Birla Institute of Technology and Science Pilani –333031, Rajasthan
2nd semester 2016-2017, Comprehensive Examination, PART-A (Closed Book)
Course Title: Photochemistry & Laser Spectroscopy, Course No. CHEM C412
Max Marks: 40, Time: 90 mints, Date: 13.05.17

Answer all questions and all parts of a question together. Answer should be appropriate with proper units. Do the rough work alongside your answer.
After completing PART-A, submit your answer copy and get the question paper for PART-B.

Q1. Explain how the width of the spectral lines is related to motions of molecules mentioning the effect of phases of samples. **3.0M**

Q2. (a) As per Einstein's theory, write the expression for (i) rate of stimulated absorption, (ii) rate of stimulated emission, (iii) rate of total emission.
0.5M+0.5M+1.0M

(b) Show that at thermal equilibrium, Einstein coefficient, $B_{un\rightarrow lm} = B_{lm\rightarrow un}$. Assume that $\nu_{lm\rightarrow un} = \nu_{un\rightarrow lm}$. Given that Planck's radiation density,
$\rho(\nu) = (8\pi h\nu^3 n^3/c^3)\{1/[(\exp(h\nu/kT) -1]\}$ **2.5M**

Q3. Considering any one spin part of wavefuction of a triplet state show that the transitions between states of different multiplicities is forbidden. **3.0M**

Q4. With the help of group theory, explain whether $n_b \rightarrow \pi^*$ transition in acetaldehyde is allowed or forbidden. Character table for C_s point group is given below: **5.0M**

	E	σ_h		
A_1	1	1	x, y	R_z
B_1	1	-1	z	R_x, R_y

Q5. Following data are given for 1-methyl naphthalene:
Fluorescence quantum yield = 0.85, Phosphorescence quantum yield = 0.044, Triplet state lifetime = 2.1 s, Singlet state lifetime = 283 ns and Triplet quantum yield = 0.15.
Calculate:
(i) Phosphorescence rate constant **1.5M**
(ii) $T_1 – S_o$ intersystem crossing rate constant **1.0M**
(iii) Fluorescence rate constant **1.0M**
(iv) $S_1 – T_1$ intersystem crossing rate constant **1.0M**
(v) $S_1 – S_o$ internal conversion rate constant **2.0M**

Q6. A donor (D) and an acceptor (A) in a protein molecule are at such a distance so that Fluorescence Resonance Energy transfer (FRET) takes place. It has been observed that the lifetimes of D in the absence and presence of A are 5.30 ns and 0.98 ns, respectively. Given that the Förster distance, $R_o = 52.6\ A^o$.
(i) Determine the energy transfer efficiency? **1.5M**
(ii) Calculate the distance between the D and the A in the protein molecule? **2.0M**
(iii) Estimate the rate of energy transfer? **1.5M**

Q7. A fluorophore shows combined static and dynamic quenching of fluorescence in presence of a quencher, Q in aqueous medium at 25^{o}C. Fluorescence quenching data are given below:

[Q] (M)	**Fluorescence intensity**
0	11100
0.045	2127
0.230	429
0.560	144

Determine the static (K_S) and dynamic (K_D) quenching constants. Given that the slope of the plot of τ_o/τ versus [Q] for the same fluorophore-quencher combination = 89.4 M^{-1}.

6.0M+1.0M

Q8. (i) How is a pulsed solid state laser more effective than gas discharge lamp for flash photolysis? Briefly explain with an example of study of a particular process. **(ii)** Explain how Nd-YAG laser can be used for flash photolysis. **3.0M+3.0M**

END

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2nd semester 2016-2017, Comprehensive Examination, PART-B (Open Book)
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Answer all questions and all parts of a question together. Answer should be appropriate with proper units. Do the rough work alongside your answer.

Q1. How the structure of a fluorophore and polarity of solvents become factors for enhancement of fluorescence intensity. Briefly explain with example and schematic diagram. **4.0M**

Q2. Calculate the relative fluorescence quantum yield of a fluorophore using the data given below:
Area under the fluorescence band of the fluorophore = 40000 cm^{-1}
Area under the fluorescence band of the standard = 10000 cm^{-1}
Absorbance of the fluorophore at excitation wavelength = 0.18
Absorbance of the standard at same excitation wavelength = 0.012
Fluorescence quantum yield of the standard in 0.1 N H_2SO_4 = 0.55
(Neglect the effect of refractive index) **2.5M**

Q3. The fluorescence intensity (in arbitrary unit) of a fluorophore in absence and presence of 0.01M concentration of a dynamic quencher are 12456 and 8890, respectively. Calculate the percentage of collisions between the fluorophore and quencher is effective in fluorescence quenching. Given that the rate constant for a diffusion controlled bimolecular process = $1\times10^{10}M^{-1}s^{-1}$ and singlet state lifetime of fluorophore in absence of quencher = 4.5 ns. **2.5M**

Q4. Tryptophan residues in a protein molecule are fractionally accessible to a water soluble quencher acrylamide. The fluorescence spectra of this protein have been recorded in presence of varying concentration of acrylamide and intensity data obtained at a given wavelength are given in the table below:

[Acrylamide] (M)	**Fluorescence Intensity**
0.0000	12300
0.0123	10944
0.0369	9780
0.1230	8052
0.4920	6648

Estimate **(i)** the Stern-Volmer quenching constant of the accessible fraction and **(ii)** the fraction of the total tryptophan being quenched? **5.0M+2.0M**

Q5. Fluorescence anisotropy of a fluorophore has been measured by a T-format method. The intensities (in arbitrary unit) measured at four different channels are given below:
I_{VV} = 10000, I_{VH} = 2000, I_{HV} = 3000, I_{HH} = 1500.

(i) Draw the schematic diagram for the T-format measurement and point out the four channels used for the measurement of above mentioned intensities. **3.0M**

(ii) Estimate the value of fluorescence anisotropy by T-format method. **3.0M**

Q6. There are two tryptophan residues in a protein molecule. One of the residues (Trp-A) is exposed to the solvent and the other one (Trp-B) is present inside the hydrophobic pocket of the protein. Trp-A absorbs at 280 nm whereas Trp-B absorbs at 310 nm. There is some absorption at 285 nm as absorption spectra are overlapped in this region. When the protein molecule is excited at 310 nm, the fluorescence anisotropy decay is found to be single exponential with a rotational relaxation or a correlation time of 100 ns. Also the time-zero anisotropy, $r(0)$ is found to be 0.20. Studies have also shown that the relaxation time is independent of excitation wavelength. Answer the following **with justification in each case**:

(i) Anisotropy data corresponds to which residue? **1.5M**

(ii) What kind of rotational motion(s) of the residue do you expect? **2.0M**

(iii) How much is the rigidity of environment around the residue? **1.5M**

(iv) What kind of anisotropy decay do you expect if the protein molecule is excited at 285 nm and why? Draw the anisotropy decay curve. **2.5M**

(v) What is the expected shape of the protein molecule and why? **2.0M**

Q7. Following anisotropy data are obtained from the time-dependent anisotropy decay of a model protein molecule with a single tryptophan residue:

$\theta_1 = 700$ ps, $\theta_2 = 2500$ ps, $g_1 = 0.25$, $g_2 = 0.75$ and $r(0) = 0.3$.

(a) Answer the following **with justification in each case**:

(i) What kind of rotational motion(s) do you expect and why? **1.5M**

(ii) What do g-values represent here? **1.5M**

(iii) What is the significance of $r(0)$ value here i.e. how this value can be correlated with other anisotropy data. **1.5M**

(iv) What is the expected location of the tryptophan residue in the protein? **1.0M**

(b) Briefly explain what steady-state and time-dependent fluorescence experiment other than fluorescence anisotropy can be carried out to support the location of the tryptophan residue in the above mentioned protein. **3.0M**

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