Physico-chemical techniques in Biotechnology
(BIOT 5103) 27

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 alternatives for the following: **10 x 1=10**

(i) 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 bind, hydrogen bonds
- (d) Disulfide bond, hydrophobic interaction, ion-ion interaction.

(ii) Which statement best describes stereoisomerism?
- (a) Stereoisomers are the isomers with same chemical composition but different molecular formulae
- (b) Stereoisomers are the isomers with same molecular formula but different structural formulae
- (c) Stereoisomers are the isomers with same molecular formula but different functional groups
- (d) Stereoisomers are the isomers with same molecular formula, same structural formula but different arrangement of atoms and groups in three dimensional space

(iii) Hydrophobic amino acid residues are likely to be found
- (a) in the interior of a protein
- (b) on the surface of a protein
- (c) both in the interior and on the surface
- (d) difficult to predict.

(iv) The major stabilizing force of the double stranded DNA is
- (a) hydrogen bonding
- (b) hydrophobic forces due to base stacking
- (c) Van der Waals force
- (d) solvation energy.

(v) Which of the following techniques would you 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) Fluorescence in-situ hybridization uses the specificity of fluorescently labelled
- (a) DNA sequences
- (b) RNA sequences
- (c) amino acid sequences
- (d) all of the above.

(vii) The factors that affect a UV/Vis absorption spectrum include
(a) pH (b) dielectric constant of the solvent
(c) orientation effects (d) all of the above.

(viii) In atomic force microscopy the most frequently used technique is
(a) tapping mode (b) contact mode
(c) dynamic mode (d) non-contact mode.

(ix) In NMR spectroscopy, in the expression $\gamma_s = \gamma_0 (1-\sigma)$, σ represents the
(a) deshielding factor (b) shielding coefficient
(c) relative sensitivity of a nucleus (d) NOE constant.

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

Group – B

2.(a) What do you mean by a kinetically stable protein and a thermodynamically stable protein? Describe with an energy diagram.

(b) Explain why ribonuclease A renatures back to the original form once the cause for denaturation is removed but egg albumin does not.

(2 +2 + 3) + 5 = 12

3.(a) Explain why Serine can be found both in the core and the on the surface of a protein molecule.

(b) "Melting point of a DNA depends on the nucleic acid composition"- explain.

(c) "Melting of a DNA is a cooperative process"- explain

4 + 4 + 4 = 12

Group – C

4.(a) Draw a labelled diagram of the optical arrangements of a UV spectrophotometer.

(b) Briefly outline two applications of UV-Vis spectrometry.

(c) 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 Lambert-Beer's law is obeyed.

4 + 4 + 4 = 12

5.(a) Using two examples explain briefly how FT-IR spectroscopy can be used for determination of secondary structures of proteins.

(b) The following data list the CD maxima and minima for β-lactoglobulin A under two different conditions:

λmax,min	Δε
In aqueous buffer, pH 5	
215	-1.7
196	+2.6
In 99% ethanol, 0.01M HCl	
220	-7.6
208	-8.0
192	+15.5

Describe qualitatively what happens to β-lactoglobulin when it is transferred into acidic ethanol solution.

(c) If the preparation time is 3 seconds, t_1 varies from 0 to 51 msec at 200 μsec 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 hours?

4 + 4 + 4 = 12

Group – D

6.(a) Define fluorescence anisotropy explaining all the terms. Explain how anisotropy varies for the fluorescence of rhodamine dye as a function of the wavelength of the exciting light.

(b) What is FRET? What is the efficiency of FRET and what are the conditions under which FRET occurs?

(c) It is found that there are two sites for the attachment of fluorescent labels to a protein X. A pair is used for which R_0 is 2.3 nm. The energy transfer efficiency is found to be 0.015. Estimate the distance between the labels.

4 + 4 + 4 = 12

7.(a) Use a Jablonski diagram to explain the difference between fluorescence and phosphorescence. Explain why the excitation spectrum of a fluorophore is always the same as the absorption spectra.

(b) Iodide quenching decreases fluorescence intensity. Would you expect there to be a change also in the shape of either the excitation or emission spectrum?

(c) It was once conventional to describe light scattering in terms of the turbidity, τ. This is defined in analogy to the extinction coefficient 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 by scattering) we define $T = - \ln I/I_0$. Show that for Rayleigh scattering, $T = (16\pi/3)R_0$

(3+2) + 3 + 4 = 12

Group – E

8.(a) Draw a labeled diagram of an atomic force microscope (AFM).

(b) Stepwise explain the operation of an AFM instrument including different modes.

(c) Use an example to highlight how AFM has been used for single molecule studies.

4 + 4 + 4 = 12

9.(a) A novel protein has been isolated from a biochemical preparation. How might biophysical techniques be used to determine the protein's subcellular distribution and possible functions in the cell?

(b) What are the differences (technique and hardware wise) between SEM and TEM?

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

4 + 4 + 4 = 12