PHYSICOCHEMICAL TECHNIQUES IN BIOTECHNOLOGY
(BIOT 5102)

Time Allotted : 2½ hrs **Full Marks : 60**

Figures out of the right margin indicate full marks.

Candidates are required to answer Group A and any 4 (four) from Group B to E, taking one from each group.

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

Group – A

1. Answer any twelve: **12 × 1 = 12**

Choose the correct alternative for the following

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

(ii) Which of the following contains only covalent interactions?
- (a) Disulphide bond and ionic interaction
- (b) Disulphide bond and peptide bond
- (c) Peptide bond and hydrogen bond
- (d) Peptide bond and ionic interaction.

(iii) How many amino acids are present in one turn of alpha helix?
(a) 4.4 (b) 10.6 (c) 3.6 (d) 5.8.

(iv) What is the molten globule during protein folding?
- (a) The fully denatured state of the protein
- (b) The native state of the protein
- (c) A partially organized globular state with mostly the secondary structure
- (d) A state where the protein loses all its secondary structures.

(v) Which of the following forces in a protein is NOT a weak force?
- (a) Interaction between a glutamic acid residue and a lysine residue
- (b) Interaction between two cysteine residues leading to disulphide bond formation residue
- (c) Interaction between a glutamic acid residue and a cysteine residue
- (d) All of these.

(vi) The CD phenomenon of a protein rises in the wavelength interval
(a) 280-350 nm (b) 260-190 nm
(c) 300-370 nm (d) 310-380 nm.

(vii) In NMR what sort of information is provided through NOE measurements?
(a) Hydrogen bonding (b) Torsion angle
(c) Dihedral angles (d) Interionic distances.

(viii) Which of the following is used in an electric microscope?
(a) Electron beam (b) Magnetic field
(c) Light waves (d) Electron beam and magnetic field.

(ix) In SEM, the electrons which are deflected back in the direction of the beam are called?
(a) Back scattered electrons (b) Secondary electrons
(c) Auger electrons (d) Characteristic x-ray.

(x) We can view the internal content of a cell by
(a) SEM (b) TEM
(c) Light microscopy (d) All of these.

Fill in the blanks with the correct word

(xi) Protein-protein interaction sites that are flexible can be mapped using _____.

(xii) Using NMR, the T_2/T_1 ratio can be used to measure the _____ in proteins.

(xiii) Dynamic quenching occurs by collision between the fluorophore in its _____ and the quencher.

(xiv) TEM uses _____ as a source for making image.

(xv) An acidic amino acid has an additional _____ group in its side chain.

Group - B

2. (a) Discuss ubiquitination and Sumoylation of proteins. Do they contribute to weak forces in stabilization of a protein structure? Explain your answer.
[(CO1)(Discuss/LOCQ)]

(b) Lathyrism is a collagen disorder caused by consumption of the leguminous plant *Lathyrus odoratus*. Symptoms include difficulty in muscle and limb movement. It may lead to paralysis if remains untreated. When investigated, it is found that there is a toxin in the plant that leads to alteration of structure of collagen. Discuss the role of the toxin in alteration of the collagen structure.
[(CO1)(Understand/IOCQ)]
(6 + 3) + 3 = 12

3. (a) Discuss the major stabilizing forces of DNA. *[(CO1)(Discuss/IOCQ)]*
(b) What is melting point of DNA? Which factors affect the melting point of DNA?
[(CO1)(Remember/LOCQ)]
(c) Design an experiment to determine the melting point of DNA. *[(CO1)(Design/HOCQ)]*
4 + (2 + 2) + 4 = 12

Group - C

4. (a) Cytosine has a molecular extinction coefficient of 6×10^3 at pH 7. Calculate the absorbance of 1×10^{-4} M cytosine solution in a 1 mm cell. Show calculations clearly. *[(CO2)(Calculate/HOCQ)]*

   (b) Why is measurement of molar extinction coefficient at 190 nm sometimes preferred for protein solutions? What are the disadvantages compared to a measurement at 280 nm? *[(CO2)(understand-/LOCQ)]*

   (c) How is purity of a protein sample with DNA contamination measured by UV spectrophotometry? Your answer should be in quantitative terms. What is the rationale behind UV-derivative spectroscopy? What is its methodology and how is it implemented? *[(CO2)(analyze/IOCQ)]*

3 + 4 + (2.5 + 2.5) = 12

5. (a) Explain the equation $R(\lambda) = LD(\lambda)/A(\lambda)$ in linear dichroism in the context of a helical biopolymer. How can IR linear dichroism be used for the measurement of base inclination in dAdT polynucleotides? *[(CO3)(Analyse/HOCQ)]*

   (b) How can FT-IR be used for ascertaining the purity of a small-molecule pharmaceutical? Use an example to highlight your answer. *[(CO4)(Remember/LOCQ)]*

   (c) Use a table to represent four NMR derived structural parameters of molecules what is the information obtained from each of them and highlight each with an example. *[(CO2)(Apply/IOCQ)]*

4 + 4 + 4 = 12

Group - D

6. (a) Use a Jablonski diagram to represent the phenomenon of emission lifetimes of absorption, fluorescence and phosphorescence at the equilibrium inter-nuclear distance of the ground state. What are the reasons that make a fluorescence spectrum independent of the wavelength of excitation? *[(CO3)(understand-explain/IOCQ)]*

   (b) What are the requirements under which Fluorescence Resonance Energy Transfer (FRET) occurs? Write out the mathematical expression for the efficiency of FRET and use a diagram to represent the efficiency as a function of the distance between donor and acceptor molecules. Name four specific applications of FRET. *[(CO3)(Remember-understand/IOCQ)]*

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

7. (a) Use a table to represent varied light scattering techniques including Rayleigh Light scattering (RLS) and Dynamic Light scattering (DLS) and the information that can be obtained from them. *[(CO3)(understand-analyse/IOCQ)]*

   (b) Write out the expression for quantum yield ϕ of a fluorophore defining all the terms. How is it typically measured? *[(CO3)(understand-explain/IOCQ)]*

6 + 6 = 12

Group - E

8. (a) Compare a light microscope and an electron microscope for the following features: (i) electromagnetic spectrum, (ii) resolving power, (iii) lenses, (iv) fixing agent. *[(CO4)(Compare/HOCQ)]*

   (b) Discuss sample preparation for SEM. *[(CO4)(Remember/LOCQ)]*

   (c) Discuss the characteristics of an object that can be studied by SEM. *[(CO4)(Understand/IOCQ)]*

4 + 4 + 4 = 12

9. (a) Define a fluorophore. Discuss the use of fluorophore in fluorescence microscopy. *[(CO3)(Analyse/HOCQ)]*

   (b) How is fluorescence microscopy used to detect expression of a protein in a genetically modified organism? *[(CO4)(Remember/LOCQ)]*

   (c) What is FRAP? *[(CO2)(Apply/IOCQ)]*

4 + 6 + 2 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	32.29	47.92	19.79

Course Outcome (CO):

After the completion of the course students will be able 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.*