Chem 310

4th Homework Set Answers

1. Cyclohexanone has a strong infrared absorption peak at a wavelength of 5.86 μm.

   (a) Convert the wavelength to wavenumber.

   $\lambda^* = 1/\lambda = (1/5.86\ \mu m)(1\ \mu m/10^{-6}\ m)(1\ m/100\ cm) = 1710\ cm^{-1}$

   (b) A solution of 2.0 mg cyclohexanone ($C_6H_{10}O$) per mL of solvent has an absorbance of 0.40 in a cell with a pathlength of 0.025 mm. Calculate the molar absorptivity of cyclohexanone.

   Convert mg/mL to molarity. MW of cyclohexanone = 72.06+10.08+16.00 = 98.14

   $(0.0020\ g/mL)(1\ mol/98.14\ g)(1000\ mL/L) = 0.0204\ M$

   $\epsilon = A/(bC) = 0.40/[(0.0025\ cm)(0.0204\ M)] = 7800\ M^{-1}cm^{-1}$

   (c) What is the LOD for this compound if the standard deviation of the blank is 0.001 absorbance units?

   Sensitivity $m = \epsilon b = (7800\ M^{-1}cm^{-1})(0.0025\ cm) = 19.6\ M^{-1}$

   $LOD = 3s_{bl}/m = 3(0.001)/(19.6\ M^{-1}) = 1.5\times10^{-4}\ M$ *(or 0.015 mg/mL)*

2. List the advantages of a FT-IR over a dispersive IR spectrometer. Which advantages are the most significant?

   (a) All wavelengths simultaneously reach the detector (Fellgett's advantage).

   (b) Throughput to the detector is large (Jacquinot's advantage)

   (c) The bandwidth is constant over the entire spectrum.

   (d) Wavenumbers and hence peak positions are known very precisely.

   (e) Stray light is much less of a problem since it is not modulated at audio frequencies.

   The first two advantages are the most signficant. Fellgett s advantage means that spectra can be acquired rapidly. Ensemble-averaging greatly increasing signal-to-noise. Jacquinot s advantage means that strongly absorbing or scattering samples can be analyzed.

3. Sketch a FT-IR spectrometer. Show and label the source, the detector, the parts of the interferometer, and the sample. Use arrows to indicate the path of the light through the spectrometer.

   The sketch should show the source (Globar), a mirror to collect the source light and produce a collimated beam, an Michelson-Morley interferometer with a beam splitter, stationary mirror, and moving mirror, a sample/sample holder, and a detector (DTGS or MCT). The beam splitter sends the source light to both mirrors. The returning beams are combined at the beam splitter. The light then passes through the sample and is focused onto the detector.

4. What is an interferogram and what is its relationship to a plot of absorbance vs wavenumber (output of a FT-IR spectrometer)? This is a discussion question and your answer should have some sketches.

   An interferogram is the output of the detector in the FT-IR spectrometer. The signal is collected while the moving mirror in the interferometer is moving. For a single wavelength (wavenumber), the interferogram is a sinusoidal oscillation (sketch of signal vs time) as the light at that wavelength undergoes periodic constructive and destructive interference in the interferometer. A Fourier transform of this data converts it to a plot of signal intensity vs frequency; for a single wavelength, the fast Fourier transform (FFT) yields a line whose amplitude is intensity of the light and whose position on the frequency axis is the frequency of the light (sketch). For all wavelengths, the interferogram is a squiggly plot with large amplitude at one time (at which point the two

mirrors in the interferometer are equal distance away from the beam splitter) and decaying amplitudes for times away from the center peak (sketch of signal vs time). The FFT of this data yields a plot of signal amplitude vs frequency, i.e., a single-beam spectrum (sketch). The computer records a blank spectrum and a sample spectrum and then transforms the output into whatever the operator desires (typically, %T or A vs λ - wavenumber) (sketch).*

5. The research FT-IR spectrometer in 473 CRL is connected to a large air purification unit mounted on the wall. The feed into the purification unit is house compressed air. What gases are being removed by the purification unit before the air is passed through the FT-IR spectrometer? Why are those gases being removed?
The purification unit is removing water vapor (H_2O) and carbon dioxide (CO_2) from the air. These gases (especially water vapor) have prominant absorption bands in the IR spectrum which interfere with the bands of the analyte. In single-beam instruments like the FT-IR spectrometers, the levels of water vapor and CO_2 can change between the blank and the sample spectra; consequently, the output of the spectrometer will show these absorption bands. The best way to avoid this problem is to purge the spectrometer with dry and CO_2-free gas. Purifying air is cheaper than using nitrogen gas (but some researchers use the boil-off of a liquid nitrogen dewar just for this purpose).

6. If the moving mirror in the Michelson interferometer has a velocity of 0.65 cm/s, what is the frequency of modulation of light of 1700 cm^{-1}?
$f = 2v\lambda^* = 2(0.65\ cm/s)(1700\ cm^{-1}) = 2210\ Hz\ (Hz = s^{-1})$

7. Box 10-1 on p. 179 of Harris shows a series of Raman spectra of nitric acid at different concentrations. The spectra demonstrate that HNO_3 becomes less dissociated as the concentration of acid increases. Why is Raman spectroscopy superior to IR absorption spectroscopy for studying vibrational spectra of species in aqueous solution?
Water is a weak Raman scatterer and a strong IR absorber. The Raman spectrum of an analyte in aqueous solution will contain a few weak peaks due to water as well as the Raman peaks due to the analyte; correction for the Raman bands of water is easy. An IR absorption spectrum will contain very large absorption bands due to water (concentration 55 M) plus the relatively small absorption bands due to the analyte. Isolating the IR absorption spectrum of the analyte is difficult.

8. Mesitylene (1,3,5-trimethylbenzene) exhibits a strong Raman line at 1000 cm^{-1}. What are the wavelengths (in nm) of the Stoke's line and the anti-Stoke's line if the Raman scattering is excited by a krypton laser at 530.9 nm?
$\lambda^* = 1/\lambda = (10^7\ nm/cm)/(530.9\ nm) = 18836\ cm^{-1}$
Stoke s line: $\lambda^* = 18836 - 1000 = 17836\ cm^{-1}$; $\lambda = 10^7/17836 = 560.7\ nm$
anti-Stoke s line: $\lambda^* = 18836 + 1000 = 19836\ cm^{-1}$; $\lambda = 10^7/19836 = 504.1\ nm$

9. What are the reasons why a Raman spectrometer with a HeNe laser (632.8 nm output) might be better to use than a Raman spectrometer with an argon ion laser (488.0 or 514.5 nm output)?
Fluorescence appears as a background signal in a Raman spectrum. A HeNe laser line (632.8 nm) is at a longer wavelength than the argon-ion laser lines (488.0 or 514.5 nm). If the sample is fluorescent, or there are fluorescent impurities present, the longer wavelength would be better because it would tend to excite less fluorescence in the sample. Fluorescence requires light absorbance, and as wavelengths increase, fewer substances tend to absorb light. Also, longer wavelengths tend to cause less photo-decomposition in the sample

(photon energy is lower).

10. Sketch a Raman spectrometer containing a double monochromator and a PMT detector; show the light path and how Raman scattering is collected from the sample.
The sketch should show a laser source with its beam focused onto the sample. Raman scattered light is collected at a right angle and focused onto the entrance slit of a double monochromator. The entrance slit of the second monochromator is the exit slit of the first monochromator. The PMT is located at the exit slit of the second monochromator. A double monochromator is needed to reduce the level of stray light. Raman scattered light is very weak, and the monochromators must reject the much more intense scattered light at the laser wavelength.

11. Discussion question: What is the difference between fluorescence and Raman scattering for a molecule in terms of:
(a) an energy state diagram?
A sketch of an energy state diagram should show the S_0 and S_1 states as heavy double lines, and vibrational excited states as light single lines above each of the heavy double lines. A photon interacting with the molecule is shown as hν (squiggly line with arrow). For fluorescence, the absorption of the photon is represented as an arrow from the S_0 double line up into the manifold of excited vibrational states of S_1. A squiggly line down to the S_1 double line represents internal conversion (loss of excess energy as heat to the surrounding medium). A solid arrow from the S_1 double line to any of the S_0 states results in fluorescence (hν photon coming out). Fluorescence is the $S_1 -> S_0$ transition.

For Raman scattering, the absorption of the photon is represented by an arrow to an empty space below the S1 double line. A dashed line at this point represents a virtual excited state. Raman scattering is represented by a solid arrow to any of the excited vibrational states of the S_0 state (hν photon coming out).

(b) the wavelengths of the two types of emitted or scattered light?
For both fluorescence and Raman scattering, the energy of the photon emitted is less than the energy of the photon absorbed. Consequently, both fluorescence and Raman scattering occur at longer wavelengths than the wavelength of the excitation light. You cannot distinguish Raman scattering and fluorescence by wavelength; they occur in the same spectral domain. This is why fluorescence in the sample is a severe interference for Raman spectroscopy. Raman peaks do appear in fluorescence spectra, but are only a problem when the fluorescence intensity is very low.

(c) the lifetimes of the two types of emission? (Think about this question).
Fluorescence emission exhibits a lifetime on the order of nanoseconds (10^{-9} s); the population of excited molecules in the S_1 state decays exponentially over 1-10 ns. Raman scattering occurs virtually instantaneously; the lifetime of the virtual excited state is approximately the frequency of the light (about 10^{-14} s). One can discriminate between fluorescence and Raman scattering if one has a very fast light detector that can be gated (i.e., turned on and off very quickly).

12. Define internal conversion and intersystem crossing in words and in an energy state diagram.
Internal conversion is the loss of energy from the molecule to the surrounding medium in the form of heat. In an energy state diagram (sketch), it is represented by a squiggly arrow from any excited state to any lower state. No light is emitted. Intersystem crossing is the conversion between two excited states with a spin change (e.g., S_1 to T_1 or back). The energy state diagram should show an arrow between the two states.

13. Harris, Problem 18-24.
Because the T_1 state in a molecule is always lower in energy than the S_1 state, phosphorescence (T_1 -> S_0) occurs at lower photon energy (longer wavelength) than fluorescence (S_1 -> S_0). A sketch of absorbance, fluorescence and phosphorescene vs wavelength should show absorbance at the shortest wavelengths, fluorescence slightly overlapping absorbance at longer wavelengths, and phosphorescence at the longest wavelengths.

14. Harris, Problem 18-25.
The fluorescence emission spectrum is obtained by exciting a sample at a constant wavelength and plotting the emission intensity as a function of emission wavelength. The excitation spectrum is obtained by observing the emission intensity at a fixed wavelength and plotting the emission intensity as a function of excitation wavelength. Because emission intensity is proportional to the amount of light absorbed by the sample, the excitation spectrum resembles the absorption spectrum of the sample.

15. One of the handouts in lecture shows an overlay plot of a UV-VIS absorption spectrum of quinine and the excitation spectrum of quinine. The two plots are similar (peaks at 350 and 250 nm) but not identical (different ratio of peak heights at the two wavelengths). Why are they different?
$F = 2.3kP_o\Phi_f\epsilon bC$. The fluorescence intensity is proportional to both the molar absorbtivity and to the intensity of the incident light P_0. Both of these quantities change with the wavelength of the incident light. In the quinine excitation spectrum, P_o was decreasing rapidly at the shorter wavelengths, so the relative peak height at 250 nm is much smaller in the excitation spectrum than it is in the absorption spectrum.

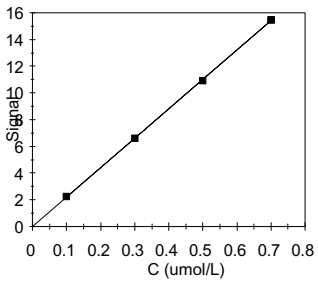
16. Sketch a scanning spectrofluorometer with both an excitation and an emission monochromator and a PMT detector. Show the light path through the instrument. Explain the reason for the right angle geometry between excitation and emission light. Explain what is happening in this instrument when one is acquiring (a) an emission spectrum and (b) an excitation spectrum.
The sketch should show the continuum source (Xe high pressure arc), the excitation monochromator, the sample isn a square cuvet, the emission monochromator and the PMT detector. Light from the excitation monochromator passes through the cuvet in one direction. Emitted fluorescence is collected at right angles and sent to the emission monochromator. This geometry helps reduce the amount of excitation light that gets into the excitation monochromator. In an emission spectrum, the wavelength of the excitation monochromator is set to the wavelength of an absorption peak in the analyte, and the wavelength of the emission monochromator is scanned. In an excitation spectrum, the reverse is true; the wavelength of the emission monochromator is set to the wavelength of peak fluorescence, and the wavelength of the excitation monochromator is scanned.

17. The reduced form of nicotinamide adenine dinucleotide (NADH) is highly fluorescent. It has an absorption peak at 340 nm and a fluorescence peak at 465 nm. Standard solutions gave the following fluorescence intensities:

[NADH] (μmol/L)	F (unitless)
0.100	2.24
0.300	6.59
0.500	10.93
0.700	15.49

(a) Generate a calibration curve from the data. Include a linear regression line of the range of data that you

judge to be linear.
See the next page. All 4 points were used in the linear regression.



(b) Give the slope and intercept of the linear regression line and the standard deviation of the slope and intercept. Use the LINEST function in Excel to get these parameters.
Slope = 22.0±0.2/(umol/L); intercept = −0.01±0.08

(c) A sample of NADH yielded a signal of 12.16. Calculate the concentration of NADH.
12.16 = 22.0C − 0.01; C = 12.17/22.0 = 0.553 umol/L

18. Quinine (found in tonic water) is one of the best known fluorescent molecules. Its structure is shown below; the structure is also shown on the handout of excitation and emission spectra of quinine. Predict the part of the molecule that is the center of fluorescence.
The methoxyquinoline part of the molecule (bottom part as shown) is the fluorescence center. The aromatic system as the rigid structure which results in high fluorescence quantum yield.