

INDIAN INSTITUTE OF SCIENCE EDUCATION AND RESEARCH, KOLKATA
SEMESTER VII, 2018

ID 4102 - Fluorescence Spectroscopy: Principles and Applications
End-Semester Examination

MAX. MARKS – 50

Time: 3 hr.

Date: 30.11.2018

Answer any five questions. Each question carries equal mark.

1. (a) Suppose you have made a reasonably high concentration solution of a particular molecule. How will you experimentally know whether the molecules remain as individual molecules or they form very stable cluster in solution? (b) How does the value of rotational anisotropy change with the relative magnitudes of rotational correlation time and fluorescence lifetime? (c) What is the maximum possible correct value of rotational anisotropy? Under what physical conditions the experimentally measured rotational anisotropy value can cross this maximum value? (2+2, 2+2, 1+1)
2. (a) How does the RET efficiency depend on the distance? Explain mathematically and graphically. What is the maximum and minimum distance which can be measured accurately using Förster formulation? (b) What is the Physical significance of R_0 ? (c) Explain the donor fluorescence decay and acceptor fluorescence decay in absence and in presence of each other. (1+2+2, 1, 2+2)
3. (a) Explain with energy diagrams what are Type I and Type II QDs. (b) Explain the following observations: In case of lanthanoids (i) there are multiple luminescence bands, (ii) the luminescence bands are much sharper than fluorescent dyes (iii) luminescence maximum is solvent polarity independent. (2+2, 2+2+2)
4. (a) Draw a schematic diagram of confocal microscope and explain the properties of the components involved. (b) Explain the superior optical properties of QD over the fluorescent dyes. (4+4, 2)
5. (a) What are the units of single and two photon absorption cross-section? (b) What is meant by numerical aperture of a microscope objective? Explain mathematically and pictorially. (c) What is the axial resolution of image obtained from a confocal microscope for excitation with 488 nm light using an objective of NA = 1.4? (d) What will be the ratio of the translational diffusion coefficient for two proteins having molecular weight of 20000 and 540000 D? (2+2, 1+1, 2+2)
6. (a) Calculate the probability of finding “no molecule”, one molecule, and two molecules for a fluorophore concentration of 1nM in a confocal microscope of observation volume of 1fL assuming Poisson distribution. (b) Explain the dependence of fluorescence intensity and the amplitude of fluorescence autocorrelation function of intensity fluctuation on the concentration of the fluorophore. (c) Draw the energy level diagram of $\text{Ru}(\text{bpy})_3^{2+}$ and its Fe and Os analogues. Explain why only the former one exhibit PL but not the latter two. Explain the effect of temperature on the PL of these complexes. (2, 1+1, 2+2+2)

(All terms have their usual meaning).

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30/11/2018