Chemistry 277
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University of Maryland, College Park
General Chemistry and Energetics
Hour Exam (100 points)

Your Name: Key

Your SID #:

Your Section # or time:

March 9, 2020

You have 53 minutes for this exam.
Explanations should be concise and clear. There is lot of extra space on the last page if you need it.
You will need a calculator for this exam. No other study aids or materials are permitted.
Generous partial credit will be given, *i.e.*, if you don't know, guess.

Useful Equations:

$\sigma_Y^2 = \lim_{N\to\infty} \frac{1}{N}\left[\sum_i (Y_i - \overline{Y})^2\right]$ $\quad$ $\mathrm{pH} = -\log([\mathrm{H^+}])$ $\quad$ $\sigma_Y^2 = \left(\frac{\partial Y}{\partial u}\right)^2 \sigma_u^2 + \left(\frac{\partial Y}{\partial v}\right)^2 \sigma_v^2 + \cdots$

The sample standard deviation for N samples is $\sigma_X = \sqrt{\frac{1}{N-1}\sum_i \left(X_i - \overline{X}\right)^2}$

For $Y = au + bv$, $\sigma_y = \sqrt{a^2\sigma_u^2 + b^2\sigma_v^2}$. For $Y = \frac{au}{bv}$, $\frac{\sigma_Y}{Y} = \sqrt{\frac{\sigma_u^2}{u^2} + \frac{\sigma_v^2}{v^2}}$

$R = 0.08206$ L·atm/mole K $\quad$ $T^2 = 4\pi^2 a^3/GM$ $\quad$ $\ln K_{eq} = -\Delta H°/(RT) + \Delta S°/R$

$R = 8.314$ J/mole K $= 1.987$ cal/mole K $= N_A k_B$ $\quad$ $SEM = \frac{\sigma}{\sqrt{n}}$

$N_A = 6.02 \times 10^{23}$ particles/mole $\quad$ zepto-atto-femto-pico-nano-micro-milli-centi-deci-base

$°C = °K - 273.15$ $\quad$ $P(v)dv = Cv^2 exp(-mv^2/2kT)$ $\quad$ $\ln k = (-E_a/RT) + \ln A$

$\mathrm{pH} = \mathrm{p}K_a + \log([\mathrm{A^-}]/[\mathrm{HA}])$ $\quad$ $K_p = K_c(RT)^{\Delta n}$ $\quad$ $K_w = [\mathrm{H^+}][\mathrm{OH^-}] = 10^{-14}$

$Absorbance = \varepsilon c \ell = \log(I_0/I)$ $\quad$ $PV = nRT$

$\left[\frac{-\hbar^2}{2\mu}\nabla^2 + V(\mathbf{r})\right]\Psi(\mathbf{r}) = E\Psi(\mathbf{r})$

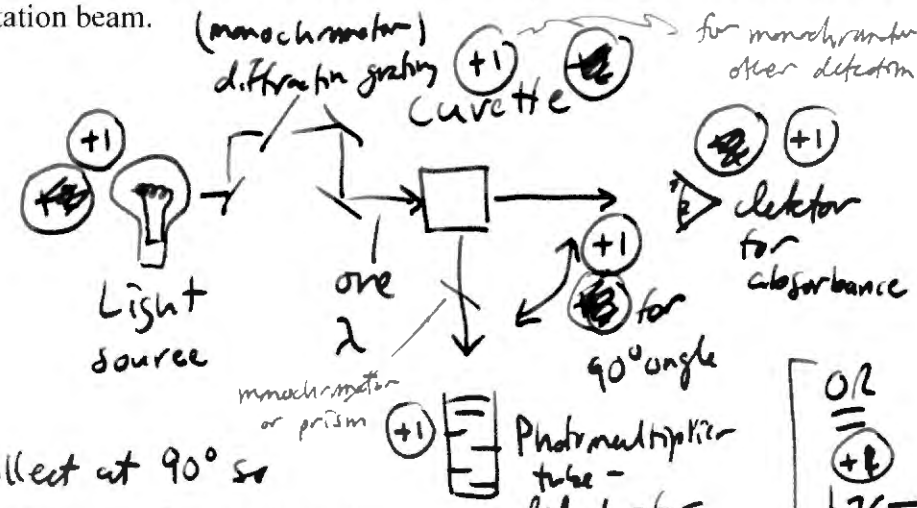
$\mathrm{p}K_a = -\log(K_a)$ $\quad$ $\mathrm{pH(e.p.)} = \frac{1}{2}(\mathrm{p}K_{a1} + \mathrm{p}K_{a2})$

Honor Pledge: At the end of the examination time, please write out the following sentence and sign it, or talk to me about it:

"I pledge on my honor that I have not given or received any unauthorized assistance on this examination."

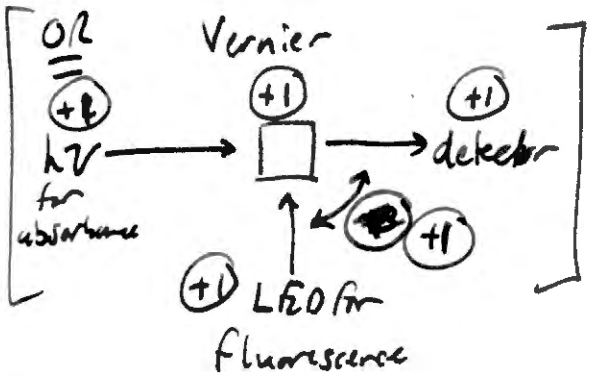
1. Absorbance and Fluorescence (38 pts):

(a; 8 pts) Sketch the different spectrometer geometries for the excitation beam and the observed light for absorbance vs. fluorescence spectrometry. Explain why we collect fluorescence emission at 90° to the excitation beam.

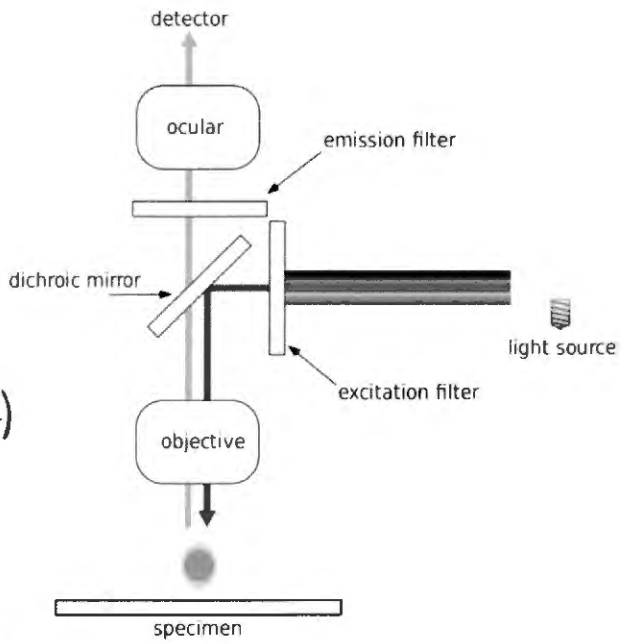


→ Collect at 90° so that we do not try to measure fluorescence against the strong incident light beam. ↑ sensitivity.

(+3)



(b; 6 pts) For simple imaging of fluorescence from a specimen, scattered light and fluorescence emerge from the sample together; only the florescence is shown in the picture at the right. What do you think the "emission filter" in the light path does, and why is it necessary?



- The emission filter blocks the light @ the incident (excitation) frequency, lets other (emitted) light through. (It's the filter in the path of emitted light, it doesn't filter out emitted frequency.)

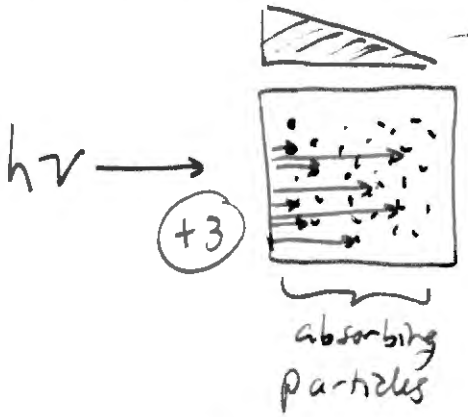
(+3)

- Needed to block the scattered light at the excitation wavelength - otherwise that could overwhelm the weak signal from fluorescence from a small number of molecules.

(+3)

Score for the page 14/14

(c; 8 pts) Draw a sketch and explain why the number of photons absorbed by a sample is NOT proportional to the number of absorbing molecules in the sample, except for very dilute samples.



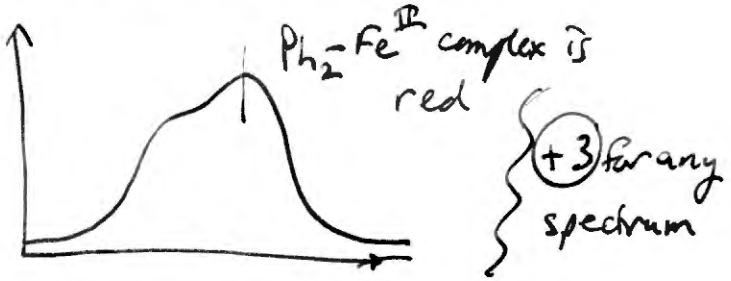
(+3) fewer photons get through as a function of distance, so the number of photons absorbed per molecule depends on concentration, it is not a constant.

(+2) At very low concentration almost all the light gets through and each particle sees the same incident light intensity. [optional - missing other points]

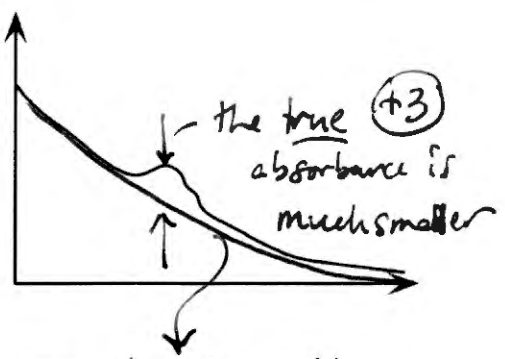
(d; 8 pts) Why is an absorbance reading >3 likely to be meaningless (for our specs)? How can you obtain an accurate absorbance reading for such a sample?

(+2) Abs = 3 means that $\frac{I}{I_0} = 10^{Abs} = 1000$ so 99.9% of the light is blocked – therefore a tiny amount of stray light makes a difference – if stray light is 0.1% of the incident beam, the absorbance can never be >3 no matter how concentrated the sample → Dilute the sample until Abs<2. (+3)

(e, 8 pts) Sketch the absorbance spectrum that your group obtained in the egg lab, in which we used phenanthroline to chelate iron and give a red color. For the sample spectrum shown, explain why the absorbance value at the top of the middle "peak" doesn't mean much and what might have gone wrong in the workup.



(+3) for any spectrum



(+3) this is mostly scattering – it is not contributed by the "ferroin" complex.

– probably protein or other gunk is left behind in the prep → particles scatter light (+2)

Score for the page 24

2.0 ± 0.3 Accuracy, precision, error analysis (25.0000 ± 0.0001 pts): → treat as exact for Part (c)

(a; 17 pts) You perform three measurements of absorbance for three 1:5 dilutions of the same stock solution of a dye, and record 0.7822, 0.7964, and 0.7633. What is the estimated mean ± SEM of the values (for σ, use the formula with the *N*–1)? Given that the original stock solution is at 1.451 ± 0.003 M, calculate the extinction coefficient of the dye and report it to the appropriate number of significant figures ± propagated uncertainty to one sig fig. Path length = 1 cm, considered exact.

(+3) $\sigma = \sqrt{\frac{\sum(x-\bar{x})^2}{N-1}} = \frac{1}{\sqrt{2}}\left[(0.01563)^2 + (0.01536)^2 + (-0.01133)^2\right]^{1/2} = \left(\frac{5.51483\times10^{-4}}{2}\right)^{1/2} = 0.01661$

(+3) $\bar{x} = \frac{1}{3}(0.7822+0.7964+0.7633) = \frac{2.3419}{3} = 0.78063$

(+3) $SEM = \frac{\sigma}{\sqrt{N}} = \frac{\sigma}{\sqrt{3}} = 0.00959$ so mean ± SEM = $0.78063 \pm 0.00959 \rightarrow 0.78 \pm 0.01$ (use this below)

(+3) $\varepsilon = \frac{A}{\lambda c} = \frac{0.7806 \pm 0.0096}{(\frac{1}{5})\cdot(1.451\pm0.003)\,M\cdot 1\,cm} = 2.6900 \pm \sigma_\varepsilon\ M^{-1}cm^{-1}$ (0.7806 ± 0.0096 → treated as exact → how was it done?)

(+3) $\frac{\sigma_\varepsilon}{2.6900} = \sqrt{\left(\frac{0.0096}{0.7806}\right)^2 + \left(\frac{0.0006}{0.2902}\right)^2} = \sqrt{(0.012298)^2 + (2.068\times10^{-3})^2} = 0.01247$

+2 for setup, +1 for answer

0.2902 or 0.003/1.451 of course

main source of error

$\sigma_\varepsilon = 3.35\times10^{-2}$

(+2) all or none

So $\varepsilon = 2.6900 \pm 0.0335 \rightarrow \varepsilon = 2.69 \pm 0.03\ M^{-1}cm^{-1}$

(b; 8 pts) From looking at the experimental values, do you think their uncertainty was due to uncertainty in the spectrometer reading or to random pipetting errors in the dilution step, and why? Describe one additional experiment you could you do to test your conclusion.

(+4) – It seems strange that the spec would report to 4 sig figs if only 2 of them are actually useful, and realistically the 1:5 dilution is probably hard to do to better than 1% accuracy → it's probably dilution.

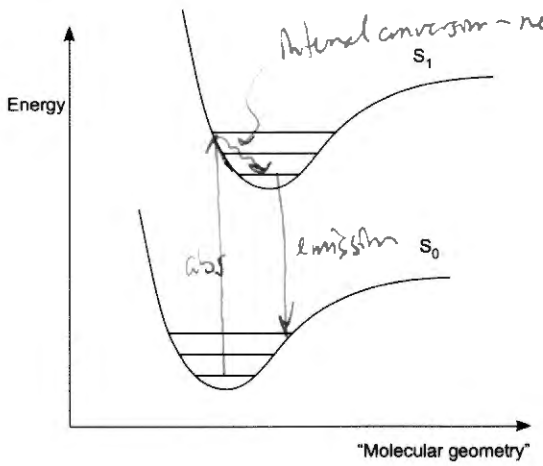
[Also OK to argue that the spec is noisy if consistent below] or to argue that dilution is assumed exact

(+4 for any)
- Could integrate the spec for longer times or do repeated measurements on the same sample to evaluate spec noise.
- Could weigh the samples removed from stock to evaluate volume uncertainty.
- Could look for a more precise method to measure dilution volumes
- Could measure stock directly far away from λ_{max} to skip dilution step entirely

Score for the page 25/25

3. Spectroscopy (18 pts)

(a; 6 pts) On the Franck-Condon diagram below, add in two lines, representing the process of absorption of a UV/vis photon followed by fluorescence. S_0 and S_1 are the ground electronic state and the first excited electronic state respectively.



internal conversion - need not show

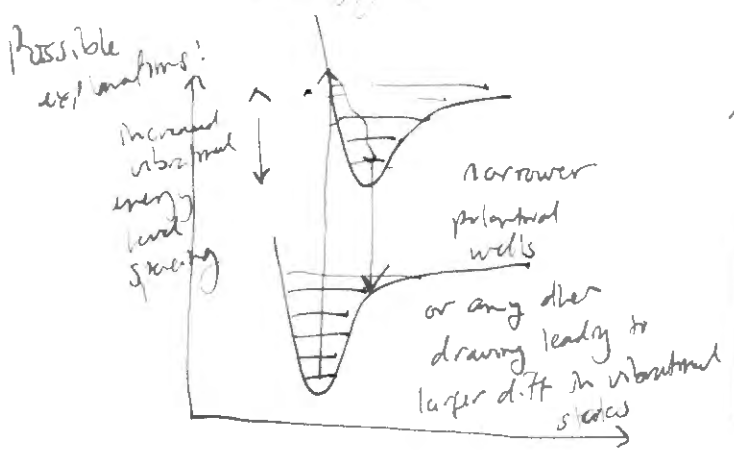
+2 for absorbance
+2 for emission
+2 for Stokes shift

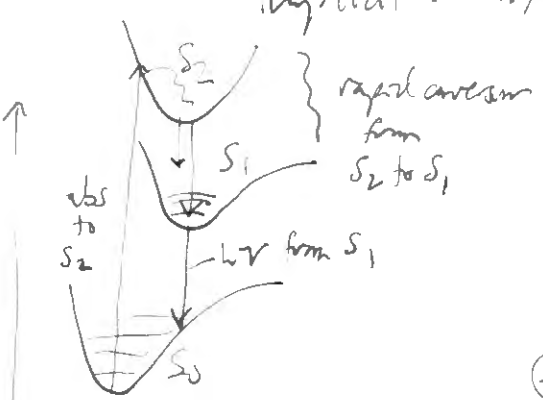
(b; 12 pts) Refer to your diagram to explain why fluorescence emission is to the red of absorbance (or fluorescence excitation). The "Stokes shift" is the energy difference between the absorbance λ_{max} and the fluorescence λ_{max}. The Stokes shift is different for different molecules. Sketch a Franck-Condon diagram for a different molecule corresponding to a larger Stokes shift than the molecule in (a). Speculate on possible physical origins of the large shift (many possible answers).

- Electronic excitation is ~ instantaneous so nuclei do not move. The ground state geometry is likely to correspond to an excited vibrational state of S_1, and vice versa. After excitation, rapid internal conversion (+1) means that the emitted photon goes from the ground vibrational state of the excited electronic state to an excited vibrational state of the ground electronic state. (+2) Therefore the energy of the emitted photon is less than the energy of the absorbed photon. (+2) - for any comparison of photon energies, light or explicit

6

Possible explanations:





(+2) for any F-C diagram
(+2) for some sort of explanation for differences
(+2) for explanation

Score for the page 18/18

4. Random lab questions (19 pts)

(a; 6 pts) If you run an enzymatic reaction until all the concentrations stop changing and then add additional substrate, explain what should happen if everything is going well. If, instead, nothing actually happens, give a possible explanation.

(+3) Substrate + Enzyme ⇌ Products + Enzyme ⇒ Substrate should be used, products should form

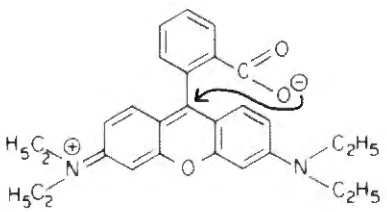
- Dead enzyme due to oxidation, denaturation, etc.
- Degraded or limiting co-substrates
- [Substrate] is too high and E is inhibited
- Enzyme is inhibited by product so relaxation is slow

(+3 for any)

(b; 5 pts) What was the basis of our measurement of the remaining NAD^+ concentration in the alcohol dehydrogenase lab? ($EtOH + NAD^+ \rightleftharpoons$ acetaldehyde + NADH)

(+3) $[NAD^+] = [NAD^+]_{initial} - [NADH]$, where $[NADH]$ is measured by absorbance at 340 nm. (+2)

(c; 8 pts) In the rhodamine lab, the measurement of concentration of the Z form shown at the right is based on absorbance, and the [L] is calculated by difference. Imagine that you used a lot more of the stock than you thought you did for one measurement. How could this give you a physically impossible number for [L]? How could we modify the experiment to fix this problem? (Hint: one possible solution involves the use of expensive and fragile quartz cuvettes that allow measurement in the UV.)



Calculated $[L] = [Rh]_{total} - [Z]_{measured}$

↓

(+3) If $[Rh]_{total}$ is actually much larger than you think it is, then the calculated [L] can be less than zero.

(+2) find a way to measure $[Rh]_{total}$ or [L] directly in the same cuvette.

- For example - [L] must absorb in the UV somewhere - do measurements at that wavelength as well as in the visible. (+3)
- or - simultaneously take an aliquot of Rh and measure its [Z] under conditions where K_{eq} is known ⇒ measure true [L]. Details at least one incorrect pipette setting mistake. (+3)

(either (other possible))

Page	Score
2	/14
3	/24
4	/25
5	/18
6	/19
Total	**/100**

Score for the page 19