M.TECH /BT/1ST SEM/BIOT 5102/2018
PHYSICO-CHEMICAL TECHNIQUES IN BIOTECHNOLOGY
(BIOT 5102)

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) Which of the following can make difference in optical isomers?
(a) heat (b) temperature (c) polarized light (d) pressure.

(ii) The surface of the membrane proteins, which interacts with the hydrophobic tail of phospholipids, is
(a) Charged
(b) Hydrated
(c) More hydrophobic than the core
(d) Less hydrophobic than the core.

(iii) The mathematical equation representing collisional quenching is given by
(a) Efficiency $\propto 1/R_0^6$ (b) $P= I_{par}–I_{perp}/I_{par}+I_{perp}$
(c) $\mu= AxM/\varepsilon Xd$ (d) $I_0/I-1=K_aC_Q$

(iv) Which of the following is used in electron microscope?
(a) electron beams (b) magnetic fields
(c) light waves (d) electron beams and magnetic fields.

(v) Which of the following techniques gives a three-dimensional picture of the specimen?
(a) Transmission Electron Microscope
(b) Scanning Electron Microscope
(c) Compound Microscope
(d) Simple Microscope

(vi) Length of an alpha helix containing 220 amino acid residues is
(a) 1188 Angstroms (b) 33 Angstroms
(c) 792 Angstroms (d) 330 Angstroms.

(vii) Rayleigh scattering is defined by which of the following choices
(a) isotropic scattering
(b) proportion of light scattered by sample has different frequency than incident light
(c) Stokes lines
(d) anti-Stokes lines.

(viii) The linear dichroism, LD, is related to the wavelength of absorption by which ONE of the following expressions?
(a) $\Delta\varepsilon lc$ (b) $A\|(\lambda)-A\perp(\lambda)$
(c) $3\sin^2\alpha\sin^2\beta_i-1$ (d) $R_{fi}=Im\ (\mu_{if}.m_{fi})$.

(ix) Which of the following combination of techniques would you use to study the stretching or mechanical properties of a protein?
(a) atomic force microscopy, optical tweezers, magnetic beads
(b) NMR, EPR, MRI
(c) LD, CD, FT-IR
(d) MS/MS, MALDI-TOF, FT-ICR.

(x) Which of the following techniques was NOT used for confirmation of the Watson-Crick structure for DNA?
(a) Infra red absorption
(b) Ultraviolet circular dichroism
(c) Nuclear magnetic resonance
(d) Atomic absorption spectroscopy

Group – B

2. (a) Optical isomers arise due to some asymmetry in their structure. Justify the statement with at least two examples.

(b) Configurational isomers differ in some of their physical properties, whereas conformers differ in their energy states. Do you agree with the statement? Justify your answer.

(c) State whether the following two pairs of compounds belong to configurational isomers or conformational isomers:
i) E and Z 2-chlorobutene, ii) R&S Serine

4+ 6 + 2 = 12

3. The following observations are made about protein structure characteristics. Explain their significance with representative examples:
(i) Anisotropic charge distribution is present in a protein
(ii) Hydrophobic amino acid residues are present on the surface of a peptide chain
(iii) A charged amino acid residue is found in the core of a protein molecule

(4 × 3 =12)

Group – C

4. (a) A solution containing the amino acids Tryptophan and Tyrosine is being analysed from their different UV spectra under alkaline conditions (0.1M KOH). Under these conditions, the extinction coefficients at 240 and 280 nm are as follows:

ε_{240}(Tyr) =11,300 $M^{-1}cm^{-1}$ ε_{240}(Trp) = 1960 $M^{-1}cm^{-1}$

ε_{280}(Tyr) =1500 $M^{-1}cm^{-1}$ ε_{280}(Trp) =5380 $M^{-1}cm^{-1}$

A 10 mg sample of the protein glucagon is hydrolysed to its constituent amino acids and diluted to 100 ml in 0.1 M KOH. The absorbance of this solution (1 cm path) is 0.7 at 240 nm and 0.256 at 280 nm. Estimate the content of Tryptophan and Tyrosine in μmol.

(i) Suppose you would like to find out how much cytochrome c is contained in one *E.coli* cell. The molar absorption coefficient at its absorption maximum is known. How would you make this measurement?

(ii) Why do peaks in absorption spectra become narrower when the temperature is lowered to 77K ?

(b) A recombinant protein has been purified. Your research project is centered around whether that purified protein has adopted a folded structure. How might you use circular dichroism(CD) to address this problem? Your answer must contain details about experimental methodology.

(4 + 4) + 4 =12

5. (a) Draw a 2D FT NMR plot for a hypothetical molecule. Annotate the plot. Draw and label the NOESY pulse sequence. What is the operative effect of that pulse sequence?
If the preparation time is 3 sec, $t_1$varies from 0 to 51 msec at 200 μ sec intervals, and the FID is collected for 1 sec, how many repetitions of each t_1 FID can one collect and complete the measurement of a COSY in about 12 hr?

(b) What UV spectral changes would accompany complete enzymatic hydrolysis of a compact protein in 0.1M NaCl? How would you determine the concentration of an aqueous solution of a DNA sample using UV spectroscopy?

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

Group – D

6. (a) Explain the phenomenon of Fluorescence resonance energy transfer (FRET) in the context of it being a quenching mechanism. What are the requirements for FRET to occur? Why is usage of FRET as a tool to measure molecular distances in biological applications referred to as "*a spectroscopic ruler*"?Explain the mode of operation of the cyclic AMP fluorosensor based on FRET measurements.

(b) A protein containing ten tryptophans shows fairly strong tryptophan fluorescence. A small molecule is known to bind tightly to the protein produces virtually no change in the fluorescence, even though it is known that there are two tryptophans in the binding site. Give several *possible explanations* for this.

(1 +2 + 1 +3) + 5 = 12

7. (a) Explain the following items with relevant equations and diagrams as appropriate (i) Jablonski diagram (ii) a spectrofluorimeter with T-geometry (iii) Fluorescence anisotropy

(b) In analogy to absorbance in spectroscopy, if a beam of incident intensity I_0 passes through 1 cm^3 of solution and emerges with a smaller intensity I (the loss being due to scattering), the parameter τ (for turbidity) is defined as $\tau = -\ln I/I_0$. Show that for Rayleigh scattering of unpolarised light, $\tau = (16\pi/3)R_\theta$. What assumptions are you making in this derivation?

(2 ×3 = 6) + 6 = 12

Group – E

8. (a) How can you determine the resolution of a light microscope? What is the maximum resolution of a light microscope?

(b) Describe the benefits of using GFP in fluorescence microscopy? Describe the basic principle of Total Internal Reflection Fluorescence Microscopy with a proper diagram.

(2 + 1) + (4 + 5) = 12

9. (a) Explain the unique optical configuration of a phase contrast microscope with a line diagram. Write down two main applications of phase contrast microscopy. How do these applications exploit the technique's advantages?

(b) Why is resolution a better measure of a microscope's effectiveness/utility than its magnification? Explain the concept of *lateral* resolution limit in microscopy by comparing this parameter between an electron and a light microscope. How can the resolution limit be improved?

(c) Why is optical contrast necessary for microscopic observations of cells and tissues? Tabulate the differences between dark field and brightfield microscopy *keeping optical contrast in mind*.

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