Answers for Exam #3---Chemistry 447----Fall 2001

1. a) False
b) True
c) False
d) True
e) False
f) False
g) False
h) True

2. See p. 357 of Skoog book for picture of energy level diagram for fluorescence and phosphorescence. The overall processes involves promotion of an electron in a bond of a molecule from a ground to an excited state orbital (antibonding orbital) via absorption of external photons. The electron promoted has a spin opposite the remaining electron in the ground state, and thus the excited state is initially a singlet excited state. Excited and ground states consist of many energy levels due to different vibrational and rotational modes. Once in the excited electronic state, the molecule can relax back to ground state in several ways. It can transfer its energy to other nearby molecules--this is called external conversion. It can relax back to ground state by so-called internal conversion. Both of these are radiationless relaxation processes. Fluorescence occurs when the relaxation involves release of a photon with energy proportional to the difference in energy between the excited singlet electronic state and the ground electronic state. Due to vibrational relaxations in the excited singlet state, the energy of the fluorescence photons will always be less than the excitation photons.

Phosphorescence occurs when the electron in the excited state flips its spin, thus creating a triplet excited state. This is called intersystem crossing. Relaxation from this triplet state to the ground state can be radiat-ionless, or it can yield photons. If photons are released, this is called phosphorescence.

The quantum yield for fluorescence is determined by the relative rate constants for the different processes that can occur once the molecule is in the excited singlet electronic state. Indeed, quantum yield is defined as the number of molecules that fluoresce divided by the number that are in the the excited state. If intersystem crossing rate, or internal and external conversion rates are faster than the rate of photon release, then the quantum yield will be low. If the rate constant for photon release from the excited singlet electronic state is greater than the rate constants for the other processes, then a high quantum yield will result.

The quantum yield will partially dictate detection limits, since

$$F = 2.3\, K' \varepsilon b c P_o$$

and K' includes the quantum yield term (φ). Thus, for a given concentration c, F will increase if quantum yield increases.

Increasing the temperature will generally decrease the quantum yield by increasing the rate of external conversions by enhancing the rate of collisions with other molecules. An increase in temperature will increase diffusion coefficients in solution phase,

3. See Fig. 20-14 in Skoog book for typical TOF-MS design

The accelerating slits and/or ionization source must be pulsed so that the instrument can measure the time it takes for an ion to travel from the source to the detector. This time is proportional to the square root of the m/z ratio. Increasing the m/z decreases the velocity of the ion being ejected into the drift tube of the TOF analyzer since the KE of all ions is controlled by the magnitude of the accelerating voltage. The resolution is poor at the higher m/z ratios, since the difference in time of arrival at the detector becomes less at high masses, owing to the arrival time being dependent on the square root of the m/z ratio. Hence, it becomes harder to distinguish small m/z differences at high m/z ratios.

The resolution required to resolve the anti-theophylline Ab alone from its complex with theophylline has two acceptable answers. If you only assume a 1:1 complex of the drug with the antibody, then

average m/z / Δm/z = 149,090 / 180 = **828**

however, most antibodies are divalent; that is they bind two antigens at the same time---Thus it is possible for a complex to form that has two theophyllines and one Ab. In this case, the resolution required would be:

149,180 / 360 = **414**

4. a) image current: This is the induced current that is detected in the receiver plates in an FT-MS instrument, due to the ions circulating within the box. For a single m/z ion, this image current will have a frequency that is inversely proportional to the m/z ratio.

b) stokes line in raman spectroscopy: This corresponds to scattered photons that have an energy less than the initial energy of the photons impinged on the sample. After momentary retention of the photon by the molecule, the molecule relaxes to a higher vibrational energy level than it was initially, Hence, the scattered photon has an energy less than the incident photon by an amount that corresponds to the difference in energy between the ground vibational state, and the final high vibrational state.

c) FAB ionization: This is fast atom bombardment ionization for MS. In this method, the sample is placed on a plate in a matrix, usually glycerol, and the surface is bombarded with high KE atom beam, usually argon atoms. Collision of the argon atoms with the surfaces causes formation of analyte ions in the gas phase above the matrix.

d) Rayleigh Scattering: momentary interaction of photon with energy that does not correspond to vibrational or electronic energy transition of the molecule, and then immediate scattering of the same photon. Hence The scattered radiation has same energy and wavelength as incident radiation

5. Figure 20-24 in Skoog shows a detailed schematic of the triple quadupole MS arrangement. To determine one species in a complex matrix, one would want to use a soft ionization source, such as electrospray, for liquid samples. The first quadrupole would be set to a allow only ions with m/z equal to the the MW of the target analyte through this first mass filter.
The middle quadrupole would be used as a reaction chamber. An inert gas such as helium would be allowed to leak into this region, and collide with all the ions that made it through the first quadrupole. The collisions would cause dissociation of the parent ions with a given m/z into smaller "daughter ion" fragments. Thus, if several species in the original sample had a m/z that was the same (within the resolution limits of the first quadrupole analyzer), then the fragmentation pattern after collision would create a given fragment that would hopefully be unique, in terms of its m/z . The third quadrupole could then be tuned to a m/z that corresponded to this unique daughter ion. The ion current measured by the electron multiplier detector would be proportional to only the concentration of the original analyte, that had a given MW m/z ratio, and created daughter ions that had the m/z set on the third quadrupole. It is unlikely that any two species present in the original sample would have the same MW and produce the exact same fragmentation pattern.

Bonus: A double beam instrument allows you to continuously correct for the background absorbance of the solvent and/or reagents that exist in the sample for which you may want to obtain an entire UV-Vis spectrum. It basically provides a P_o or I_o value continuously at all the wavelengths as the monochromotor is scanned. In addition to allowing for background absorbance correction, a double beam instrument eliminates errors and noise in absorbance measurements due to source lamp fluctuations. Indeed, if source intensity changes suddenly, this is detected via a new I_o or P_o value for the reference beam. This is valuable not only when you scan to record an entire spectrum, but also when you measure absorbance at a single wavelength.