

Instrument components, Signal to Noise, and Absorbance Spectra

Introduction

Analytical instruments are made of various components. A UV/Visible spectrometer consists of a light source ---> monochromator ---> sample ---> detector. The overall performance of the instrument is dependent on the characteristics of the individual components. It is much easier to make sense of analytical instruments if you think of them as consisting of individual components working together. Part A of this laboratory should help you in thinking of the characteristics of visible spectrometers in terms of individual spectrometer components; namely source intensity and detector sensitivity as a function of the wavelength of visible light.

In order to understand this laboratory a full appreciation for how absorbance spectra are measured must first be obtained. This understanding is critical and a lack of understanding will be clearly evident in the written report. A measurement of % transmittance (from which absorbance can be readily calculated) actually requires 2 measurements.

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

where I = intensity of light at a given wavelength that the detector senses when the sample is in the source beam, and I_0 = intensity of light at the same wavelength that the detector senses when a "blank" is in the source beam. There are at least two ways to obtain a %T or an absorbance measurement at a given wavelength which correspond to two fundamentally different types of instruments: single-beam and double-beam instruments. The fundamental difference between a single beam and a double beam instrument is how you record an absorbance spectrum or a spectrophotometric measurement. In single beam instruments (like the Spec 20 and Ocean Optics CCD array spectrometers) you have a blank, you put it in the instrument, make adjustments or measurements, take the blank out, and then obtain a measurement on your sample. In a double beam instrument, your reference ("blank") goes in one sample compartment, and your sample goes into an adjacent compartment. The measurement of both the sample and the blank is then made automatically by the instrument without switching cuvettes. This type of instrument splits the source light into two separate beams – hence the term double-beam. It is hoped that you understand the differences, both practical and fundamental aspects, between single beam and double beam spectrometers. It is also important that you understand the differences between single beam and double beam

spectra, and how the acquisition of absorbance spectra differ using the two instrument types as demonstrated in Part B of this laboratory.

Part C of this laboratory relates more to part A, instrument components, than part B. In part A you will determine how the characteristics of different instrument components, namely source output and detector sensitivity as a function of wavelength, affects overall instrument response as a function of wavelength. In part C instrument response will be related to signal-to-noise ratio. Signals and Noise is the title of chapter 5 in the Skoog text, and the concepts described in the first section (pp. 110-111) are important to understanding signal-to-noise ratio. In most measurements the amount of noise is constant and independent of signal, so increasing the signal will increase the signal-to-noise ratio. These concepts seemingly come from nowhere but should be quite familiar. Remember that all analytical measurements have error associated with them – called random error. This is the origin of discussions concerning the Gaussian distribution, standard deviation, and so on. In wet chemical analyses, like titrations, these errors resulted from the inability to read burets perfectly, and other such uncontrollable events. In instrumentation we use the term noise to describe the random error associated with an instrumental measurement. This noise, or random error, originates primarily from electronics in the instrument. The result is the same however. Signal on the other hand is the response one obtains from the analyte of interest. Thus signal-to-noise ratio is a very useful figure of merit, as it describes how easily the measurement is able to distinguish analyte signal from the ever present noise associated with any measurement. The greater the signal-to-noise ratio, the more easily the analyte can be detected from the noise or random error in the measurement. A more thorough investigation into other parameters that affect signal-to-noise ratio is performed in the signal-to-noise ratio trading rules lab.

A. Single Beam Instrument Response – Spectrometer Components

The Spec-20

The spectronic-20 spectrophotometer, an old mainstay in teaching labs for decades, is turned on by rotating the left-hand knob clockwise. This should be done at least 20 min before measurements are made. After the instrument has warmed up, the knob is used to adjust the phototube amplification so that the meter will read 0 %T when no light is striking the phototube (which is the case when no cuvette is in the sample holder--without an inserted cuvette a shutter blocks the optical path). This sets the dark current of the phototube.

The right hand knob regulates the amount of light passing through a second slit to the phototube. The need for this light-control knob arises because the light source does not emit light of equal intensities at different wavelengths and the phototube is not equally responsive to light of varying wavelengths. In addition, the "blank" solution (the solvent) may itself absorb at certain wavelengths. To measure the absorbance due to only the analyte in solution, extraneous parameters which affect the absorbance reading must be compensated for. Therefore, after the spectrophotometer has been zeroed (by means of the amplifier control knob), a blank solution is placed in the light path, and the light-control knob is rotated until the dial reads 100 %T to achieve the desired compensation. If a sample solution is now placed in the light path, any change in the absorbance (or % Transmittance) is due to the analyte.

Here is a table of relative phototube detector response versus wavelength. You will need this data for calculations in your write-up.

<u>Wavelength (nm)</u>	<u>Relative Sensitivity</u>
350	0.90
375	0.98
400	1.00
425	0.98
450	0.91
475	0.81
500	0.68
512	0.61
525	0.53
550	0.37
575	0.21
600	0.10
612	0.07
625	0.05

Table 1. *Spectronic 20 relative vacuum phototube detector response as a function of wavelength.*

The phototube is ten times more sensitive to light of wavelength 400 nm than to light of 600 nm. This means that the phototube will require a tenfold greater flux of 600 nm light than that of 400 nm light for the same detector output.

The Ocean Optics CCD Array Spectrometer

Although it appears at first that the Spec-20 and CCD array instruments are entirely different, and their instrument components differ by quite a bit, they perform the same function: the acquisition of visible spectra. Both instruments even perform the acquisition of spectra in fundamentally the same way in that they are both single beam instruments. The main difference is that the Spec-20 has a single vacuum phototube detector which detects each wavelength (or band of wavelengths) sequentially as the wavelength selector is manually scanned. The CCD array spectrometer has a 1-dimensional array of tiny charged coupled device detectors, each detector simultaneously sensing a single small band of wavelengths. This provides the ability of the array spectrometer to obtain the entire spectrum simultaneously. The figure below is a plot of relative CCD detector response as a function of wavelength.

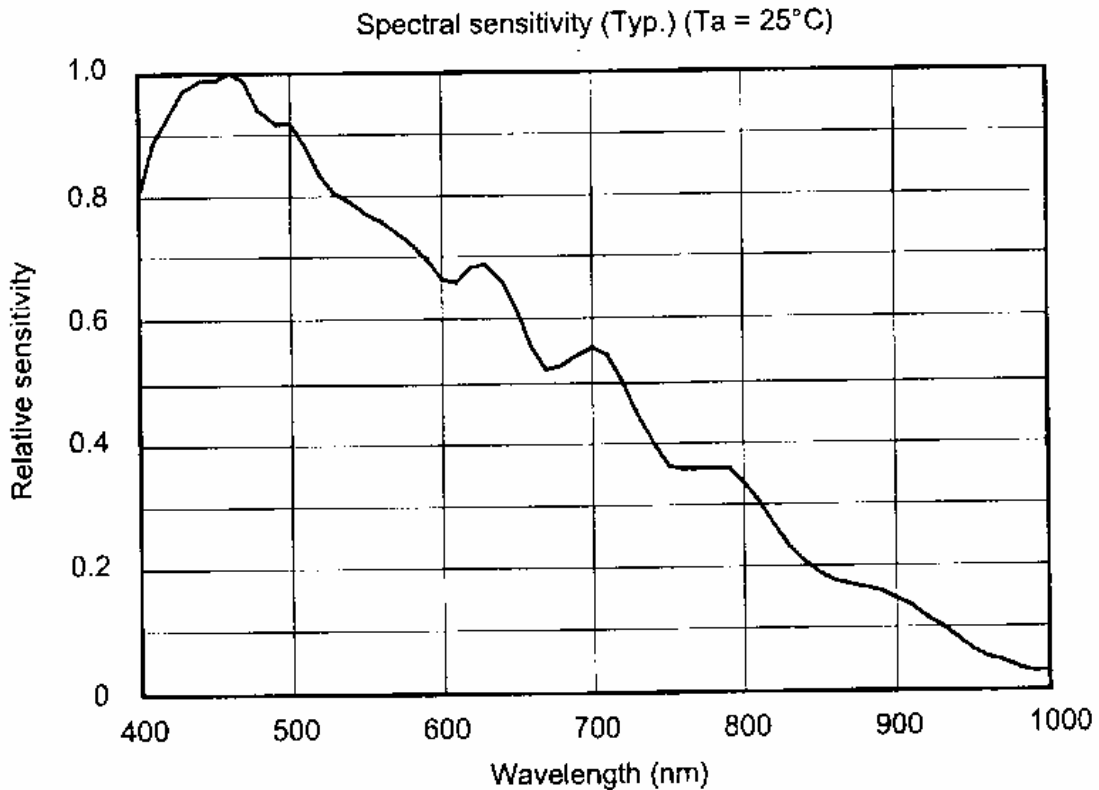


Figure 1. *CCD array detector response as a function of wavelength for the Ocean Optics array spectrometer.*

To obtain an absorbance spectrum with the CCD array spectrometer an equivalent procedure must be followed as with the Spec-20. First a correction must be made for “dark current”, detector signal in the absence of light, then a blank solution must be placed into the sample compartment and stored before an absorbance spectrum can be acquired. The second difference between the two instruments is the light source. The

Spec-20 uses a traditional blackbody radiator (an incandescent light bulb), while the CCD array spectrometer, to make it small and portable, uses an LED light source. Following the procedure below one can calculate and compare the source outputs of these two instruments.

Procedure (for instrument response section A)

With the spec-20 on and warmed up, adjust the amplifier control knob until the meter reads 0 %T. Place about 3 mL of distilled water into a rinsed cuvette and wipe with a Kimwipe. Insert the water filled cuvette (note the rules for cuvette use in the single beam absorbance spectrum section). Close the cover to eliminate stray light, set the wavelength control knob to 510 nm, and rotate the light control until you read about 90 %T. By rotating the wavelength knob, scan the visible spectrum and note how the response (measured by needle position) varies with wavelength. Determine the exact wavelength to which the instrument is most responsive (near 510 nm), and adjust the light control to read 100 %T at this wavelength. *Without readjusting* either the amplifier or light control knobs, determine the %T readings at the wavelengths given in Table 1. A plot of %T versus wavelength of your data collected constitutes a single beam visible spectrum of water. The use of %T on the y-axis is misleading however, since that is not what was really measured. (Remember $\%T = I/I_0$, and only one measurement was made at each wavelength, not two.) What you have really measured is the single beam water spectrum, which is the same as the instrument response as a function of wavelength, since distilled water does not absorb in the visible region.

A single beam spectrum of water (or instrument response since water has no visible absorbance) is readily visualized and demonstrated using the CCD array spectrometer. In scan [S] mode, place a cuvette of distilled water into the spectrometer. The spectrum seen when you do this with the CCD array spectrometer (wavelength in nm on the x-axis vs. detector response or counts on the y-axis) is the single beam water spectrum or instrument response. Using the CCD array spectrometer software find the instrument response at the same wavelengths as in Table 1 for the Spectronic 20.

For your interest only – don't write up the results

Now place the special cuvette into the sample holder to observe the color of the light beam. Rotate the cuvette until the light path strikes the sloping surface of the chalk. Observe and record the color of the beam every 50 nm from 650 to 350 nm. It may be necessary to rotate the light control knob to increase the intensity of the light, but do not

allow the meter needle to go off scale. Adjust the wavelength to 600 nm and note the variation in color across the band of light. What range of wavelengths of light comprises the band you see?

For your report, here are things to consider – part A.

1. Plot the Spec-20 phototube response with relative response on the y-axis and wavelength on the x-axis from Table 1 data. On the same plot do the same for the CCD detector response by estimating from data provided in Figure 1.
2. Plot the source intensity versus wavelength as % relative intensity on the y axis and wavelength on the x-axis. This can be done by taking the instrument response and dividing by the phototube response at the various wavelengths, normalizing to the largest value and putting in percentage terms. Overlay these data for the two instruments in another figure.
3. Plot and compare the instrument response curves for these instruments with wavelength on the x-axis. Although you read %T with the Spec-20 and counts with the array spectrometer, the y-axis is more properly labeled instrument response or emittance in both cases. Since the scales are different, normalize them both to 100 so they can both be compared on the same scale. Overlay these plots on a figure and label them.
4. Compare the instrument response single beam spectra (water spectra) of the array spectrometer to that obtained with the Spectronic-20. Do these single beam spectra look the same or different? Explain.
5. To what wavelength of light is each instrument most responsive? How does this correlate with the source intensity and detector response? Explain these results.

The answers to these last two questions should be put in your Results & Discussion section, or the Discussion section if that is separate. It should be written so that this flows directly and not written to read as a direct answer to a posed question.

B. Absorbance Spectra

Absorbance Spectrum Procedure

Here is some practical advice on handling cuvettes, which is very important. Often two or more cuvettes are used simultaneously, one for the blank solution

and the others for samples. Any variation in the cuvette (such as a change in cuvette width or the curvature of the glass, stains, smudges, or scratches) will cause varying results.

- A. Do not handle the lower portion of a cuvette (through which the light beam passes).
- B. Always rinse the cuvette with several portions of the solution before taking a measurement.
- C. Wipe off any liquid drops or smudges on the lower half of the cuvette with a clean lint-free wiper (like Kim-wipes) before placing the cuvette in the instrument. Never wipe cuvettes with towels. Inspect to make sure that no lint remains on the outside and that air bubbles are not present on the inside walls.
- D. When inserting a cuvette, line up the index lines exactly.
- E. When using two cuvettes simultaneously, use one of the cuvettes always for the blank solution and the other cuvette for the various samples to be measured. Mark the tubes accordingly and do not interchange the cuvettes.

Single Beam Spec-20

1. Since this is a single beam instrument only one cuvette is required, however it is much more convenient to use two matched cuvettes to obtain an absorbance spectrum with this instrument. Finding matched cuvettes involves finding two test tubes which have the most similar response in the spectrometer. Label the test tubes near the top and half fill each clean test tube with CoCl_2 solution.
2. Set the Spec20 wavelength to 510 nm, adjust for the dark current, and place a tube in the spectrophotometer. Use the vertical line on the test tube for alignment purposes. Adjust the light control so the meter reads 50 %T. Check the other tubes and record the %T of each. Use the two tubes which match best as your matched set.
3. Obtain about 25 mL of approximately 0.075 M Co(II). Record the actual concentration of the solution you ended up with.
4. Place about 3 mL of D.I. water in one of the matched cuvettes, and about 3 mL of the Co(II) solution in the other matched cuvette. Set the spectrometer to the lowest reasonable wavelength (around 375 nm) available. Zero the dark current (with nothing in the sample holder), then place the D.I. cuvette in the sample holder. Adjust the light

control knob until you get a 100 %T reading on the meter. The instrument is now ready for a measurement.

5. Place the Co(II) cuvette into the sample holder. Record the %T reading indicated on the meter.

6. Turn the light control adjustment counterclockwise (to protect the phototube), adjust the wavelength control to 400 nm, and repeat step 4 (you will need to readjust for dark current and blank for each new wavelength). Repeat every 10 nm from 400 nm through 800 nm.

7. Convert the %T values for each wavelength to absorbance values ($Abs = -\log T$, and $\%T = T \times 100$) and plot absorbance vs. wavelength. This is an absorbance spectrum.

Single Beam CCD Array

1. It is convenient to use a single cuvette for this single beam instrument since all wavelengths will be analyzed simultaneously with the detector array. Blocking the light source from reaching the detector, store a “dark” spectrum. Then check the correct for electrical dark box on the spectrum screen. Put distilled water in the cuvette and store a reference spectrum. This will make the [A] button for acquiring absorbance spectra active, thus making it possible to collect absorbance spectra. Collect and store an absorbance spectrum of the $CoCl_2$ solution.

Double Beam

1. Obtain two matched cells for use in the double-beam UV/VIS instrument. Use the Shimadzu UV160U. The instrument should be started, your instructor will show you how to use the instrument when you need to. Using good cuvette protocol (see above), put D.I. water into one of the cells to serve as the reference cell and put your Co(II) solution into the other. Place each cell into the appropriate location in the instrument.

2. Acquire an absorbance spectrum from 400-800 nm. Plot the spectral data for inputting to a spreadsheet for your report.

For your report, here are things to consider – part B.

1. Compare the CoCl_2 absorbance spectra acquired on the three instruments (include figure or figures), not only the spectral results but also the process of acquiring the spectra using the single beam instruments and the double beam instrument.
2. Assuming the CoCl_2 absorbance spectra appear similar or even essentially identical with the CCD array and the Spec-20 instruments, do you think that the single beam spectra would also be similar? Explain. The answer to this question can be placed in the results and discussion or the discussion section if separate from results. If there is no place to do it and make it sound OK in the body of your report, pose the question at the end as an addendum and answer it there.

C. Signal to Noise Ratio and Instrument Response

To a first approximation noise is independent of signal strength. It may thus be instructive to compare the signal-to-noise ratio in two regions of the spectrum: one where instrument response is quite high and another where instrument response is relatively low.

Procedure

Using the CCD array spectrometer obtain a reference. There is no need to use a cuvette, although if it makes you feel better a cuvette can be filled with distilled water and placed into the spectrometer for all measurements. Once the single beam reference spectrum is obtained, the other buttons to obtain absorbance, transmittance spectra, etc. become active. Press the [T] button to acquire a transmittance spectrum. If the world and the spectrometer were perfect, you would get a perfectly straight line at 100% transmittance, since $\%T = I/I_0 \times 100$, and both I and I_0 are acquired under identical conditions. You will not, because the world is not perfect and the spectrometer exhibits noise or random error.

1. Acquire a “spectrum” in % Transmittance and store it to export the data to a spreadsheet. Calculate the signal-to-noise ratio in the region from 400-500 nm and from 650-750 nm.

For your report, here are things to consider – part C.

1. Calculate the RMS S/N ratio of the instrument in the two spectral regions ($S/N = \text{Mean}/\text{Standard Deviation}$). Export the data to a spreadsheet to perform the calculations more easily. Comment on why the signal-to-noise is greater in one region than the other, and how that might affect a quantitative spectrophotometric analysis of an analyte at a low concentration.