M.TECH/BT/1ST SEM/BIOT 5103/2016

PHYSICO-CHEMICAL TECHNIQUES IN BIOTECHNOLOGY
(BIOT 5103)

Time Allotted : 3 hrs **Full Marks : 70**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 5 (five) from Group B to E, taking at least one from each group.

Candidates are required to give answer in their own words as far as practicable.

Group – A
(Multiple Choice Type Questions)

1. Choose the correct alternative for the following: **10 × 1 = 10**

(i) In AFM, the force experienced by the tip on the cantilever is given by
(a) $F = K.\delta z$ (b) $y = ax^2 + bx + c$
(c) $f = Z \sin\theta/\lambda$ (d) $T = 0.9\ \lambda/\ \beta\cos\theta_B$.

(ii) The linear dichroism parameter LD (λ) depends on the
(a) absorbance of the sample (b) emission wavelength
(c) solvent effects (d) emission lifetime.

(iii) The N-H group has a fundamental stretching frequency of around
(a) 1700 cm^{-1} (b) 3400 cm^{-1}
(c) 1400 cm^{-1} (d) 2000 cm^{-1}.

(iv) The turbidity τ to describe "conventional" light scattering is given by
(a) $\tau = -\ln I/I_o$ (b) $\tau = I - I_0$
(c) $\tau = 4\ \pi\ \rho_0/r^3$ (d) $\tau = KC/R_0$

(v) Which of the following techniques you would apply to analyze the changes of a cellular organelle?
(a) Scanning electron microscopy
(b) Transmission electron microscopy
(c) Phase contrast microscopy
(d) Fluorescence microscopy.

(vi) The approx λ_{max} for protein samples is around
(a) 260 nm (b) 280 nm
(c) 275 nm (d) 290 nm.

(vii) FRET experiments with single molecules allows which of the following?
(a) Observing conformational fluctuations in real time
(b) Observing macromolecular behavior in solution
(c) Steady state macromolecular behavior
(d) All of the above.

(viii) Optical isomers which do not have the object image relationship are called
(a) enantiomers (b) diastereoisimers
(c) racemic compound (d) meso compounds.

(ix) Which statement is true for a DNA double?
(a) Bases are stacked inside and the phosphate groups are protruded
(b) Bases are protruded outside and the phosphate groups are stacked inside stabilized by magnesium ions
(c) Both the bases and the phosphate groups are stacked
(d) Both the bases and the phosphate groups are protruded outside.

(x) The set that includes components of weak interaction is
(a) Van der Waals force, ion-ion interaction, ion-dipole interaction
(b) Peptide bonds, hydrophobic interaction, ion-ion interaction
(c) Peptide bond, disulfide bond, hydrogen bonds
(d) Disulfide bond, hydrophobic interaction, ion-ion interaction.

Group – B

2. (a) Describe the structure of an alpha-helix. What do you mean by melting of an alpha helix?

(b) Discuss the forces that stabilize the conformation of a protein molecule.
(4 + 2) + 6 = 12

3. (a) Amino acid sequence is important to maintain the conformation of a protein molecule. Justify the statement.

(b) Hydrophobic amino acid residues are usually found in the core of protein molecules. Discuss the exceptional cases where they are found on the surface.

(c) What do you mean by isotropic and anisotropic charge distribution on a protein molecule? Discuss why anisotropic charge distribution is common in membrane proteins.
4 + 4 + (2 + 2) = 12

Group – C

4. (a) Explain Lambert-Beer's law giving the relationship, definition of the parameters and the units.

   (b) A solution at a concentration of 32 μg/ml of a substance having a molecular weight of 423 has an absorbance of 0.27 at 540 nm measured in a cuvette with a 1 cm light path. What is the molar absorption coefficient at 540 nm? Assume that Beer's law is obeyed.

   (c) Draw a line diagram of a dual beam spectrophotometer labeling the various components.

4 + 4 + 4 = 12

5. (a) Use two graphs to explain the T1 and T2 relaxation times in NMR. Proton spectra of proteins invariably show pronounced changes on denaturation. Would you expect the appearance or disappearance of splitting to be one of the changes?

   (b) Explain how FT-IR spectroscopy is used diagnostically in proteins by measuring the vibrations associated with functional groups and highly polar bonds within proteins?

   (c) How can CD of polyd(GC).polyd(GC) be used to distinguish between B- and Z-DNA forms in solution?

(4 + 2) + 3 + 3 = 12

Group – D

6. (a) Define the quantum yield of a fluorophore. Write down the mathematical expression for quantum yield explaining all the terms. What is the importance of measuring quantum yield?

   (b) What are the types of fluorescence quenching mechanisms and explain their differences.

   (c) What is the Stern-Volmer equation? Explain its/their implication(s) with respect to the different quenching mechanisms.

(2 + 2 + 1) + 3 + (1 + 3) = 12

7. (a) How does Stokes shift in a fluorophore assist in its high sensitivity? Explain mathematically how Rayleigh scattering (elastic light scattering) can be used in the determination of M, the molecular mass of a biological macromolecule? What correction factors have to be introduced for scattering measurements in non-ideal solutions? Explain your answer.

   (b) To visualize the effect of dust contamination on light scattering experiments, perform the following calculation. A solution, containing 5 mg/ml of protein of M = 100,000, is contaminated to the extent of 0.001 percent of the protein weight by dust. The dust particles are 0.1 μm in radius, with density = 2.00. By what per cent will the 90^0 scattering be changed by this contamination, assuming Rayleigh scattering for all particles?

(2 + 3 + 3) + 4 = 12

Group – E

8. AFM is a technique that has become widely used for both the observation and manipulation of biological macromolecules at the single molecule level.
   (a) Draw a schematic diagram of an atomic force microscope labeling the different parts and listing essential operational details. What are the different modes of operation of an AFM instrument?

   (b) How is the motion of RNA polymerase on DNA observed by AFM? How are questions regarding transcription answered on the basis of these results?

   (c) What are the relative advantages/disadvantages of AFM versus electron microscopy?

(4 + 2) + (2 + 2) + 2 = 12

9. (a) What are the differences between SEM and TEM?

   (b) Pointwise describe how sample specimens are prepared for SEM and TEM measurements.

   (c) Calculate the wavelength of electrons travelling at a velocity of (i) 0.2×10^{-6} ms^{-1} and (ii) 20×10^{6} m s^{-1}. Comment on these values with respect to the resolution that can be obtained by an electron microscope ($m_e = 9.109 \times 10^{-31}$kg).

4 + 4 + 4 = 12