500 nm, respectively). Under the “Scan Control” settings select manual. Record the emission spectra of the anthracene sample for each of the following settings of the averaging time: 0.0125, 0.05, 0.1, 0.5, and 1.0 s. Save the data so that you can plot these data on a common set of axes for comparison.

- Reset the sampling time to 0.1 s and record the anthracene emission spectrum again. Using the CAT (computed average of transients) settings which follow, obtain the average emission spectrum for 4, 9, 16, 25 and 100 scans. Save the data so that you can plot these data on a common set of axes for comparison.

Part 2. Quantitative analysis of quinine in diet and non-diet tonic water

Using the “Scan” application

- Prepare a 1000 PPM stock solution of quinine sulfate (formula weight (FW) = 782.96 g/mol) in 0.1 M sulfuric acid (*Note: your TA may have already prepared this for you to save some time. Check in with them before making this solution.*).
- From the stock solution you prepared in the previous step, prepare a 100 ppm solution. (Note: All dilutions must be made with 0.1 M sulfuric acid). Consider the absorbance spectrum of quinine shown in this handout. Using the wavelength of maximum absorbance shown in that spectrum as an excitation wavelength, acquire an emission spectrum for your quinine solution.

Using the “Simple Reads” application

- Design and conduct experiments to document the effects of the slit width of the excitation and analyzer / emission monochromators on the intensity and appearance of the quinine fluorescence spectrum for the 100 ppm solution. You will need to think about the optical block diagram of the spectrofluorometer used in this experiment (Cary Eclipse) as discussed above, and plan your experiments carefully to explore these effects.
- Prepare a calibration curve for quinine sulfate in 0.1 M sulfuric acid over the range 0 to 50 ppm. Given the information in the introduction about self quenching, you may want to include more points at the lower end of this range on your calibration curve.
- Using your calibration curve, determine the concentration of quinine in commercial, sugar-free tonic water.

Part 3. Effect of quenching by heavy atom components of a sample

- Document the effect of adding potassium iodide to the quinine sulfate solution on the fluorescence intensity. A suggested range of solution concentrations to explore is 0.00 to 0.50 g of KI per 100 mL quinine sulfate solution, with a quinine sulfate concentration of 5 - 10 ppm.

Waste Disposal

All the waste solutions are to be disposed of in the waste bottle(s) dedicated to this experiment. Clean any glassware or other supplies or materials you used and return it to where you found it. Discard of any pasteur pipettes in the sharps waste container.

Data Export

1. Open “Scan” software.
 2. When collecting data, save as a .BSW or .DSW.
 3. Re-open .BSW or .DSW data and “Save As” a .CSV.
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8. Report Guidelines

Your report should consist of a short introduction and methods section, but then primarily be focused on the results and discussion. General “results and discussion” guidelines: Report all results, calculations and plots, along with explanatory prose as guided by the following. Your report should also include an “author contributions” section describing the role each member played in this experiment and the contributions to writing.

Part 0

Include a general discussion of the instrument design by addressing these questions:

- Fluorescence spectrophotometry is inherently more sensitive than absorbance spectrophotometry for compounds which show appreciable fluorescence. What structural factors influence the extent to which a compound is likely to exhibit fluorescence?
- Consider the design and optical layout of a spectrofluorimeter vs an instrument designed to measure absorbance at the same nominal wavelength resolution. Why is a monochromator required in the emission leg of the optical design?
- Fluorescence spectrophotometry is inherently more sensitive than absorbance spectrophotometry for compounds which show appreciable fluorescence. From a measurement perspective, using the same type of detector for both measurements (e.g. a PMT), why is fluorescence inherently more sensitive than absorbance?

Part 1

- Using Excel, plot the data you obtained in experiment 1-1, above. Your plot should show the emission spectrum for the anthracene sample over the range of 350 – 600 nm. All five data sets covering the range of 1.5 – 20 nm exit slit width should be presented on the same plot. What is the effect of changing slit width on sensitivity? What is the minimum slit width required to adequately resolve the 3 emission peaks in this wavelength range? By what factor is sensitivity (in terms of limit of detection or limit of quantification) improved by choosing this setting vs the smallest slit width (which provides highest resolution)?
- Again, using Excel, plot the fluorescence intensity obtained at 423.1 nm vs PMT voltage for the data obtained in experiment 1-2, above. Is this nonlinear dependence expected? Why is the PMT the detector of choice for fluorescence measurements compared to a photodiode or and avalanche photodiode?
- Generate a composite plot which compares the smoothed data obtained in experiment 1-3 to unsmoothed data acquired under the same instrumental settings. What is the primary disadvantage to box car smoothing compared to the other techniques investigated (moving average and Savitsky-Golay)? Comment on the results from the application of a moving average technique compared to the Savitsky-Golay approach.
- For the results from experiment 1-4 quantify the effect of averaging time on the signal to noise ratio obtained from each experiment. Show your results in an appropriate plot. Do your results agree with sampling theory?

Part 2

- Your report must include the calibration curve, as well as your value for the concentration of quinine in the tonic water. Also include an explanation of your choice of operating parameters for the instrument, and choice of dilution scheme for the tonic water. Explain any observed nonlinearity in the calibration curve.
- Discuss your calibration curve for quinine fluorescence intensity. Carefully explain any observed nonlinearity in the calibration curve. In your discussion consider the theoretical response for fluorescence: $P_F = P_0 K (1 - 10^{-A})$, and your measurement of the extinction coefficient for quinine at the excitation wavelength you used. Recall that:

$$A(\lambda) = \varepsilon(\lambda) b c$$

- Dust off your math books, and expand the expression: $P_F = P_0 K (1 - 10^{-A})$ to at least 4 terms. How do the additional terms affect the linearity of the calibration relationship for samples which have an extinction coefficient path length product > 0.02 ? To explore the conditions which lead to nonlinear calibration relationships (so-called “working curves”).
- What are the limit of detection and limit of quantification predicted by your results?

Part 3

- Discuss and interpret your results for the experiments involving the addition of KI to quinine solutions. How important is it to consider heavy atom quenching in quantitative fluorescence measurements? Discuss

a strategy (experimental design) which would automatically compensate for such artifacts in a sample which may contain quenchers.

9. References

1. Chapters 13 & 15 in Skoog et al.

Client-Based Projects

1. Overview

In the second half of the semester, your group will take on a real analytical problem provided by a community or industry client. You will interpret the client's question from an analytical chemistry perspective, design a feasible strategy using the instrumentation and resources available in the department, and carry out experiments to generate defensible results. The project is meant to mirror how analytical chemistry is practiced outside of class: define a problem, justify a method, collect and analyze data, and communicate findings to a non-specialist client.

The project culminates in four deliverables that move from planning to execution to communication. You will first submit a methods/reagents proposal to clarify your approach and allow time for feedback and ordering. You will then produce a written report and give a formal presentation aimed at your client, and complete a peer review. Details and rubrics for each component appear below.

Products of this Project and As a Group:

- Method/reagents proposal.
 - Client project presentation.
 - Written project report.
 - Client project peer review.
-

2. Client Project Methods Proposal

Your group must put forth a proposal of the instrumental methods you plan to use to answer your client's question. To be clear, the client problem that you are given does not necessarily mean that they have asked the question in a way that allows you to answer the problem as an analytical chemist. Part of your job is interpreting the question from a molecular and analytical perspective, determining what is and/or is not possible, and choosing a course of action. The purpose of this proposal assignment is to push you to start planning, but also give the TA and instructor time to order reagents/help you hone the independent work you will be pursuing.

To develop the appropriate methodology, you should use primary literature. We are asking you to specifically detail the experimental methodology you plan to use. For example, if you choose to use an HPLC method, you must specify the specific mobile phases you propose to use. If you choose a spectrophotometric method, you should indicate which wavelength(s) you plan to use. Your proposed methodology should be aligned with the equipment that you have access to as part of the Department of Chemistry and Biochemistry. You may consider methodology beyond the six rotational experiments that you did in the first half of the semester, though you should discuss the feasibility of using non-rotation experiment equipment with your instructor first. Methods must be feasible with departmental instrumentation and resources; ask before proposing off-menu equipment.

The writing style for this report should be similar to a research proposal, which basically means that you need to justify the choice of methodology you choose. A specific structure is outlined in the rubric for this project. Aim for about two pages, submitted as a single PDF on Canvas. Due dates are listed on Canvas.

Rubric (100 pts total):

- Introduction (10 pts): 5 pts for stating the client question and what it means from an analytical chemistry perspective; 5 pts for detailing why the question is important.
- Methods (50 pts): 15 pts for detailing sample preparation (for example, whether you need extraction or pre-concentration); 30 pts for detailing instrumental methods; 5 pts for describing expected results (for example,

predicted elution order); 5 pts for explaining potential problems or pitfalls (for example, what could go wrong with careless sample preparation).

- Equipment list (12.5 pts): Bullet list of specific equipment needed; be specific about glassware and stationary phases.
 - Reagents list (17.5 pts): Bullet list of reagents needed.
 - Safety (5 pts): Detail the safety considerations you must address for the reagents and methods you are proposing to use; for example, identify air-sensitive reagents and any special setup required.
 - References (5 pts): Include in-text citations and primary literature. Please cite at least three peer-reviewed journal articles. Format references the way journal articles do.
-

3. Written Report

Your group must write up the work from your independent experiments as a client-facing report. The report should be written for scientifically literate readers who are not necessarily chemists, so clarity and context matter. Structure your report like a journal article with clear sections (Introduction, Materials and Methods, Results and Discussion, Conclusions, and References). Figures and tables must be publication-quality with clear captions, labeled axes, units, and appropriate error reporting. Submit a single PDF on Canvas (one report per group). Due dates are listed on Canvas.

Rubric (170 pts total):

- Introduction (15 pts): Restate the analytical chemistry questions you tackled.
 - Materials and Methods (45 pts): Detail the sample preparation you actually performed and the instrumental methods used; use articles from *Analytical Chemistry* as guidance for this section.
 - Results and Discussion (55 pts): Present results in high-quality figures, tables, or graphics and interpret them using appropriate statistical analysis.
 - Conclusion (30 pts): Reflect on the answers to the client's questions; discuss what was problematic in your experiments and how that limited certainty; describe future work or directions to address remaining gaps.
 - Writing Quality (25 pts): Grammar and spelling are correct; writing is clear to a general audience; references to primary literature are included.
-

4. Presentation

Your group must present your work to the class and invited clients. The presentation should be aimed at scientifically literate non-specialists, and it should clearly articulate the analytical question, justify the methods used, and interpret results in a way that answers the client's question. Include the same core components as the report (background/significance, methods, results and discussion, conclusions, and references). Figures should be publication-quality and legible at presentation scale. All group members must present a portion of the work. Submit your slide deck on Canvas. Due dates and presentation schedules are listed on Canvas.

Rubric (110 pts total):

- Content (70 pts): 8 pts for laying out the analytical question clearly, including background and significance; 12 pts for clearly articulated methods with justification and explanation of what you actually used; 30 pts for interpreting results with high-quality figures and appropriate statistical analysis; 17 pts for conclusions that address uncertainty, sources of error, and how results compare to literature; 3 pts for using citations appropriately (including slide citations).
 - Presentation and style (40 pts): 10 pts for slides that are aesthetically pleasing and free of grammar or spelling errors; 8 pts for logical flow (chronological is not always best); 10 pts for clear speaking with all group members presenting; 12 pts for answering questions well.
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5. Peer Review

You will be asked to peer-review your group member(s) via a Google Form. We realize that everyone has different strengths but that you all should be contributing to the group efforts. This is an opportunity to reflect on your group's performance.

Rubric (20 pts total):

- Complete the peer review thoughtfully and on time (20 pts).
-

6. Client Project Assignments

Group(s) A

Explanation: Your client is interested in capsaicin—the compound responsible for heat in chili peppers. They want a clear, data-driven comparison of how capsaicin levels vary across different chili varieties, and whether the fruit and seeds differ in capsaicin content. There is also a real-world connection to topical pain-relief products that use capsaicin. Your client is Dr. Sabrina Russo (srusso2@unl.edu) at the University of Nebraska. She will be invited to your final presentation, and you are encouraged to reach out to her and/or Dr. Busta with any questions.

Client question: How do capsaicin levels compare across different chili varieties and between fruit and seeds, and how do those levels relate to capsaicin content in common pain-relief products?

Group(s) B

Explanation: Your client works with buckthorn ink and wants to understand why its color shifts from purple to green to yellow under different conditions. They are also curious about practical performance (how the ink flows, spreads, and bleeds on paper). There is an optional ink-making workshop where you can learn more and see the process firsthand—please mark your calendars for Monday, February 23, 2026, 2:00–3:50 pm in Chem 238 (location subject to confirmation). You can contact Dr. Krista Twu (ktwu@d.umn.edu) for a sample of the ink. She will be invited to your final presentation, and you are encouraged to reach out to her and/or Dr. Busta with any questions.

Client question: What causes buckthorn ink to change color (purple → green → yellow), which components are most responsible for that shift under different conditions?

Group(s) C

Explanation: Your client works with biochar and wants to quantify how well it removes metal ions from solution. They already have a reliable way to measure concentrations, but a second approach has been less consistent. Your job is to evaluate how feasible that second approach is for detecting changes in metal ion concentration, and what factors limit its reliability (such as concentration range and sample turbidity from biochar particles). Your client is Dr. Jacob Wainman (jgauer@d.umn.edu) at UMD. He will be invited to your final presentation, and you are encouraged to reach out to him and/or Dr. Busta with any questions.

Client question: How small of a change in metal ion concentration can we reliably detect with the alternative measurement approach, and what limits its accuracy (e.g., concentration range, signal noise, or light scatter from biochar particles)?

Group(s) D

Explanation: Your client works in art conservation and uses menthol as a temporary consolidant for fragile objects. They need to buy large quantities and are considering lower-grade menthol to reduce cost. Your task is to compare different grades of menthol for performance (how long they take to sublime) and quality (whether they leave any detectable residue after sublimation). Your client is Megan Randall (MRandall@preserveart.org) at the Midwest Art Conservation Center in Minneapolis. She will be invited to your final presentation, and you are encouraged to reach out to her and/or Dr. Busta with any questions.

Client question: How do different grades of menthol compare in sublimation time and residue left behind, and are lower-grade options acceptable for conservation work?

Group(s) E

Explanation: Your client is working on a method for testing lead in drinking water and wants to improve the consistency of a derivatization-based workflow. Your task is to evaluate where variability is introduced in the derivatization step and which conditions most affect reliability, so the method can be optimized for consistent results.

Client question: How do derivatization conditions (reagent choice, time, temperature, and reagent age) affect the reliability and variability of lead measurements? Your client is Dr. Busta and you are encouraged to reach out to him with any questions.
