

## Spectroscopy Final

Part I (32 points). An important part of understanding spectroscopy is a good working understanding of the terminology used by spectroscopists. Briefly define four of the following five terms. Limit your responses to no more than two or three sentences each and be sure to indicate clearly the definitions you wish me to review.

a. period

The period is the time it takes for one cycle of a wavelength to pass a given point. It is the reciprocal of the frequency.

b. thermal noise

Thermal noise is present in all electronic circuits and is a fluctuation in current due to the thermal agitation of electrons. Thermal noise is present even in the absence of an analytical signal.

c. chopper

A chopper is a device included in the optics of some spectrometers. It can serve a couple of purposes, one of which is to block periodically the source from reaching the detector. It also can be used to send the source radiation along different paths.

d. spectral bandwidth

The spectral bandwidth is a measure of the width of radiation (in nm) that makes it through the spectrometer's optics to the sample (or detector). Typically the bandwidth is measured at half-height.

e. response time

The response time is the amount of time it takes the input transducer (detector) to respond to a change in the analytical signal. For example, if the source radiation is turned off, the response time is the time it takes for the detector to reflect that  $P_T$  is zero.

Part II (48 points). Shown below are four general discussion questions. Please develop answers to three of these questions, limiting each response to 5 – 10 sentences (use the back of the page if your writing style is too large for the available space). You may wish to organize your thoughts on a piece of scratch paper before you begin writing. To make good use of the limit on sentences your answers must concisely address the question's main points. Be sure to indicate which answers you wish me to review

- a. Although atomic absorption spectroscopy operates in the UV/Vis range, it uses a very different type of light source than a typical molecular UV/Vis spectrometer. Explain why these two types of instruments must use different types of sources.

In UV/Vis absorption the analyte is undergoing a change in electronic energy levels. For a molecule this transition also involves changes in vibrational and rotational energy levels, producing a fairly broad absorption band that typically is greater than 20 nm. In the case of an atom, however, the transitions are purely electronic, producing narrow absorption bands with widths of approximately  $10^{-3}$  nm. Molecular UV/Vis uses a continuous source that emits at all wavelengths and then uses a monochromator to narrow the width to approximately 1 nm. Even this width, however, is too wide for an atomic absorption line as it would produce a  $P_T$  that is indistinguishable from  $P_0$  because light is absorbed at such a narrow width of wavelengths. Instead, atomic absorption uses a hollow cathode lamp, which is a line source with bandwidths that are approximately the same as that for the absorption lines.

- b. One of the three general limitations to Beer's law is that due to the instrumentation. Briefly explain the two types of instrumental limitations, the effect that each has on a Beer's law calibration curve, and how the design of molecular UV/Vis instrument can minimize these limitations.

The two instrumental limitations are the presence of stray radiation and the absence of monochromatic light. Beer's law assumes that the absorbed radiation is purely monochromatic; that is, that the radiation is of a single wavelength. Even the best instrument, however, passes a finite bandwidth that encompasses a range of wavelengths. The effect of non-monochromatic radiation is a non-linear Beer's law calibration curve at larger concentrations of analyte. Using the monochromator's smallest possible slit width minimizes this problem by decreasing the bandwidth. Stray radiation is any radiation that reaches the detector without passing through the sample. This has the effect of increasing both  $P_T$  and  $P_0$  and is a problem at higher concentrations of analyte when  $P_T$  is small. The effect is to increase the apparent transmittance and to decrease the apparent absorbance. Making the instrument light tight and measuring the dark current are the best ways to minimize this problem.

- c. Two common designs for a UV/Vis spectrometer's optics are the single-beam and the double-beam. Explain the difference between these two designs. What advantages are there in choosing a double-beam design over a single-beam design? What are the disadvantages, if any, of the double-beam design?

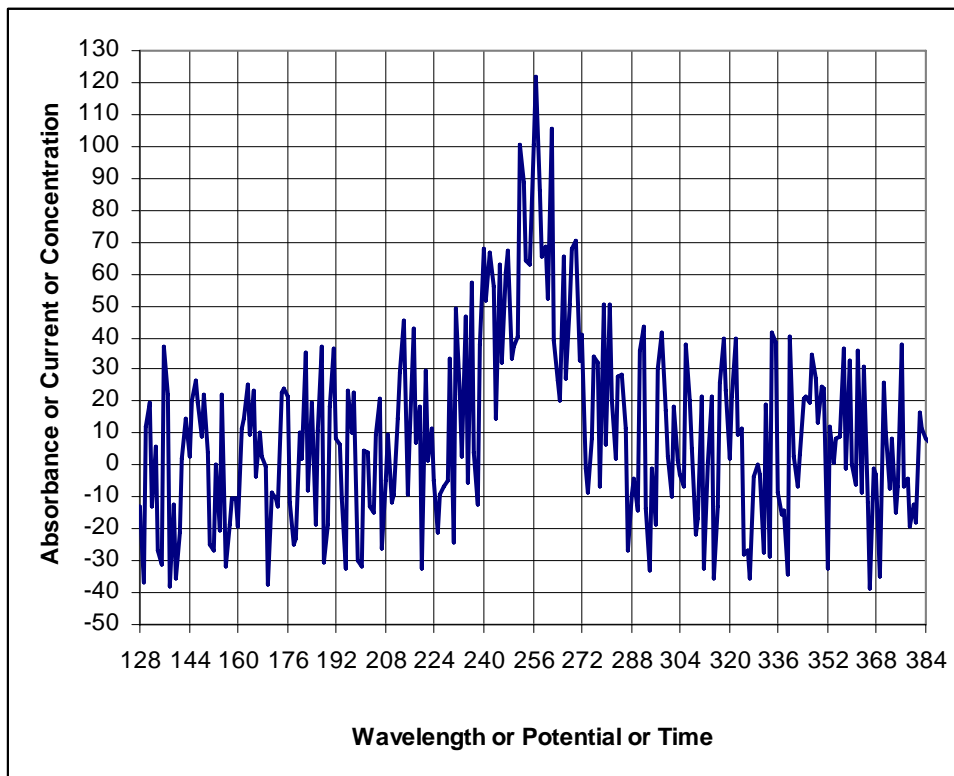
In a single-beam spectrometer there is one path from the source to the sample to the detector. In a double-beam spectrometer there are two paths from the source to the detector, with one path passing through the sample and the other path passing through a reference sample. A chopper is used to alternately direct the light between the two paths; the chopper also serves as a shutter. The biggest advantage of a double-beam spectrometer is that the two paths allow for a constant measurement of  $P_0$  using the reference, which limits flicker noise resulting from drift in the source radiation's intensity. The use of the chopper as a shutter also allows for a constant determination of the dark current. The main disadvantages are that the instrument is more complex, which increases both the original cost and the expense of maintenance.

- d. Atomic absorption spectroscopy is susceptible to two serious matrix interferences: the presence of non-volatile species and ionization. For each, explain the source of the interference, the effect it has on the quantitative analysis of an analyte, and how it can be corrected or minimized.

In atomic absorption spectroscopy it is essential that the analyte be converted into its free atom form. In some cases the analyte is in a matrix that favors the formation of a non-volatile species that is so stable that it cannot be broken down into free atoms by the energy of the spectrometer's flame. This suppresses the formation of free atoms in the flame and, therefore, results in an underestimation of the analyte's concentration. Adding a releasing agent or a protecting agent helps solve this problem. For example, EDTA forms strong complexes with many metal ions that will successfully atomize in the flame because EDTA, being organic, is combusted in the flame. Some atoms are easily ionized in the flame, which presents a problem because the ion's absorption lines will not be the same as that for the free atom. Again, this leads to an underestimation of the analyte's concentration. Adding a species to the sample, such as Li, that is more easily ionized will limit this problem by increasing the concentration of electrons in the flame, which shifts the analyte's ionization equilibrium back toward the free atom.

Part III (40 points). Please provide solutions to all three of the following quantitative problems. Be sure to neatly organize your work so that it is easy to follow and to show enough detail in your work so that partial credit can be assigned. You may use a spreadsheet or other software to help with the calculations; be sure, however, to attach appropriate copies of your work and to explain what your work.

- a. Shown below is a noisy signal obtained in a single scan. Estimate the signal-to-noise ratio for the largest peak showing all of your work. What signal-to-noise ratio would you expect if you were to co-add 16 spectra?



A variety of answers are acceptable here due to the need to estimate the mean signal and the standard deviation of the noise. The mean signal is **not** the maximum signal, which includes some contribution from noise; any estimate between 80 and 100 is acceptable. The peak-to-peak noise is 80 (maximum of 41 and minimum of -39), but values close to this are acceptable. The standard deviation is 1/5 of the noise, or 20. Your reported signal-to-noise ratio, which is the ratio of the mean signal to the standard deviation of the noise, should be between 5 and 6.25.

The signal-to-noise ratio improves by the  $n^{1/2}$ , where  $n$  is the number of co-added scans. For 16 scans, therefore, your reported signal-to-noise ratio should be 4x larger, or between 20 and 25.

- b. Phosphorous in urine can be determined by treating a sample with molybdenum (VI) and reducing the resulting phosphomolybdo complex with aminonaphtholsulfonic acid to give the characteristic molybdenum blue color that absorbs at 690 nm. A patient excreted 1270 mL of urine in 24 hours. A 1.00-mL aliquot of the urine was transferred to a 50-mL volumetric flask and treated with the molybdate reagent and aminonaphtholsulfonic acid. After diluting to volume its absorbance was found to be 0.625 in a 1.00-cm cell. A series of standard phosphate solutions containing 1.00, 2.00, 3.00, and 4.00 ppm P were prepared and analyzed in the same manner as the urine sample giving absorbencies of 0.205, 0.410, 0.615, and 0.820, respectively. Calculate the number of grams of P excreted by the patient during the 24-hour sampling period.

From the calibration data you should obtain a calibration curve of

$$\text{Abs} = 0.205[\text{P}] - 4 \times 10^{-16}$$

Using the sample's absorbance of 0.625, gives the concentration of P in the sample, as analyzed, 3.05 ppm. Since the sample and the standards were treated identically, this also is the concentration of P in the urine (note: there is no need to account for the dilution of the 1.00 mL aliquot of urine since the standards were diluted in the same manner). The mg of P in the urine, thus, is

$$\text{mg P} = 3.05 \text{ mg P/L} \times 1.270 \text{ L} = 3.87 \text{ mg P}$$

- c. Many pharmaceutical compounds absorb strongly UV radiation. For example, tetracycline and epitetracycline have the following molar absorptivities (assume three significant figures):

compound	$\epsilon$ ( $\text{M}^{-1} \text{cm}^{-1}$ )	
	254 nm	267 nm
tetracycline	16000	19000
epitetracycline	16000	15000

A mixture of tetracycline and epitetracycline give absorbencies of 0.402 at 254 nm and 0.432 at 267 nm in a 1.00-cm cell. What are the concentrations of these drugs in the mixture?

To find the concentrations of each we set up the following pair of simultaneous equations:

$$0.402 = 16000[\text{T}] + 16000[\text{E}]$$

$$0.432 = 19000[\text{T}] + 15000[\text{E}]$$

Solving the two equations gives

$$[\text{E}] = 1.13 \times 10^{-5} \text{ M and } [\text{T}] = 1.38 \times 10^{-5} \text{ M}$$

Part IV (48 points). The following three questions are based on the paper “The SLIM Spectrometer” by K. M. Cantrell and J. D. Ingle, Jr. Please answer all three questions. There is a link to the paper on the course web site’s archives page.

- a. At the beginning of the course we noted that an instrument consists of a signal generator, an input transducer, a signal process, and an output transducer. In addition, the instrument generates an analytical signal, an input signal, an output signal, and a final result. Using this list of components, signals and result, deconstruct the SLIM spectrometer. Be sure to clearly explain your reason(s) for each assignment.

There are two answers you could provide. The first possibility, which includes the eventual need to use an external computer to retrieve the data, is:

signal generator: LED’s, sample and flow cell  
analytical signal: attenuation of photons  
input transducer: photodetector  
input signal: frequency  
signal processor: microcontroller (an analog-to-digital converter)  
output signal: digital data that is stored in EEPROM  
output transducer: external computer  
final result: absorbance values

The second possibility focuses solely on the SLIM spectrometer and ignores the eventual need to use an external computer to retrieve the data; thus:

signal generator: LED’s, sample and flow cell  
analytical signal: attenuation of photons  
input transducer: photodetector  
input signal: current  
signal processor: current-to-frequency converter  
output signal: frequency  
output transducer: microcontroller (analog-to-digital converter)  
final result: digital data that is stored in EEPROM

- b. An important part of any spectroscopic instrument that measures absorbance is the need to establish a dark current and an instrument blank (or reference). Briefly define each term, explain why it is necessary to determine its value and explain how its value is established with the SLIM spectrometer.

The dark current is any current at the detector that is present even in the absence of the source radiation. There are two important contributions to the dark current: stray light and thermal noise. The SLIM spectrometer measures the dark current once per cycle when all three LEDs are turned off. In addition, the SLIM spectrometer is wrapped in black felt to minimize stray light.

Because the flow cell and the sample's matrix can affect the throughput of radiation from the source to the detector, it is necessary to establish  $P_0$  by replacing the sample with a blank. In the SLIM spectrometer the instrument blank is determined by measuring  $P_0$  for each LED for the first five cycles and then averaging the result for each. Presumably the flow cell is filled with distilled water (or some other suitable blank) so that the sample is not in the flow cell during this process.

- c. Briefly critique the SLIM spectrometer with respect to its usefulness as a visible spectrometer. Among the issues you should address are whether it can be used throughout the entire visible region, and whether there are regions where it is more or less susceptible to noise. Be sure to clearly justify your data by pointing to specific information or details in the paper's text, tables, and/or figures.

There are many possible responses to this question. A good critique of the SLIM spectrometer, however, provides an evaluation of both its weaknesses and its strengths. A key summary of the spectrometer's performance is provided by the authors on page 34: "In the design of the SLIM spectrometer, selectivity, detection limit (calibration slope), and linearity are somewhat compromised in order to gain simplicity, low cost, small size, low power consumption, remote data-logging capability, and versatility." Your response should pick up on several of these, from each category, and discuss them using evidence from the paper.

Part V (32 points). On the following page is a description of the neocuproine method for the spectrophotometric determination of copper in wastewater. Briefly answer four of the following five questions in the space provided. Be sure to clearly indicate which answers I should evaluate.

- a. How would you prepare a blank for this analysis?

The best blank takes 100 mL of water through all steps in the procedure.

- b. The first step of the analysis removes any cyanide, sulfide and soluble organic material from the sample. Why is this step necessary? You might try to draw on your knowledge of inorganic chemistry from Chem 130.

All of these species bind with  $\text{Cu}^{2+}$  and prevent, or limit, its reduction to  $\text{Cu}^+$ , its complexation with neocuproine, and its extraction into  $\text{CHCl}_3$ . The extent to which this happens will reduce the reported concentration of  $\text{Cu}^{2+}$  in the sample.

- c. Although not specifically mentioned, this method is subject to interferences from oxidizing agents. What effect would the presence of an oxidizing agent have on the analysis?

An oxidizing agent will prevent the complete reduction of  $\text{Cu}^{2+}$  to  $\text{Cu}^+$  either by reoxidizing the copper or by reacting with the hydroxylamine hydrochloride. The effect of the oxidizing agent is to reduce the reported concentration of  $\text{Cu}^{2+}$ .

- d. The minimum detectable absorbance that can be measured with confidence is 0.010. What is the smallest concentration of copper, in  $\mu\text{g/L}$ , that can be determined by this method?

An absorbance of 0.010 corresponds to a concentration of  $1.25 \times 10^{-6}$  M in the 25 mL extract. The copper in the extract was in the 50 mL aliquot placed in the separatory funnel; thus, the concentration in the aqueous sample is  $\frac{1}{2}$  of this, or  $6.25 \times 10^{-7}$  M. This also is the concentration of the copper in the 100-mL volumetric flask and in the original sample. Converting to ppb gives the concentration as 39.7  $\mu\text{g/L}$ .

- e. Suppose that you are to analyze a sample of seawater for which the concentration of copper is expected to be greater than 40 mg/L but less than 60 mg/L. What problem does this create and how can you modify the procedure so that you can successfully analyze the sample?

The maximum concentration of copper in the extract is 0.2 mg in 25 mL. Since the copper comes from the original sample, the portion of the sample that is analyzed must contain no more than 0.2 mg. Half of the copper in the original 100-mL sample is taken into the separation step; thus, the maximum concentration of copper in the original solution is 0.4 mg/0.1 L, or 40 mg/L. To modify the procedure the analyst begins by taking a sample containing less than 0.4 mg and diluting it to 100 mL. The remainder of the procedure can remain unchanged.

**The neocuproine method for copper in wastewater.** In this method copper in a +1 oxidation state reacts with the ligand neocuproine (2,9-dimethyl-1,10-phenanthroline) to form a complex of  $\text{Cu}(\text{neocup})_2$ . The complex is extracted into a chloroform-methanol mixture, giving a yellow solution with a molar absorptivity of  $8000 \text{ M}^{-1} \text{ cm}^{-1}$  at a wavelength of 457 nm. Beer's law is obeyed up to a concentration of 0.2 mg Cu/25 mL of extraction solvent. Full color development occurs when the sample's pH is between 3 and 9. A typical procedure is provided here:

A 100.0-mL sample is placed in a 250-mL beaker, acidified with 1 mL of  $\text{H}_2\text{SO}_4$  and 5 mL of  $\text{HNO}_3$ , and boiled to destroy any traces of cyanide, sulfide, or organic material that may be present. The remaining sample is transferred to a 100-mL volumetric flask and diluted to volume. A 50-mL portion of this sample is transferred to a 250-mL separatory funnel and 5 mL of a hydroxylamine hydrochloride solution is added to reduce the  $\text{Cu}^{2+}$  to  $\text{Cu}^+$ . A 10-mL portion of a sodium citrate solution is added to complex any metal ions in the sample that might precipitate when the sample's pH is adjusted. A solution of 5 M  $\text{NH}_3$  is added in 1-mL increments until the pH is between 4 and 6. A 10-mL portion of neocuproine is added along with 10 mL of  $\text{CHCl}_3$ . The contents of the separatory funnel are shaken and the layers are allowed to separate. The  $\text{CHCl}_3$  layer is drained into a 25-mL volumetric flask and diluted to volume with methanol. The absorbance of the  $\text{CHCl}_3$ - $\text{CH}_3\text{OH}$  solution is measured at 457 nm in a 1.00 cm cell. A stock solution of copper is prepared by dissolving Cu wire with  $\text{HNO}_3$ . Working standards of appropriate concentration are prepared from this stock solution.