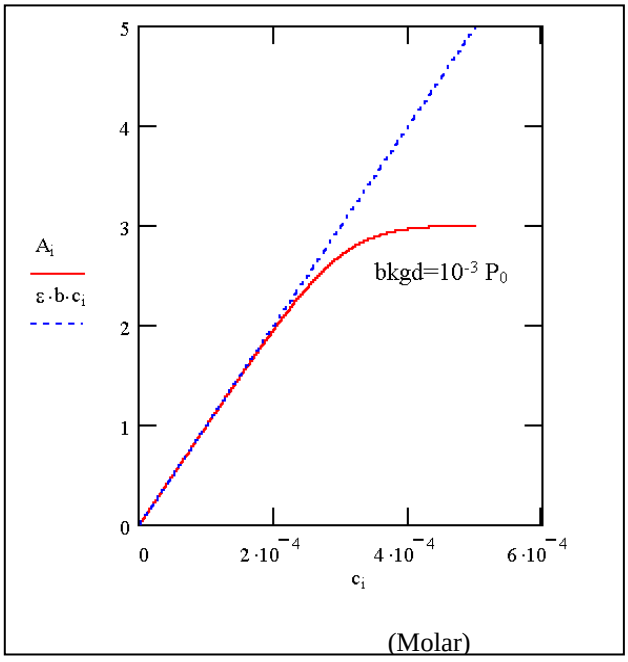
Noise and Detection Limits

Introduction. This laboratory will allow you to gain experience observing the behavior of noise and the estimation of detection limits. The experiment will use the Agilent (formerly Hewlett-Packard) model 8452UV-Vis absorbance spectrometer, although what we learn here is completely relevant to any absorbance measurement. The HP-8452 is a single-beam diode-array spectrophotometer. A diode-array instrument disperses the whole range of spectral wavelengths λ across an array-type detector, so that all wavelengths can be recorded simultaneously, or in parallel. There is no need to pass the different wavelengths of light over the detector sequentially, giving a great time advantage over an instrument based on a single-channel detector such as a photomultiplier. As you may recall, to make an absorbance measurement, you first put a "blank" sample (i.e. usually a sample without the analyte of interest, but otherwise the same as your test sample) into the sample compartment of the instrument, which then records P_0, the light power that passes the blank sample at all wavelengths. You then replace the blank sample with your test sample, and the instrument again records the light power P at all wavelengths. The computer controlling the instrument then performs the following calculation to determine absorbance at each wavelength.

$$A = -\log\left(\frac{P}{P_0}\right) \qquad \text{(eq 1)}$$

The **Beer-Lambert Law**, also commonly called "**Beer's Law**" allows concentration c to be determined by $A \equiv \varepsilon\, b\, c$, where ε is the molar absorptivity (sometimes called the "extinction coefficient"), and b is the sample thickness. A plot of ε vs. the wavelength λ is called the spectrum.



According to Beer's Law, a plot of A or $-\log(P/P_0)$ vs. concentration predicts an infinite range of concentrations over which the instrument gives a linear relation between A and c. In reality, when A is high, the amount of light being absorbed by the sample is high, and P is low. The amount of light falling on the detector is therefore low, making it more difficult to measure P accurately because of non-sample contributions to the measured signals.

What the instrument actually senses is the *apparent* absorbance:

$$A_{apparent} = -\log\left\{\frac{P + P_{bkgd}}{P_0 + P_{bkgd}}\right\} \approx -\log\left\{10^{-\varepsilon bc} + \frac{P_{bkgd}}{P_0}\right\} \qquad \text{(eq 2)}$$

where P_{bkgd} represents an amount of signal power that is *not* related to the sample (for example, detector background response from "dark current," the current flows in the *absence* of light striking the detector, and the power of any stray light). Notice that the first term in the right bracket of Eq. 2 is the same as the ideal case described in Eq. 1 above.

There are several limitations to Beer's Law. We will discuss two in this lab:

1. Limit of accuracy at high absorbances. At very high absorbance, the detector signal due to P (i.e. the light power not absorbed by the sample) will be lower than the detector signal due to the background P_{bkgd}. We say that the signal-to-background (S/B) ratio is less than one. The signal-to-noise ratio is also less than one because the signal power is very small in this case, and the signal from the instrument is dominated by noise. A plot of Eq. 2 for a range of concentrations is shown in Fig. 1 for an ideal case of zero background (dotted blue line), and for the more typical case (solid red line) of a background that is 0.1% of the size of the detector response from P_0, i.e., $P_{bkgd}/P_0 = 0.001$.

Notice that the presence of the detector background limits the upper end of the absorbance that can be measured accurately. This happens in Fig. 1 where there is a significant deviation of the solid red line from the dotted blue line. Notice also that this upper limit or concentration corresponds to a *predictable* limit.

$$\left(A_{apparent}\right)_{\lim P \to 0} = -\log\left(\frac{P_{bkgd}}{P_0}\right) \qquad \text{(eq 3)}$$

In this lab, you will determine the upper limit for absorbance measurements on this spectrometer.

2. Limit of Detection: Effect of noise due to the light source. Noise on the light source (i.e. fluctuations in P_0) also affects the appearance of the calibration curve in important ways. Below in Figs. 2 and 3 is the same plot as in Fig. 1, but with 0.01% random noise added to P_0. On the left (Fig. 2) is the high-concentration part of the calibration curve, showing the limit still at $A \sim 3$. But now the red curve looks noisy, due to the added random noise. On the right (Fig. 3) is the

Figure 2

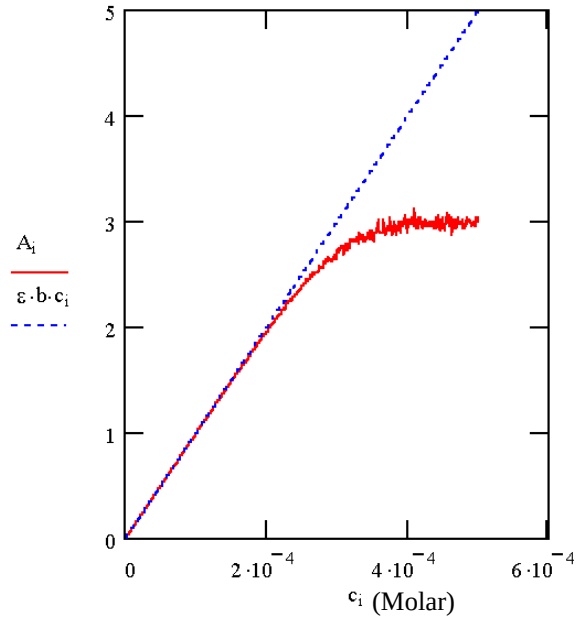
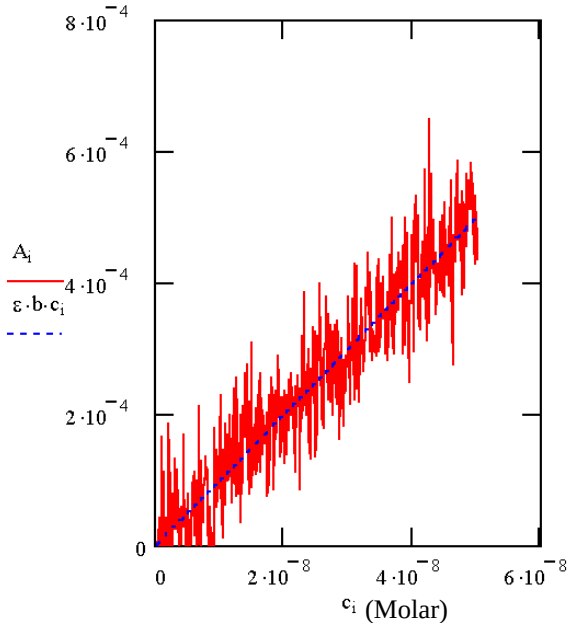


Figure 3



low-concentration part of the calibration curve on an expanded vertical axis. The noise on P_0 will not only affect the appearance of the calibration curve at high concentration, but, more importantly, it will affect the detection limit, which is at the low-concentration end of the calibration curve, as shown in Fig. 3.

As the analyte concentration becomes lower and lower and approaches zero, the absorbance decreases and $P \rightarrow P_0$, and any noise in P_0 limits the ability to determine A and also the concentration accurately. Eq. 4 below shows that the absorbance A also becomes noisy as the analyte concentration approaches zero, or as $P \rightarrow P_0$.

$$A_{\lim P \rightarrow P_0} = -\log\left(\frac{P_0 + P_{noise1}}{P_0 + P_{noise2}}\right) \approx \frac{P_{noise2} - P_{noise1}}{2.3 P_0} \qquad \text{(eq 4)}$$

To be thorough in this discussion, A in Eq. 4 describes noise having a standard deviation of σ_{blank} $= 0.707 \cdot \sigma / 2.3 \bullet P_0$, where σ_{blank} / P_0 was 0.0001 in this example. Normally you would not know σ_{blank} without measuring it, so this formula is of little practical use except to demonstrate that $A_{\lim P \rightarrow P_0}$ is controlled by noise. In practical terms, it means that the analyte in a sample can sometimes be too dilute to measure by absorption spectrometry.

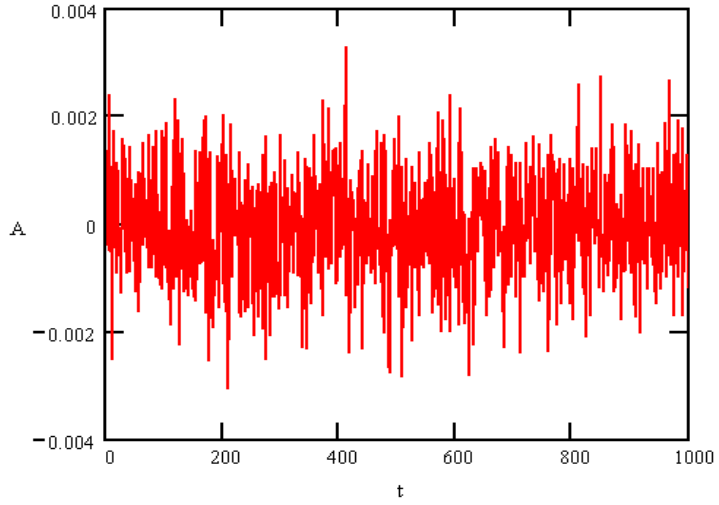
The important idea is that the limit of detection (*LOD*) is defined by the noise on the blank absorbance measurement, so in units of signal

$$LOD = \langle blank \rangle + 3\sigma_{blank} \qquad \text{(eq 5)}$$

where <*blank*> is the mean of the blank signal. Since the noise is random in this case, the fluctuations are equally as likely to be greater than zero as they are less than zero, <*blank*> is assumed to be zero in this case, and the limit of detection is $3\sigma_{blank}$, which we can calculate from the data. The factor of 3 is a factor commonly used in the analytical chemistry community, and it can be loosely interpreted as "My signal should be at least 3 times as large as the noise in order to be able to confidently say that it is a real signal and not due to noise." That value of 3 comes from the statistics of detection and presumes random noise.

Note that you can convert the LOD to concentration units by knowing the path length and the molar absorptivity.

Figure 4



You will measure absorbance for a blank solution as a function of time. An example of this is shown in Fig. 4 below. The instrument will allow you to collect these data and export them. You can then calculate the standard deviation using Excel or whatever software you prefer.

The noise on the blank sample is due to fluctuations in lamp intensity or P_0. The noise in Fig. 4 was created from hypothetical data using a Gaussian random number generator. In reality, the average intensity of a light source can drift over time, in addition to the fast fluctuations shown in Fig. 4. The behavior of noise in the instrument determines what strategy is used for attaining the best detection limit. In NMR, for example, lengthy signal-averaging runs improve signal-to-noise ratio (S/N) in proportion to the square root of time. This is the case because the noise in an NMR spectrometer is dominated by the detector and it behaves like pure random noise. In the absorbance spectrometer, much of the noise is from lamp intensity fluctuations, which are random on short time scales, but slowing drifting over long time scales. The purpose of this part of the experiment will be to observe the lamp noise of the absorbance spectrometer and calculate a detection limit (LOD) in absorbance units and in concentration units. Your Teaching Assistant will show you how to monitor absorbance for the blank as a function of time. Inspecting how A varies over time will reveal whether the fluctuations behave simply as random noise or if long-term drift of the light intensity is a significant problem. Calculating the standard deviation of A_{blank} from these data allows you to determine the detection limit.

PRE-LAB ASSIGNMENT

Do the pre-lab assignment in your laboratory notebook. This assignment is NOT always a guideline for the lab you will do; instead, the assignment will help you think about issues that will be important in the laboratory. You can use this assignment, and ones that follow, to prepare for the laboratory work.

Come to lab prepared to show this to your TA at the beginning of your lab period. Be prepared to discuss the issues with your teammates and with the TA.

1. Decide ahead of time what concentrations of quinine solutions you will prepare to obtain a calibration curve like that of Fig. 2, and what wavelength λ you will use to collect this curve. To minimize the amount of sample preparation time, choose 5 concentrations. For you to choose the right concentrations, you need some basis for guessing at the upper limit of A for the spectrometer and you also need to know the molar absorptivity ε for the analyte. Both of these can be figured out from Figs. 5 and 6. In Fig. 5, we provide an absorbance spectrum of tonic water, which contains quinine and sodium benzoate. Notice that above about 3.0 on the absorbance scale, the noise in Fig. 5 becomes quite large. In Fig. 6, you can calculate the molar absorptivity ε of quinine at its absorbance maximum λ_{max}. The pathlength b of the sample cell is taken as 1.00 cm. ***Do all of this ahead of time!***
2. Decide ahead of time what concentrations you will prepare to determine the detection limit of the spectrometer. Decide whether you even need to make any solutions or whether you can just repeat the blank many times. Explain in your notebook what you have decided to do.
3. To prepare your solutions, you will need to weigh out quinine sulfate and dissolve it in an aqueous solution of 0.05 M H_2SO_4. ***As always, use your best quantitative technique, and be sure to record in your lab notebook what you do and what glassware that you use***. We recommend that you prepare 50.00 mL of a quinine stock solution that is 25 times more concentrated than your lowest solution concentration. Given the formula weight for quinine sulfate, $(C_{20}H_{24}N_2O_2)_2\ H_2SO_4\ 2H_2O$ (782.96 g/mol), calculate how many grams of quinine sulfate you will need. *Note that quinine is a dimer in this salt.* You will also be given five

25.0-mL volumetric flasks for your five solutions, and 1.0-, 2.0-, 3.0-, 4.0-, 5.0-, and 10.0-mL volumetric pipettes. Calculate the volume of stock solution you will put into each flask before diluting to the mark with 0.05M H_2SO_4.

4. Using the two text file data sets **RANDOM_NOISE.TXT** and **DRIFT.TXT** available from the Canvas site (bullet 13 in the homepage) for Chem 438, plot the data and calculate the standard deviation for each. Think about this: if P_0 is drifting, will this hurt the detection limit because there is more fluctuation? How can one recognize drift in the spectrometer source? How can one correct for long-term drift?

EXPERIMENTAL

Part 1. Use your five solutions to make absorbance measurements to determine the upper limit of the absorbance spectrometer experimentally, as per your pre-lab work. Be sure to make all of your dilutions using the 0.05 M sulfuric acid solution as diluent so that the pH is low and constant. This ensures that the quinine sulfate will be soluble.

Part 2. Study the behavior of noise to determine the limit of detection for various integration times. Your TA will instruct you on the use of the instrument software so that you can measure absorbance and record your data on a USB memory stick (*please bring one to lab*) for analysis. (In the instrument settings, use a cycle time equal to the integration time.) Study the noise on the blank. This will use "kinetics mode" in the software, which allows you to store absorbance data for one wavelength as a function of time. You will only use a blank.

a. Chose a wavelength of 340 nm and an integration time of 1 s. Record a blank measurement, then record a signal for the same blank sample for 64 s. Save the data set, using your last name as the file name, in the proper subdirectory. You can later transfer it to your thumb drive and read it in Excel or whatever your software of choice is. First edit the text file in Notepad to remove the header at the beginning of the file.
b. Record a blank measurement for an integration time of 7 s, but acquire for 448 s. This gives you the same number of data points as above.
c. Repeat (b) using a 15-s integration time, but with a 960-s acquisition time (16 minutes) so that you have the same number of points in all three data sets.

SAFETY AND DISPOSAL

All solutions are to be disposed of in the non-organic waste disposal jug in the hood.

***Warning:* Sulfuric acid solutions, even though dilute, are corrosive. Wear gloves when handling the solutions.**

WRITTEN REPORT

1. Provide a plot of A vs. c and report your *graphical* estimate of the limiting absorbance. Fit the data in the plot to the functional form of Eq. 2, and report the more exact value of the limiting absorbance and also the ratio of P_{bkgd}/P_0.

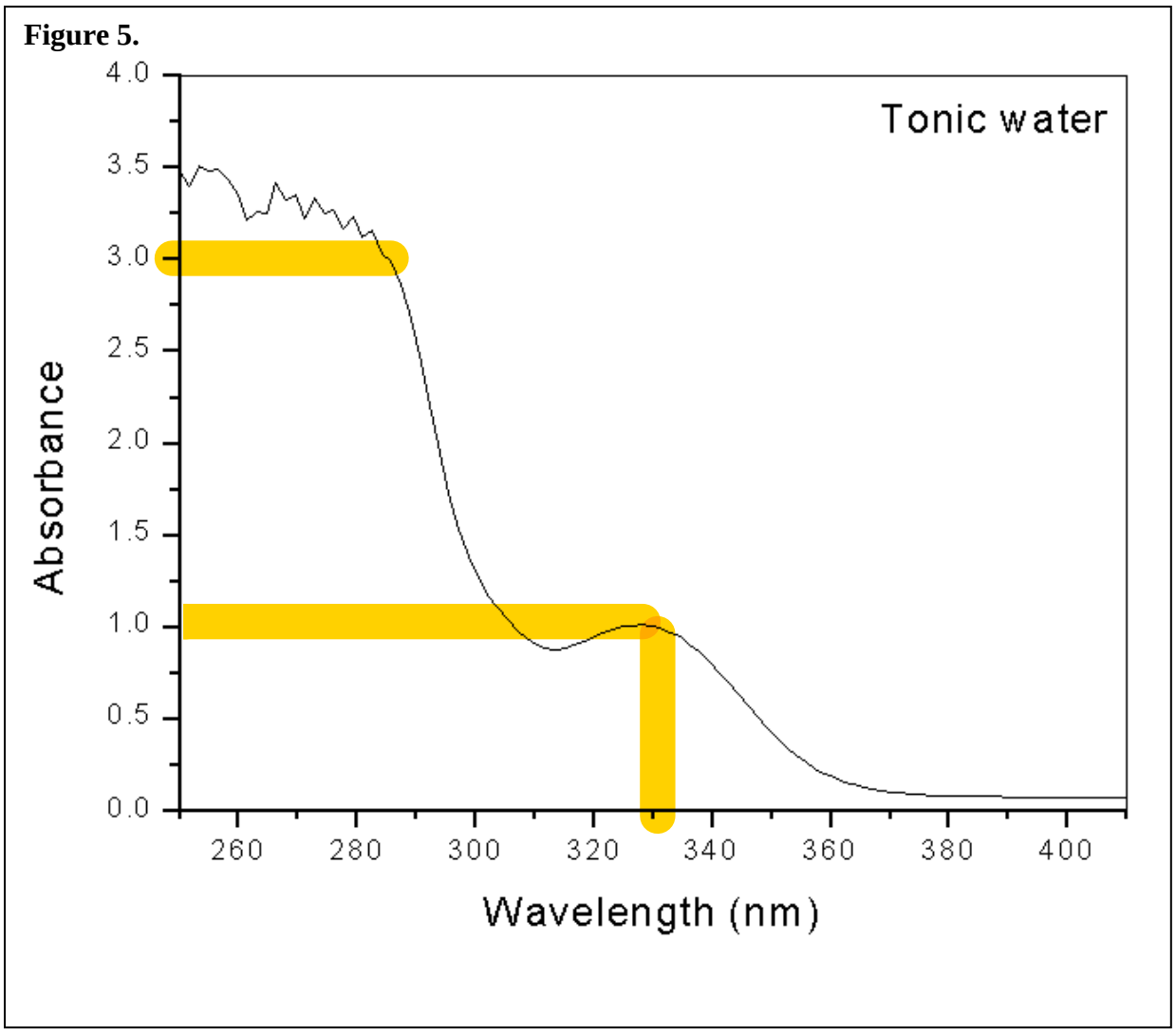
2. Plot the data for each of the three integration times in **Part 2**. Answer the following questions.
   a. For each data set, what is the standard deviation, and is it what you expect? What is the detection limit for each? How does the length of the acquisition affect the amount of drift?
   b. Do the data behave as random noise in each case? You can answer this by seeing if the value of absorbance at one time is influenced by or correlated with the value at the

previous time. Typically, there is some slow drift of P_0, giving correlation from point to point, but randomness over long periods of time.

c. Use Excel or other software to calculate a standard deviation for each of the three integration times. Calculate the limit of detection in absorbance units for each integration time. Summarize your results in a table.
d. Calculate the detection limit in molar concentration units for quinine.
e. Do longer integration times improve the detection limit? Please comment.

This absorbance spectrum was obtained on the same absorbance spectrometer you will be using.

Figure 5.



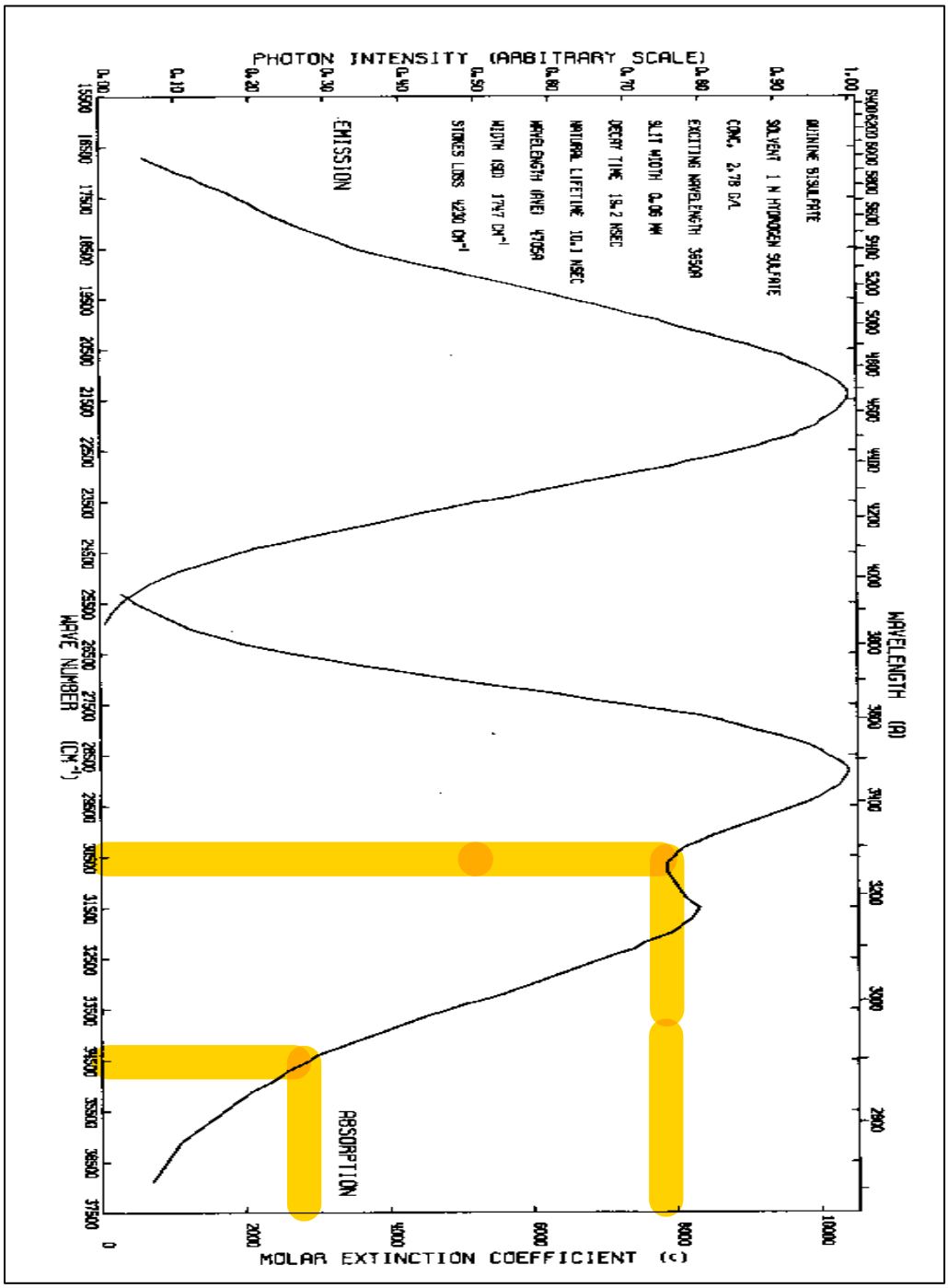


Figure 6. Excitation and Emission Spectra for quinine sulfate, from "Handbook of Fluorescence spectra of Aromatic Molecules",
I.B. Berlman, Academic Press, 1971.