M.TECH/BT/1ST SEM/BIOT 5102/2021

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) In 2D NMR the combination of COSY/TOCSY and NOESY is useful for which one of the following reasons?
(a) COSY/TOCSY assigns protons to different spin systems representing individual amino acid side residues and their side chains
(b) NOESY helps to determine the spatial relationships between the spin systems
(c) such homonuclear techniques can determine the number of residues
(d) both (a) and (b)

(ii) The following equation
$\rho^* = (!.52 \text{ X } A_{280} - 0.75 X A_{260})$ mg cm^{-3} is used for which of the following reasons
(a) can be used for measuring the concentration of a protein if the molar extinction coefficient ε is known
(b) can be used for measuring the concentration of DNA by ratiometric measurement
(c) can be used for the measurement of the concentration of a protein if the molar extinction coefficient is not known
(d) none of the above

(iii) The equation $I(\nu) = c/n\ \rho(\nu)$ represents
(a) a way to describe the dynamics of a radiation induced transition
(b) a relationship where ρ(ν) represents the radiation energy density
(c) a relationship where ρ(ν) represents the frequency of radiation
(d) both (a) and (b)

(iv) For purification of mRNA binding proteins, which of the following ligands can be used for affinity chromatography
(a) polyadenylic acid linked via N6 amino group
(b) NADP/NAD
(c) protein G derived from streptococci
(d) Iminodiacetic acid

(v) Which one of the following equations represents the quantum yield of fluorescence?
(a) $k_f=1/\tau_0$ (b) $\Phi_f=\tau/\tau_0$ (c) $\tau=1/K_d$ (d) Eff = $r_o^6/r_0^6+r^6$

(vi) Which of the following combinations of wavenumbers represent Amide I and Amide II frequencies in a FT-IR spectrum of a protein?
(a) 2900 cm^{-1}, 2980 cm^{-1} (b) 3280 cm^{-1}, 3050 cm^{-1}
(c) 1300-1350 cm^{-1},667 cm^{-1} (d) 1600-1700 cm^{-1}, 1500-1600 cm^{-1}

(vii) Melting of DNA gives a
(a) liner graph (b) hyperbolic graph
(c) Sigmoidal graph (d) none of the above

(viii) Hydrogen bond often plays an important in stabilization of different molecules. Select the molecule where hydrogen bond does not contribute to the stability.
(a) Proteins (b) DNA (c) water (d) lipid micelle

(ix) Flourescence microscopy is best technique for observing
(a) three dimensional structure of a cell (b) dynamicity of macromolecules of a cell
(c) intracellular organelles of a cell (d) surface phenomenon of a cell

(x) Which amino acid pair cannot form a salt bridge structure
(a) Aspartic acid and Lysine (b) Aspartic acid and Leucine
(c) Histidine and Aspartic acid (d) Glutamic acid and Arginine.

Group- B

2. (a) Draw the structures of the following compounds: D-glyceraldehyde, 2, E-Butene, L-threonine. [(CO1) (Remember/LOCQ)]
(b) Analyze the following observations
(i) Lipid forms micelle in water
(ii) Membrane proteins often contain segments with large helical structures. [(CO1) (Analyze/IOCQ)]
(c) Evaluate the importance of GC content in determining the melting point of a DNA molecule. [(CO1)(Evaluate/HOCQ)]

3 + 6 + 3 = 12

3. (a) Differentiate between conformation and configuration. Illustrate different conformations of n-butane with proper description. [(CO1) (Analyze/IOCQ)]
(b) Evaluate the importance of the proximal His residue of myoglobin in binding of oxygen. [(CO1) (Evaluate/HOCQ)]
(c) Describe the cooperative nature of protein denaturation. (CO2)(Analyse/IOCQ)]

(2 + 4) + 3 + 3 = 12

Group - C

4. (a) UV absorption spectroscopy is particularly valuable for determination of the concentration of biological macromolecules. Itemize the reasons for this. Explain

in *quantitative terms* the relative advantages/disadvantages of measuring the concentration of a protein at 190 nm vs 280nm.
[(CO3) (Understand-analyze/IOCQ)]

(b) A solution at a concentration of 30 μg/ml of a substance having a molecular weight of 428 has an absorbance of 0.29 at 540 nm measured in a cuvette with a 1 cm light path. What is the molar absorption coefficient at 540 nm? Assume that Beer-Lambert's law is followed. [(CO3)(Analyze/LOCQ)]

(c) Use a labelled diagram to represent the different optical components of a FT-IR spectrometer. What is the specific role of a Michelson interferometer? How is a sample prepared for IR? [(CO3) Remember-understand/LOCQ)]

(d) For a sample to be IR-active there should be a transition dipole associated with the bond vibration. What is the mathematical expression for the transition dipole moment μ^{q}_{10} for the normal coordinate mode q of *a particular* fundamental vibration? Explain the significance of this mathematical expression. [(CO3)(Analyze/HOCQ)]

3 + 3 + 3 + 3 = 12

5. (a) (i) The relaxation mechanisms in nuclear magnetic response (NMR) are analysed through the two parameters T_1 and T_2. Define the two parameters. How are they measured and what are the applicable equations?

(ii) What type of processes in biological macromolecules can be probed by measurement of these relaxation times? Cite at least four such processes.

(iii) As a biomedical application, how has multidimensional NMR been used to study the structure of the ribosome that is the target of many antibiotics? [(CO3) (Remember-Analyze/IOCQ)]

(b) (i) What are the specific conditions for a molecule to be IR-active?

(ii) Inanalysing the FTIR-spectrum of a protein for its 3-state secondary structure (alpha helices, beta strands, turns/random coil) what is the standard band-narrowing deconvolution method in operation? Outline the steps in this methodology in detail using IR-band assignments that are necessary.

(iii) Give a representative example of how FT-IR and time resolved FT-IR techniques have been used in the analysis of a peptide/protein that is of biochemical and biomedical importance. Your answer should have specific details of the system, process/reaction being monitored and relevant aspects of the technique and spectrum.
[(CO3) (Remember-Understand/LOCQ)]

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

Group – D

6. (a) (i) Why are fluorescence maxima observed at larger wavelengths than absorbance maxima? [(CO4) (Remember-understand/IOCQ)]

(ii) Bioluminescence occurs in a wide variety of biological organisms. Illustrate and explain this phenomenon using examples, its *unique* characteristics and its *varied* purposes. [(CO4) (Understand-Analyze/IOCQ)]

(b) To visualize the effect of dust contamination on light scattering experiments, the following calculation is performed. A solution, containing 5 mg/ml of protein of M=100,000, is contaminated to the extent of 0.001% of the protein weight by dust. The dust particles are 0.1 μm in radius, with density =2.000 gm/cm^3. By what percent will the 90^0 scattering be changed by this contamination, assuming Rayleigh scattering for all particles? Is this a good assumption? Is the true situation apt to be more, or less serious than your estimate?
[(CO4)(Analyze/HOCQ)]

(2 + 5) + 5 = 12

7. (a) Define fluorescence anisotropy (FA) using a labelled diagram of the experimental setup and a mathematical expression. Based on your knowledge about the technique and the dynamics of proteins answer the followingquestion: A fluorophore is coupled to a protein by a covalent bond. A FA experiment is conducted where the anisotropy is measured as a function of the ionic strength of the suspension buffer; it was observed that the FA decreases sharply as the ionic strength increases. Explain the *effects of increasing ionic strength* of the buffer on the protein based on the anisotropy results.
[(CO4) (understand analyse/IOCQ)]

(b) A protein has been labelled with a dye and the latter has a fluorescence lifetime of 7.0 nsec. This same protein has another site to which a fluorescent label can be attached. Given that this pair has a R_0 of 2.3 nm and the energy transfer efficiency is 0.015 , estimate the distance between the labels. Use this problem to establish the use of FRET as a "molecular ruler". What characteristics of FRET make this application feasible and widespread?
[(CO4) (Understand-analyze/IOCQ)]

(c) Explain the phenomenon of fluorescence quenching using the expression of quantum yield of a fluorophore and other relevant expressions. What are the types of quenching and their respective mathematical expressions? Cite two major applications of each type of quenching mechanism.
[(CO4)(Remember-understand/LOCQ)]

4 + 4 + 4 = 12

Group - E

8. (a) Define a fluorophore. What is fluorescencemicroscopy?
[(CO4) (Remember /LOCQ)]

(b) Justify the following statements.

(i) Fluorescence microscopy can only be applied where we have a suitable fluorophore.

(ii) Dynamic behaviour of cellular components can be studied by fluorescent microscopy. [(CO4) (Analyze/IOCQ)]

(2 + 2) + (4 + 4) = 12

9. (a) AFM was developed to overcome a limitation of STM. What was that limitation? [(CO4) (Remember/LOCQ)]
   (b) Discuss the principle of AFM. [(CO4) (Understand/LOCQ)]
   (c) Discuss the application of AFM for studying biological samples. [CO4)(Apply/IOCQ)]

2 + 4 + 6 = 12

Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	30%	43 %	27%

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

Department & Section	Submission Link
BT	https://classroom.google.com/c/NDQ1NDMyMDcwNjU5/a/NDc0ODcwNjkzNzI3/details