PHYSICOCHEMICAL 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) Dipole-dipole interactions are stronger than _____ interaction but weaker than _____ interaction.
(a) van der Waals, ion-ion
(b) ion-ion, van der Waals
(c) ion-dipole, van der Waals
(d) disulfide, ion-ion

(ii) Which of the following statements is incorrect?
(a) Hydrogen bonds are formed between an ion and a dipole
(b) ΔG is negative for melting of ice
(c) Sodium and potassium ions can undergo solvation in water
(d) Hemoglobin has more than one polypeptide chains.

(iii) Which of the following bonds are not involved in stabilizing the tertiary structure of protein?
(a) Disulfide bond
(b) Hydrogen bonding
(c) Salt bridges
(d) Phosphodiester bond.

(iv) In a Fluorescence Correlation Spectroscopy (FCS) experiment which of the following parameters are relevant measurables?
(a) hydrodynamic radius
(b) diffusion coefficient
(c) (a) and (b)
(d) none of the above.

(v) Circular Dichroism (CD) is an optical phenomenon that is accurately represented by which one of the following choices?
(a) Occurs when molecules in solution are exposed to circularly polarized light
(b) Occurs when molecules in solution are exposed to only left circularly polarized light
(c) Occurs when molecules in solution are exposed to only right circularly polarized light
(d) Occurs when molecules in solution are exposed to only monochromatic light.

(vi) Which of the following methods are able to detect *complex* protein interactions (more than 2 proteins) at molecular scale resolution?
(a) X-ray and NMR
(b) Surface plasmon resonance
(c) FRET analysis
(d) All of the above.

(vii) The rate of decay of a fluorescence signal is represented by which one of the following mathematical relationships?
(a) An exponential relationship
(b) A parabolic relationship
(c) A hyperbolic relationship
(d) None of the above.

(viii) Iodide quenching decreases fluorescence intensity. This is also accompanied by which one of the following changes?
(a) Change in the excitation spectrum
(b) Change in the emission spectrum
(c) Formation of a charge transfer complex between ion and salt
(d) None of the above.

(ix) Which one of the following component choices IS NOT a part of a dual beam UV spectrophotometer?
(a) Sample, reference cuvette
(b) Beam selector
(c) Prism and grating
(d) Michelson Interferometer.

(x) All the following statements are true both for SEM and TEM, except
(a) The illuminating source is an electron beam
(b) The microscope is focused using electromagnetic lenses
(c) Can be used to view specimens smaller than 0.2 microns
(d) Samples shoud be cut into thin sections.

Group - B

2. (a) Detergent and ethanol both interfere with the hydrophobic interactions of proteins. But their mechanisms of action are different. Justify the statement. [(CO1)(Justify/HOCQ)]
(b) Design an experiment to describe that some proteins like RNAseA can come back to their native structure once the denaturing agents are removed. [(CO1)(Design/HOCQ)]
5 + 7 = 12

3. (a) Describe the major weak forces that maintain the three dimensional structure of a protein molecule. [(CO1)(Understand/IOCQ)]
(b) Discuss the role of disulfide bonds to stabilize the three dimensional structure of a protein. [(CO1)(Understand/IOCQ)]
7 + 5 = 12

Group - C

4. (a) What spectral changes would probably accompany complete enzymatic hydrolysis of a compact globular protein in 0.1 M HCl?
(b) (i) A solution at a concentration of 35 μg/ml of a substance having a molecular weight of 430 has an absorbance of 0.27 at a wavelength of 540 nm measured in a cuvette with a 1-cm light path. What is the molar extinction coefficient at 540 nm? Assume that Lambert-Beer's law is being followed. (ii) Lambert-Beer's Law is valid

for low concentrations. What are the complications in its application at higher concentrations? Your answer should include sample and optical measurement related reasons. How might you devise an effective accurate solution for this problem? [(CO2)(Calculate-Analyze/IOCQ)]

4 + (4 + 4) = 12

5. (a) Use a table to represent the signature bands and corresponding vibrational frequencies (cm^{-1}) in FT-IR for secondary structure determination in proteins. Use an example of a polypeptide to illustrate how FT-IR can be used to detect hydrogen bonding with α-helices. What are the special features involved in preparing a protein sample for FT-IR? [(CO2)-remember-understand-LOCQ]

   (b) Use representative figures and mathematical equations to explain the significance of T_1 and T_2 in NMR. Use a table to report six important NMR derived structural parameters of molecules and the specific information that they provide. [(CO2)(Understand- Analyze/IOCQ)]

(2 × 3) + 6 = 12

Group - D

6. (a) What are the factors that determine the fluorescence properties of a molecule? Define the quantum yield Ø of a fluorophore. Write out the mathematical relationship for Ø defining all the terms. What is the importance of the fluorescence quantum yield parameter and how is it actually measured for a molecule? [(CO3)(Understand-analyze/IOCQ)]

   (b) A protein causes ANS to fluoresce. If the protein concentration is increased before adding ANS, the fluorescence decreases. Give two possible explanations for this decrease. [(CO3)-Analyze/IOCQ)]

(2 + 1 + 3 + 2) + 4 = 12

7. (a) A protein is labelled at two positions with an acceptor-donor FRET pair for surface topology mapping of the protein. This particular pair has a R_0 of 2.4 nm. The energy transfer efficiency is found to be 0.018. Estimate the distance between the labels. If FRET were the only tool available to you, how would you go about mapping the surface topology of this protein by extending this measurement?

   (b) What are the three conditions that need to be satisfied for an extrinsic fluorophore to be used satisfactorily for fluorescence measurements? Use one common extrinsic fluorophore that is conjugated to proteins as an example to support your answer. Give all relevant absorption, emission conditions for this fluorophore. [(CO3)(Understand-apply/IOCQ)]

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

Group - E

8. Describe the arrangements and roles of the following components of SEM.
   (i) Electron gun, (ii) Condensation lenses, (iii) vacuum system, (iv) specimen chamber. [(CO4)(Describe/IOCQ)]

(4 × 3) = 12

9. (a) Define resolution. Electron microscopy increases resolution of an image. Justify the statement. [(CO4)(Remember, Justify/LOCQ, HOCQ)]
   (b) SEM is used to study the surface phenomenon of a sample while TEM provides information about morphological and compositional information of the cell with a three dimensional image. Justify the statement. [(CO4)(Justify/ HOCQ)]

8 + 4 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	6.25	68.75	25

Course Outcome (CO):

Upon completion, this course should prepare registered students to:

1. Learn and apply principles of molecular interactions, classical thermodynamics and statistical mechanics to biological macromolecules viz. proteins and nucleic acids.
2. Learn the principles and instrumentation behind optical absorption techniques (e.g. UV-Vis, FT-IR) and magnetic absorption techniques (e.g. NMR) and their applications in the domain of biological macromolecules (e.g. UV bioassays, NMR of peptides/small proteins)
3. Learn the principles, instrumentation and applications of various sub-techniques of fluorescence emission spectroscopy (e.g. quenching, anisotropy) and Rayleigh scattering towards basic and applied functions with respect to proteins and nucleic acid (e.g. fluorescence biosensors, size of macromolecules)
4. Learn the principles, instrumentation and applications of single molecule techniques like confocal, atomic force, phase contrast and electron microscopies (application examples include single particle FRET and motion of RNA polymerase on DNA)

*LOCQ: Lower Order Cognitive Question; IOCQ: Intermediate Order Cognitive Question; HOCQ: Higher Order Cognitive Question