Chemistry 524--Final Exam--Keiderling

May 4, 2011 – 3:30 - ?? pm -- 4286 SES

Please answer all questions **in the answer book** provided. Calculators, rulers, pens and pencils are permitted. **No open books or extra sheets allowed.** If you need a specific fundamental constant, conversion factor or definition ask for it; but everything needed should be in the exam, unless I made an error!

Strategy – there are lots of small questions, if you do not know one, skip ahead, leave a space in your answer book, it may come to you later and you will not waste time. *You do not need to answer everything to get a good grade.*

There is some possibly helpful information at the end of the exam. GOOD LUCK!

In the second half of the course we surveyed a number of different optical spectroscopic techniques and for each we reviewed the technologies used for building the spectrometers used for different kinds of analytical applications. Questions 1 – 7 are organized like the end of the course. All answers should be short and to the point, best answers are a few sentences. Unless clearly indicated to choose between them, all parts should be answered:

1. ***[10]*** ***Atomic spectroscopy*** is very valuable but got a short attention in lecture, although a large component of the text. It comes in several flavors: Atomic Absorption (AA), atomic emission (AE), resonance fluorescence, and I am sure there are several others.

a. Compare AA and AE in terms of types of instrumentation needed, ability to discriminate analytes, costs, and sensitivity

Answer only two (2) of b, c, d or e:

b. What advantages does a graphite furnace have over flame atomization, problems?

c. Describe the major steps the sample goes through for flame or plasma atomization.

d. What are the advantages of using inductively coupled plasma (ICP) atomization?

e. How is AA data quantified for determination of concentration?

2. ***[6]*** ***Molecular spectra*** are in general broader than atomic spectra and, if they were resolved to individual transitions, would be much more complex

Answer only two (2) of a, b, c or d:

a. Explain the apparent mirror symmetry between absorbance and fluorescence bandshapes and note why it is not a true mirror image

b. Explain why fluorescence is normally seen from the S_1 excited state and often from just its v=0 vibrational state. How does this impact analyses?

c. From which state do vibrations appear in the absorption spectral band shape, why?

d. Why are rotational components of the molecular spectra relatively unimportant in analytical spectroscopy?

3. ***[22] Absorbance spectroscopy*** in the ***uv-vis*** region underlies the most commonly used analytical techniques

a. Absorbance requires two intensity measurements as opposed to one for luminescence. Explain why you need two, what they give you and how they are obtained

Answer only one (1) of b or c:

b. Explain a common way to create a double beam spectrometer (i.e. what kind of optical design is used)

c. Explain some advantages of double beam spectrometry, disadvantages?

Answer only two (2) of d, e, f or g:

d. Some spectrometers scan from vis to uv but stop for a short time at ~350 nm. Explain why

e. Since many spectrometers use gratings to scan from 200-800 nm, how do they avoid second and third order diffraction interference? Give one or two example solutions

f. Some spectrometers vary the slit width as the scan proceeds. Give a plausible reason why

g. What range of absorbance is reasonable for a commercial uv-vis? What are the limiting noise sources at high and low absorbances?

Answer only one (1) of h or j:

h. What is important about isobestic points for analyses? How can it be used analytically?

~~***i.*** Why use the wavelength corresponding to the band maximum for analyses if the whole band absorbance varies linearly with concentration?~~

j. What impact can the slit width have on concentration determination with absorbance?

Answer only one (1) of k or l:

k. Xe arcs make very good uv-vis sources. Why are they not used in most commercial spectrometers?

l. Why do you think are there not commercial CCD based uv-vis absorption spectrometers, but there are incredible numbers of digital cameras and telephones with CCDs?

4. ***[22] Molecular luminescence*** is the counterpart to absorbance and consists of ***fluorescence*** and ***phosphorescence*** (and other less analytically relevant methods)

a. Many molecules do not fluoresce, even though they have absorbance. Explain why

b. What kind of information is obtained by scanning the excitation wavelength and fixing the emission wavelength? How is it different from absorbance?

c. Propose advantages and disadvantages of using a broad band (thermal) detector for normalization of the total source intensity as compared to excitation of a rhodamine dye with the output of the excitation monochromator and detection of its fluorescence.

d. Describe how you would detect fluorescence lifetimes (there are two ways, pick one). Specify the types of detectors and electronics you would need

e. Why can fluorescence for an isotropic sample be polarized but not absorbance?

Answer only one (1) of f or g:

- ***f.*** Explain how you might discriminate phosphorescence from fluorescence in a sample
- ***g.*** How would you discriminate Raman from fluorescence in a sample

Answer only one (1) of h or i:

h. Explain sampling or experimental design changes that can enhance phosphorescence.

i. What is quenching and give an example of using it for analysis of structure or properties?

5. ***[30] IR absorbance spectra*** are primarily used to monitor molecular vibrations

- ***a.*** Modern FTIRs dominate the market, partly due to the three "advantages" of FTIR. List the advantages and explain why each leads to more reliable spectra with FTIR as compared to dispersive IR
- ***b.*** For near-IR spectra the bands are usually broad and not uniquely assigned. Plus they are very weak. Explain why near-IR has none-the-less become a useful analytical tool and how it can gain discrimination (selectivity).
- ***c.*** Why is IR used more for qualitative analyses than for quantitative analyses?
- ***d.*** My lab uses a dispersive IR for measuring VCD of single broad bands in proteins. Propose another experiment where dispersive IR is (or could be) used.

Answer only one (1) of e or f:

- ***e.*** Water and CO_2 in the atmosphere pose interference issues in the IR spectrum. How is this interference normally corrected in FTIR? Where are these bands spectrally?
- ***f.*** To correct for the solvent absorbance I can subtract the spectrum measure in a similar cell. Why might this be in error? Why might I still have an error if I use the same cell?

Answer only one (1) of g or i:

- ***g.*** Explain why most FTIRs are used (default scan) from 400-4000 cm^{-1}, what limits scan?
- ~~***h.*** What modifications are needed to extend to the Far IR region (e.g. 400-100 cm^{-1})?~~
- ***i.*** If I want to modify my FTIR to a new region, which might be more difficult, far IR or near IR and explain why (many possible answers)

Answer only one (1) of j or k:

- ***j.*** Old dispersive spectrometers used thermopile detectors but FTIRs use DTGS detectors, why is this? How do they differ in fundamental detection mechanism?
- ***k.*** Photon detectors for the IR, like MCT and InSb are normally cooled with liquid N_2. What is gained and what is lost in this cooling? Why do routine FTIRs (e.g. Mattson in 4315 SES) not have a cooled detector?

Answer only one (1) of l or m:

- ***l.*** Explain why ATR is surface sensitive and transmission IR is bulk sensitive
- ***m.*** Describe some materials that are useful for sampling (making cells) water-based solutions of proteins, and explain why they are chosen.

6. ***[16] Raman spectroscopy*** also is focused primarily on vibrational spectra.

a. IR and Raman are viewed as complementary but both allow $\Delta v = \pm 1$ transitions, what makes them different (i.e. complementary)?

b. Explain the difference between Stokes and anti-Stokes Raman spectra, where you find these transitions and why Stokes is usually more intense.

c. Raman and fluorescence spectrometers are conceptually alike, however Raman spectrometers almost always use a laser source for excitation and fluorimeters often use a broadband source with monochromator. Explain

d. Raman spectrometers originally used double monochromators, why? Now they often use single spectrographs. Why did they change and how did they solve the problem?

Answer only one (1) of e or f:

e. How can you use an internal standard to get Raman cross-section (intensity) measurements from a single measurement?

f. Why can Raman sample smaller volumes than IR, but often need more sample?

7. ***[13] Circular dichroism*** is measured as an absorption modulation spectroscopy.

a. CD is actually measured as ΔA in absorbance. Biochemists in particular are interested in CD expressed as $[\theta]$. How is the conversion made?

b. Most CDs use a photoelastic modulator (PEM, often from Hinds Inc.). Explain how it works for CD measurements

c. Why is CD so useful in biochemical analysis studies?

Answer only one (1) of d or e:

d. JASCO CDs are popular, but use “old fashioned” prism monochromators and do not seem to have an obvious linear polarizer. Please explain these observations.

e. OLIS has a different CD detection design. Explain some aspects of its differences

Answer only one (1) of f or g:

f. What criteria are needed for a CD to be used for kinetics with stop-flow mixing?

g. CD in the IR is vibrational CD (VCD). Describe how a typical VCD instrument differs from a UV CD instrument?

8. ***[6] Answer only two (2) of the following,***

a. My MCT detector for my FTIR spectrometer has 1/f noise. How do I know this is the source and how can I adjust the experimental conditions so that this is unimportant?

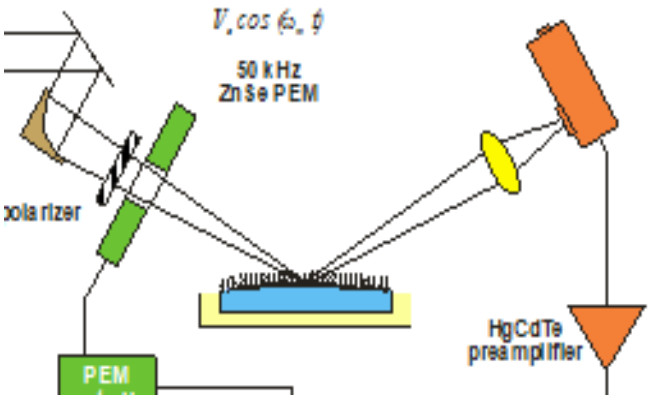
b. Once 1/f noise is reduced, the detector is limited by Johnson noise. What is the source of this noise and what can I do about it?

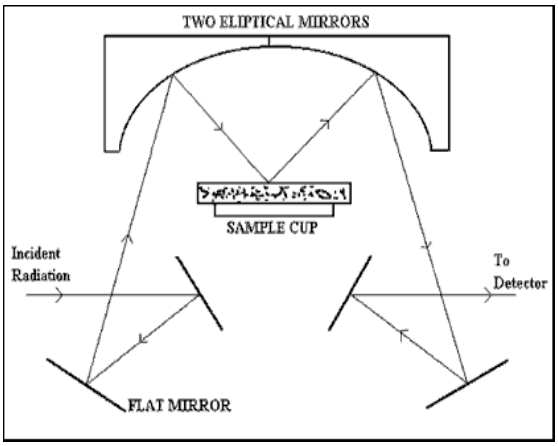
c. If it were perfect, the detector would be shot noise limited. What is the source of this noise and how can it be reduced or how can the S/N be improved?

9. ***[6]*** I have a fluorescence lifetime experiment which has a pulsed laser excitation, with pulses at 10 Hz. However the fluorescence decay data after each pulse are noisy. Describe/ design a detection scheme that I could use to improve the S/N and determine the lifetime if the pulse were 10 ns and the lifetime were 300 ns. Be specific about detectors and electronics and explain your choices.

10. ***[5]*** We described several filters and amplifiers based on op amps. Describe the characteristics of the input and outputs of a typical op amp and how it can be used to control gain or band pass.

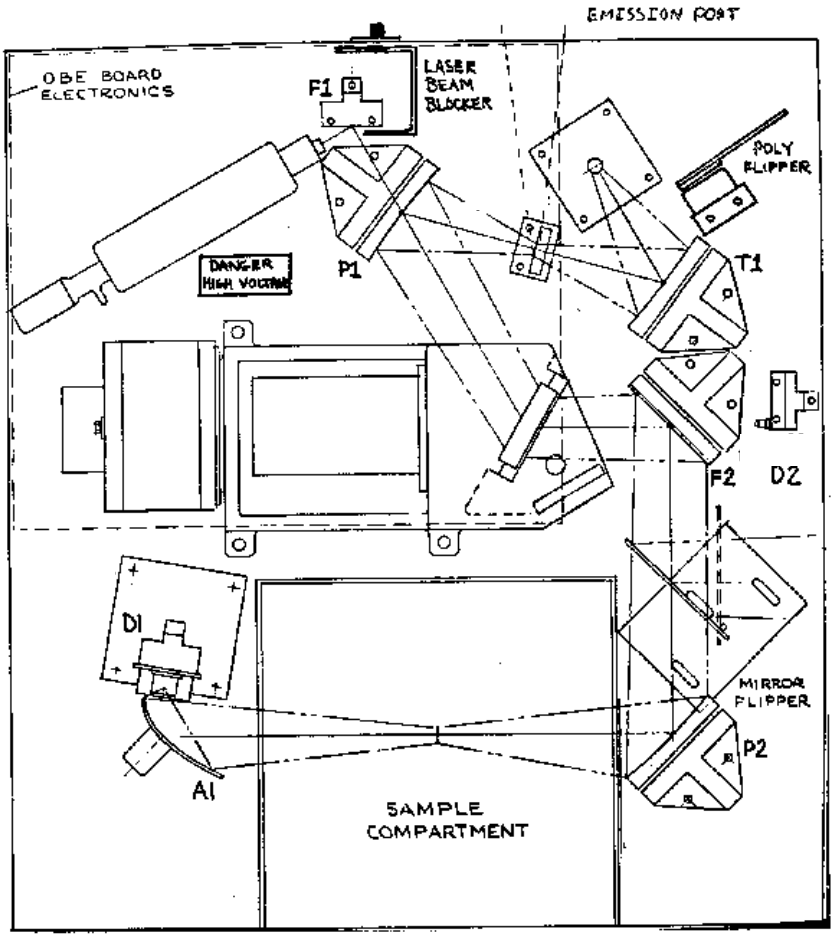
11. ***[10]*** For the two reflection IR sampling accessories below, identify the main components, explain what kind of measurement they are used for and contrast the elements and flexibility (adjustments) needed in the design. Specify what kind of information they yield



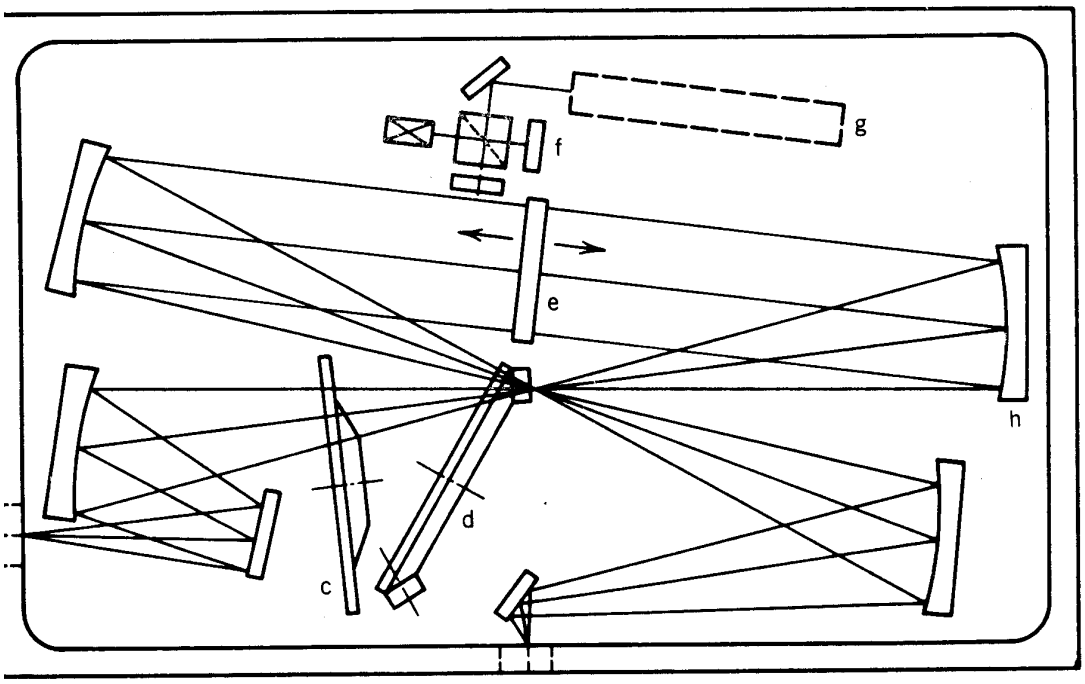


12. ***[43]*** For the **attached instrument** diagrams *(starting next page)* ***choose either A or B and then do C and D and E (next two pages).*** Identify the possible function and type of each of the major parts that are hand-labeled with a number in a circle. You may make a table with numbers coding the objects in the diagram and for each identified item propose specifically which type of component (e.g. "source: quartz-halogen or W-I lamp" or "detector: PMT") is being used (note: for optical components, just put what it is and purpose, e.g. lens, focus beam on slit). For the source and detector, briefly justify the choice. ***Finally state the purpose of the instrument shown and why you think you are right.***

A


EMISSION PORT
OBE BOARD ELECTRONICS
F1
LASER BEAM BLOCKER
POLY FLIPPER
DANGER HIGH VOLTAGE
P1
T1
F2
D2
D1
MIRROR FLIPPER
P2
A1
SAMPLE COMPARTMENT

B


g
f
e
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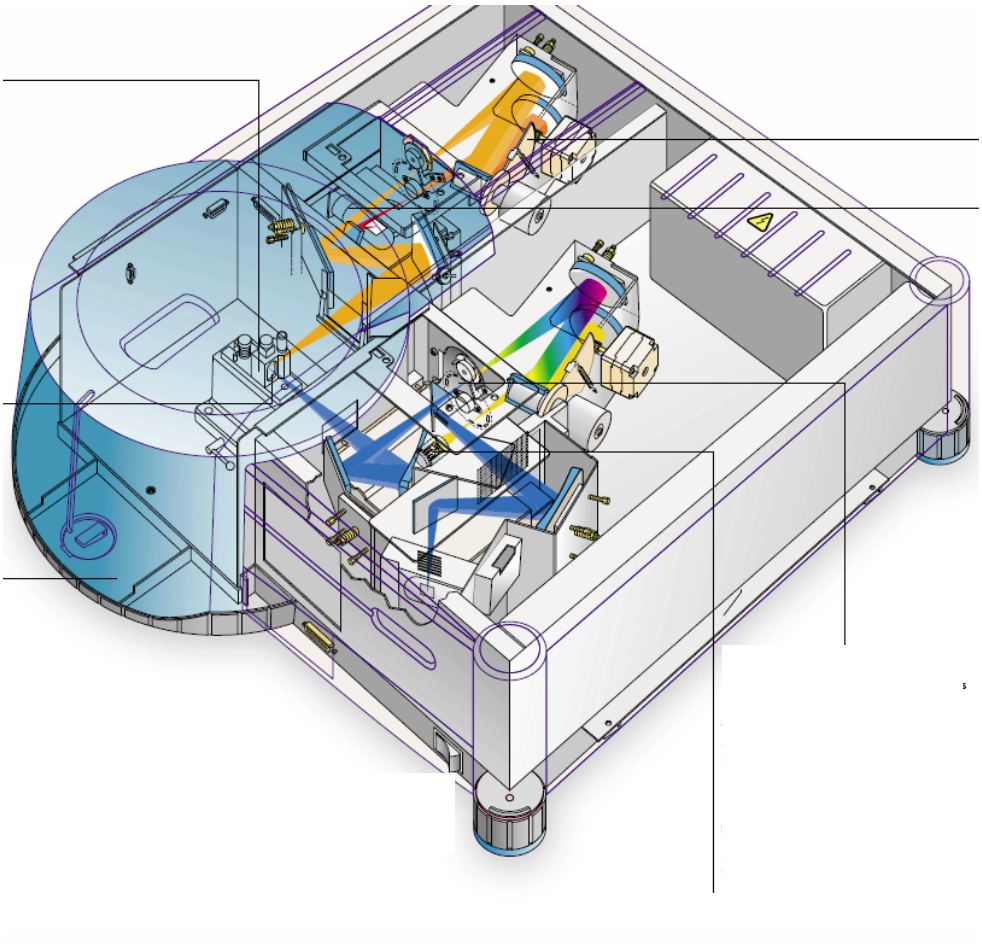
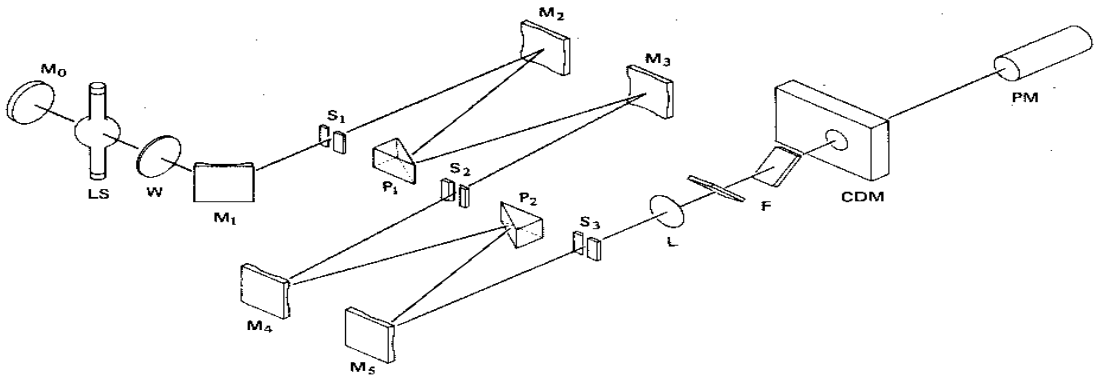
C



D



E