Key for Spectroscopy Final

The final exam for spectroscopy consists of three parts: questions that require short written responses; questions that require longer written responses; and questions that require calculations. You are free to use your textbook, the library, web resources, your work on take-home assignments, handouts, and your notes as you work on this exam. You are not free to discuss any portion of this exam with other students or with faculty members other than the instructor.

Your responses are due by 4:00 pm on Friday, September 29th. As you have access to lots of resources and ample time to work on the exam, your final product should be organized and neat. Answers to questions that require a written response must be typed, thoughtful, rich in detail, and written in complete sentences and, where required, in well-developed paragraphs. You may wish to annotate your response with appropriate figures drawn from resources available to you.

For questions that require calculations, organize your work so that the logic of your approach is clear. Please be sure that your work is neat and easy to follow, and that your final answer is clear. You may wish to annotate your work with short written descriptions that explain your approach to the problem.

Questions that draw on the paper "Molecular Absorption Measurements with an Optical Fibre Coupled Array of Ultra-Violet Light-Emitting Diodes" by D. A. Bui, K. G. Kraiczek, and P. C. Hauser refer to the BKH paper. If you need a copy of the paper, you can access it by entering the following DOI into a browser: https://doi.org/10.1016/j.aca.2017.07.007.

The exam has 200 points, distributed as follows: Part I has 32 points; Part II has 96 points; Part III has 72 points.

Part I: Questions with Short Written Responses (2–4 Sentences Each)

Question IA. In the study area section of the Chem 260 lab you will find three items extracted from old instruments that no longer are in use. Examine these items and answer the following questions. Be sure that each response explains why you are confident in your identification.

- Item 1. What is the role of the part identified by the pink post-it-note? The item itself is a portion of a detector that was used with an HPLC.

  Answer. The key to identifying this item is to note that it is located between the light source and the detector. It clearly is designed to move up and down. In its down position, it blocks light from reaching the detector; in its up position, light can pass through an optical window and reach the detector. This is a shutter that allows the instrument to continuously adjust for the dark current; that is, to keep 0 %T properly adjusted.

- Item 2. From what type of instrument was this part taken?

  Answer. This optical bench includes two monochromators. The only instrument we examined that requires to monochromators is one for monitoring fluorescence, where the first monochomator is used to select the excitation wavelength and the second monochromator is used to select the wavelength for measuring the fluorescent emission.

- Item 3. What is this part, which was recovered from an old atomic absorption spectrometer?

  Answer. An atomic absorption spectrometer needs a light source, a monochromator, a flame or furnace for atomizing the sample, and a detector. Of these options, the only possibility is a detector. Indeed, this is a photomultipier, which is used to convert photons to electrons and, as a result, into a current that we can measure.

Question IB. At the beginning of the course, we examined two broad ways to characterize an instrument: as a block diagram and as a flow of information. We described the block diagram as consisting of a *stimulus*,

a *sample*, and *analytical information*, and we described the flow of information as moving from an *input transducer*, to a *signal processor*, to an *output transducer*. Map each of the italicized terms to Figure 1 in the BKH paper, which shows a schematic diagram of the spectroscopic instrument described in this paper.

Answer. A block diagrams considers analytical information as the result of applying a stimulus to a sample, where a stimulus is the result of fundamental laws of chemistry and physics. For the instrument in the BKH paper, the *stimulus* is the absorption of light from the LED by the *sample*, which is in the cuvette or flow cell, and the *analytical information* is a measurement of absorbance by the photodiodes and the log-ratio amplifier.

When considering the flow of information, the *input transducer* is that part of an instrument that converts a chemical or physical property into an electrical signal, which for the instrument in the BKH paper includes the LED array, the optical fibers, the ball lenses, the sample cell, and the photodiodes; that is, everything but the log-ratio amplifier. The *signal processor* converts the signal from the input transducer into a more useful form; in this case, the log-ratio amplifier converts the intensities of light from each path—expressed as currents—into a voltage that is proportional to absorbance: $V = \log(i_0/i)$. The *output transducer* converts the output from the signal processor into a readable result; although not shown in Figure 1, the paper notes that they used an "e-corder data acquisition system (model ED401) and the Chart software package from EDAQ (Denistone East, NSW, Australia) running on a personal computer were used for the digitization and recording of signals."

Question IC. An important part of any instrument that measures absorbance is the need to establish the limits for transmittance. What are these limits and how are they defined? How are these limits established for the instrument described in the BKH paper?

Answer. The two limits are 0% transmittance, which is the signal at the detector when the photons from the light source are not allowed to reach the detector, and 100% transmittance, which is the signal at the detector when photons from the light source pass reach the detector after passing through the instrument's optics and passing through an analyte-free sample. The former often is called the dark current and the latter is called the reference.

For the instrument in the BKH paper, the dark current is measured by turning off the LED light sources and recording the current at the detector. The instrument allows for a continuous monitoring of 100%T as a portion of the light from the LEDs is split into two beams, with one beam passing serving as a reference (5, 8, and 10 in Figure 1).

Part II: Questions with Longer Written Responses (2–5 Paragraphs Each)

Question IIA. An atomic absorption spectrometer requires a very different light source than does an instrument for molecular absorption. Describe the light source used by each instrument and explain why these two instruments need such different light sources. As part of your answer, explain the consequences if you interchange the two types of sources.

Answer. A strong answer to this question will discuss the following points:

- an instrument for molecular absorption usually uses a continuous source and an instrument for atomic absorption always uses a line source
- a continuous source emits light at all wavelengths over its spectral range; a line source emits just a few, discrete wavelengths over its spectral range
- atomic absorption requires a line source because the width of an analyte's absorption band is very narrow because free atoms do not have vibrational and rotational energy levels superimposed on their electronic energy levels
- if we used a continuous source for atomic absorption, then almost none of the source radiation will absorb; thus, P_T and P_0 are essentially identical, which gives $T = 1$ and $A = 0$.

Question IIB. Two common designs for a spectrometer's optics are single-beam and double-beam. Explain the difference between the 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?

Answer. A strong answer to this question will discuss the following points:

- a single-beam instrument has a single optical path between the source and the detector; a double-beam instrument uses two optical paths, one that passes through the sample and one that passes through a reference
- with a double-beam instrument it is possible to continuously adjust the instrument's setting for 100%T, which minimizes problems from changes in the source's emission over time
- the main disadvantages of a double-beam instrument is the initial cost to purchase, the increased number of parts, each of which is subject to breaking, and a larger optical bench that makes the instrument less portable

Question IIC. We discussed three general limitations to Beer's law. Explain the source of each limitation and its effect on a calibration curve using the instrument described and the information presented in the BKH paper as an illustrative example.

Answer. A strong answer to this question will discuss the following points:

- fundamental limitation: the requirement that the absorbing particles act independently of each other and that the refractive index of the sample is independent of concentration; at high concentrations, neither condition remains true and ϵ becomes concentration-dependent, giving positive or negative deviations from Beer's law
- instrumental limitations, which takes two forms:
  - non-monochromatic radiation: Beer's law requires that ϵ is constant for all wavelengths of light that pass through the sample; when this is not true, there is a negative deviation from Beer's law
  - stray light: when light—either from the instrument's light source or from another source—reaches the detector without passing through the sample, then it is not accounted for in either P_{T} or P_0, which means that the transmittance becomes $T = (P_{\text{T}} + P_{\text{stray}})/(P_0 + P_{\text{stray}})$; at high concentrations of the analyte, P_{stray} becomes sufficiently large relative to P_{T}, resulting in negative deviations from Beer's law
- chemical limitations: if the analyte's concentration depends on the position of an equilibrium, then changes in the equilibrium's position will result in a change in absorbance even though there is no change in the analyte's total analytical concentration; the result is a negative or a positive deviation from Beer's law

Part III: Questions that Require Calculations

Question IIIA. Iron is transported in serum by the serum protein transferrin, which contains two sites that bind Fe(III). When too much iron is bound to transferrin it can contribute to a variety of physiological complications. Desferrioxamine is a strong chelating agent that is used to bind Fe(III) and remove it from the body. Each molecule of desferrioxamine binds a single Fe(III). The relative amounts of Fe(III) in transferrin and in desferrioxamine B are determined by visible absorbance spectrophotometry at wavelengths of 428 nm and 470 nm. Transferrin's molar absorptivity when saturated with Fe(III) is 3540 M^{-1} cm^{-1} at 428 nm and 4170 M^{-1} cm^{-1} at 470 nm; for desferrioxamine the molar absorptivity when saturated with Fe(III) is 2730 M^{-1} cm^{-1} at 428 nm and 2290 M^{-1} cm^{-1} at 470 nm. In the absence of Fe(III), neither transferrin nor desferrioxamine absorb at these wavelengths.

- Suppose a sample of tranferrin that is saturated with Fe(III) has an absorbance of 0.463 at 470 nm when using a 1.00 cm cell. What is the concentration of transferrin in mg/mL and the concentration of Fe(III) in µg/mL? The molecular weight of transferrin is 81,000 g/mol.

  Answer. Using Beer's law, the concentration of transferrin, C_{T}, is

$$C_{\text{T}} = \frac{A_{470}}{\epsilon_{470} b} = \frac{0.463}{4170\ \text{M}^{-1}\text{cm}^{-1} \times 1.00\ \text{cm}} = 1.11 \times 10^{-4}\ \text{M}$$

The concentration of Fe(III) is twice the concentration of transferrin, or 2.22×10^{-4} M, as transferrin binds two irons. These are, of course, molar concentrations. To calculate the concentration of transferrin in mg/mL, we note that

$$\frac{1.11 \times 10^{-4} \text{ mol}}{\text{L}} \times \frac{81000 \text{ g}}{\text{mol}} \times \frac{1000 \text{ mg}}{\text{g}} \times \frac{1 \text{ L}}{1000 \text{ mL}} = 8.99 \text{ mg/mL}$$

and the concentration of Fe(III) is

$$\frac{2.22 \times 10^{-4} \text{ mol}}{\text{L}} \times \frac{55.85 \text{ g}}{\text{mol}} \times \frac{10^{6} \text{ µg}}{\text{g}} \times \frac{1 \text{ L}}{1000 \text{ mL}} = 12.4 \text{ µg/mL}$$

- What is the absorbance of this solution at a wavelength of 428 nm?

  Answer. Given the concentration of transferrin and its molar absorptivity at 428 nm, the absorbance is

  $$A = 3540 \text{ M}^{-1}\text{cm} \times 1.00 \text{ cm}^{-1} \times 1.11 \times 10^{-4} \text{ M} = 0.393$$

- After adding desferrioxamine to the sample, the absorbance is measured at both wavelengths, giving a value of 0.424 at 470 nm and 0.401 at 428 nm. Calculate the concentration of transferrin and of desferrioxamine in the sample and the percent of Fe(III) that is bound to transferrin.

  Answer. The following two equations show how the absorbance at each wavelength is related to the concentrations of transferrin and of desferrioxamine

  $$0.424 = 4170 \times C_{\text{T}} + 2290 \times C_{\text{D}}$$

  $$0.401 = 3540 \times C_{\text{T}} + 2730 \times C_{\text{D}}$$

  With two equations and two unknown concentrations, we can solve for both concentrations. Muliplying through the bottom equation by $\frac{2290}{2730}$ leaves us with the following two equations

  $$0.424 = 4170 \times C_{\text{T}} + 2290 \times C_{\text{D}}$$

  $$0.336 = 2969 \times C_{\text{T}} + 2290 \times C_{\text{D}}$$

  Subtracting the second equation from the first equation gives

  $$0.088 = 1201 \times C_{\text{T}}$$

  Solving for the concentration of transferrin gives 7.33×10^{-5} M. Substituting back into the either of the original equations gives the concentration of desferrioxamine as 5.17×10^{-5} M. The total concentration of Fe(III) is 2× the concentration of transferrin plus the concentration of desferrioxamine; thus, the concentration of iron bound to transferrin is 1.47×10^{-4}M, the total concentration of iron is 1.98×10^{-4} M and the percentage bound by transferrin is

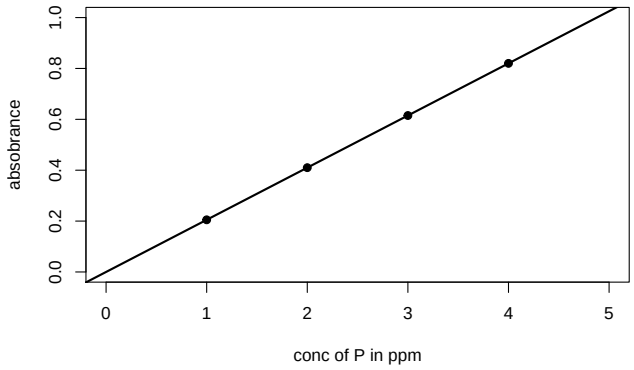
  $$\frac{1.47 \times 10^{-4} \text{ M}}{1.98 \times 10^{-4} \text{ M}} \times 100 = 74\%$$

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

Answer. This is an example of a multi-point external standardization. Because the sample and the standards are treated identically—a 1.00-mL aliquot is treated and then diluted to 50.00-mL after treating with

reagents to develop the characteristic molybdenum blue color—we can use the concentrations of phosphate in the standards prior to their preparation to construct the calibration curve and then take the resulting concentration of phosphorous in the sample as its concentration in the original urine sample.

The calibration data and resulting calibration curve, from a linear regression analysis, is shown here



The calibration equation is

$$A = 0 + 0.205 \times C_{\text{P}}$$

where C_{P} is the concentration of phosphorous. Substituting the sample's absorbance of 0.625 into the calibration equation and solving for C_{P} gives its value as 3.05 ppm P, or 3.05 mg P/L. This is, as noted above, the concentration of P in urine; thus, the total grams of P excreted by the patient is

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

Question IIIC. Nitrate in water is determined by reacting a sample with phenoldisulfonic acid, which produces a yellow-colored species that absorbs strongly at 410 nm. A 100-mL sample is stabilized by adding 80 µL of H_2SO_4 and treated with Ag_2SO_4 to remove any chloride that is present in the sample (as it interferes with the analysis). Any precipitate of AgCl is removed and the supernatant is adjusted to a pH of 7 with NaOH and evaporated to dryness. A 2.0-mL aliquot of phenoldisulfonic acid is added to the residue and heated in a hot water bath to aid in the residue's dissolution. When the residue has dissolved, 20 mL of distilled water and 6 mL of ammonia are added to develop the color. The resulting solution is transferred to a 50-mL volumetric flask and diluted to volume. A standard addition is prepared by spiking 1.00 mL of a standard solution that contains 0.722 g KNO_3/L to a 100-mL portion of the sample and carrying it through the same procedure. The absorbances of the sample and the sample plus standard addition are 0.238 and 0.822, respectively. What is the concentration of nitrate in the sample (in g NO_3^-/mL)?

Answer. There are several ways to go about working this problem, but all will get to the same place at the end. Here is one approach. First, this is a one-point standard addition where we know that

$$\frac{A_{\text{sam}}}{C_{\text{sam}}} = \epsilon b = \frac{A_{\text{std}}}{C_{\text{sam}} + C_{\text{std}}}$$

where A_{sam} is the absorbance of the sample, A_{std} is the absorbance of the sample with the standard addition, C_{sam} is the concentration of the analyte in the sample, and $C_{\text{sam}} + C_{\text{std}}$ is the combined concentration of the analyte in the sample and the standard following the standard addition.

The concentration of the standard is given as 0.722 g KNO_3/L, or 722 µg KNO_3/mL. We need this in terms of NO_3^-, not KNO_3, so the concentration is 443 µg NO_3^-/mL.

Letting x be the µg NO_3^- in the sample and substituting this and other know values into the previous equation gives

$$\frac{0.238}{x/50.00 \text{ mL}} = \frac{0.822}{(x + 443 \text{ µg} NO_3^-)/50.00 \text{ mL}}$$

Rearranging and solving for x gives its value as 181 µg NO_3^-; as this is in a 100.0 mL sample—the original sample taken through the procedure—the concentration of nitrate in the sample is 0.181 µg NO_3^-/mL.