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)   A fluorescence decay curve is mathematically represented as
            (a) an exponential decay   (b) a parabolic decay
            (c) a linear decay   (d) all of the above

    (ii)   Which of the following represents a set of weak forces
            (a) Hydrogen bond, peptide linkage, base stacking interaction
            (b) Solvation, disulfide linkage, hydrogen bonds
            (c) Phosphodiester bond, ionic interaction, ion-dipole interaction
            (d) Hydrogen bond, solvation, hydrophobic interaction

    (iii)   Melting of a DNA is influenced by
            (a) salt concentration   (b) Base composition of the DNA
            (c) Nature of the cation   (d) All of the above

    (iv)   Presence of both beta sheets and alpha helices is a must for which motif?
            (a) Beta barrel   (b) Greek key motif
            (c) Beta-alpha-beta motif   (d) None of the above

    (v)   A 2D FT NMR plot has the following parameters on the two axes
            (a) δ, δ (b) I, ν
            (c) J, θ (d) I, λ

    (vi)   Which of the following are chromophores relevant for UV/Vis spectroscopy?
            (a) peptide bonds (amide bond)
            (b) certain amino acid side chains (viz. Trp and Tyr)
            (c) certain prosthetic groups and coenzymes (e.g. porphyrin groups in heme)
            (d) all of the above

(vii)   Change in sugar pucker for nucleic acids occur in the timescale of
(a) 1 to 100 psec   (b) 10 µsec-1 sec
(c) 1-10 psec
(d) 10-100 fsec

(viii)   The approach used to calculate protein structures from NMR data involves which one of the following choices
(a) generation of an ensemble of "low energy" conformations
(b) generation of structural conformations from torsion angle only
(c) generation of structural conformations from distance restraints only
(d) generation of an ensemble of "high energy" conformations

(ix)   Which statement regarding protein folding is NOT correct?
(a) Protein folding starts with hydrophobic amino acid residues
(b) Protein folding can be monitored by following absorbance at 280 nm
(c) After folding, charged amino acid residues are usually placed in the core of a protein
(d) Protein folding is spontaneous

(x)   The Rabi two level system is typically used to explain which of the following optical methods
(a) Laser excitation   (b) Hg arc lamp excitation
(c) UV spectroscopy   (d) FT-IR spectroscopy

Group – B

2. (a)   A protein remains in the folded form in biological systems though there is a decrease in entropy. What forces do you think keep the protein in the folded state?

    (b)   How does ΔG of the process become negative even when there is a decrease in entropy?

                                                                    8 + 4 = 12

3. (a)   What is the melting point of DNA? Derive the relation between Tm, ΔH and ΔS.

    (b)   What is Cot curve? Draw a typical Cot curve for a higher eukaryote. Explain the regions referring to the structural features of the DNA.

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

Group – C

4. (a)   Draw schematically the diagram of optical arrangements in a double beam spectrophotometer. When does Rayleigh light scattering interfere in spectrophotometric measurements of biological macromolecules?

    (b)   A protein with extinction coefficient $E^{1\%}=16$ yields an absorbance of 0.76 when measured in a 0.5 cm cell. Calculate the weight concentration.

(c) Explain stepwise the band narrowing approach of analysis of FT-IR spectra for secondary structure of proteins. Cite two advantages of FTIR with respect to protein secondary structure determination. What is one general methodological disadvantage of IR as a spectroscopic technique?

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

5. (a) Depict the in plane bending vibrational modes for 3 nonlinear atoms. What is the expression for the dipole moment operator using a Taylor series expansion? What are the primary signature regions for α-helices and β-strands in IR/FT-IR spectroscopy?

(b) Use any FT-IR spectral plot to depict hydrogen-bonded association of two bio-macromolecular entities.
   (i)   Explain the nature of the interaction that causes the Nuclear Overhauser effect (NOE) with applicable equations.
   (ii) How can you use NMR terms and parameters to determine and explain the identification of protein-protein interaction sites. Why is this important?
   (iii) Define the constituent terms in the following equation of relevance in NMR measurements:
        $^{x}J_{yz} = A\cos^{2}\theta + B\cos\theta + c$. What does this equation represent?

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

Group – D

6. (a) Draw a Jablonski diagram with emission lifetimes of absorption, fluorescence and phosphorescence. Write the mathematical expression for fluorescence quantum yield, Φ, defining all the constituent parameters. How is quantum yield generally measured in biochemical measurements?

(b) A protein causes anilino-napthalene sulfonic acid (ANS) to fluoresce. If the protein concentration is increased before adding ANS, the fluorescence decreases. Give two viable explanations for the decrease.
Iodide quenching decreases fluorescence intensity. Briefly explain the mechanism of quenching. Would you expect there to be a change also in the shape of either the excitation or emission spectrum?

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

7. (a) Describe the two types of solvent effects that influence a fluorescence spectrum. What are the dependent variables for such shifts?

(b) Define fluorescence polarization (FP) with its corresponding mathematical relationship. A fluorophore is covalently coupled to a protein. FP is is measured for this sample as a function of ionic strength of the suspending buffer; it is found to decrease markedly as the ionic strength increases. What effect does increasing the ionic strength have on the protein?

(c) Is the excitation spectrum of a fluorophore always the same as the absorption spectrum? Explain.

(d)   Name and define the two quenching mechanisms by which a quencher molecule decreases the quantum yield of a fluorophore by nonradiative processes. Write down the mathematical expressions for the two types of quenching. How can you distinguish between the two types of quenching experimentally?

3 + 3 + 2 + 4 = 12

Group – E

8.   (a)   Define resolution and magnification of a microscope. State the principle used to improve the resolution power of a microscope using an electron beam.

(b)   Compare three key features of an optical microscope and an electron microscope.

(2 + 2 + 2) + 6 = 12

9.   (a)   In a scanning electron microscopy (SEM) measurement, energy exchange between the electron beam and the sample results in reflection of high energy back scattered electrons and emission of low energy secondary electrons and Auger electrons. Define the three types of electrons involved.

(b)   Itemize the steps for sample preparation of SEM.

6 + 6 = 12

Department & Section	Submission Link
BT	https://classroom.google.com/c/Mjc0ODQxMTU1NTk5/a/Mjg4NDMxMTEwMTQ0/details