HERITAGE INSTITUTE OF TECHNOLOGY

…M.Tech 1styr 1st Semester Examination. 20 14……   Session   : …2015…………………

Discipline   : …Biotechnology……………………………….

Paper Code : BIOT 5103…Paper Name :Physico-Chemical Techniques in Biotechnology
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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 x 1=10

(i)   The major stabilizing force of a DNA double helix is:
(a)hydrophobic interaction   (b) ionic interaction
(c) hydrogen bond   (d) ion-dipole interaction

(ii)   Which one is not an example of a weak force?
(a) Ion-ion interaction   (b) dipole-dipole interaction
(c) disulfide linkage   (d) van der Waals force

(iii)   The C=O group has a fundamental stretching frequency of around
(a) 1700 cm^{-1}
(b) 1500 cm^{-1}
(c) 1400 cm^{-1}
(d) 2000 cm^{-1}

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

(v)   Fluorescence spectroscopy can be used to
(a) probe dynamic processes of excited electronic states
(b) measure distances in biological structures
(c) follow reaction kinetics
(d) all of the above

(vi)   Which one is not an example of configurational isomers:
(a) D and L glyceraldehyde   (b) Threonine and allothreonine
(c) D tartaric acid and meso tartaric acid   (d) A and B forms of DNA

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(vii)   Serine residues are stable
    (b) On the outer surface of a protein   (b) in the core of a protein
    (c) Both on the outer surface and core   (d) extremely unstable within a protein

(viii)   FRET experiments with single molecules allows which of the following
    (a) observing conformational fluctuations in real time
    (b) observing macromolecular behaviour in solution
    (c) steady state macromolecular behaviour
    (d) all of the above

(ix)   In an alpha helix, side chains of the amino acids are

    (a) All residues are protruded outside
    (b) all residues are stacked inside
    (c) polar residues are protruded outside
    (e) non-polar residues are protruded outside

(x)   The circular dichroism (CD) is defined as
    (a) difference in extinction coefficient
    (b) difference in emission wavelength
    (c) difference in emission frequency
    (d) all of the above

Group - B

2.(a)   State the differences between conformation and configuration. Changes in the tertiary structure of a protein molecule are conformational changes. State whether the statement is true or false. Justify your answer.

(b)   Explain why:
i) Polyglutamic acid cannot form a stable helix at physiological pH but can do so at pH 11.
ii) Proline is considered a helix breaker.   2+4+(4+3) = 12

3.(a)   Discuss the major stabilizing forces of an alpha helix.

(b)   What do you mean by melting of alpha helix? Which forces are disturbed during this process?   7+(2+5)= 12

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Group - C

4.(a) What is Lambert Beer's law? Cytosine has a molecular extinction coefficient of 6×10^3 at 270 nm and pH 7. Calculate the absorbance of 1×10^{-5} and 1×10^{-4} M cytosine solutions in a 1 mm cell.

(b) Draw a labelled diagram of a double cell absorption spectrophotometer.

(c) Cite two applications of UV spectrophotometry.   6+4+2 = 12

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

(b) If the reduced dichroism (R) for single stranded poly (rA) at perfect alignment is -1.25 at 260 nm and the bases are inclined $28^{°}$, what is the relative angle between the axis of inclination and the transition dipole (β)? Explain your answer   6+6 = 12

Group - D

6.(a) Using a diagram explain the difference between fluorescence and phosphorescence.

(b) Why is a fluorescence spectrum independent of the wavelength of excitation?

(c) Calculate the intrinsic lifetime τ_0 from the following decay curve if the quantum yield is 0.5

t (sec)=0	2	4	6	10	14	20	30
I =1.0	.82	.67	.55	.37	.25	.14	.05

3 +3 +6 = 12

7.(a) Describe the criteria of FRET.

(b) A protein has two sites for attachment of fluorescent labels. A pair is used for which R_0 is 2.3 nm. The energy transfer efficiency is found to be about 0.015.Estimate the distance between the labels.

(c) What are the differences between dynamic and static quenching?   4 +4 +4 = 12

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Group - E

8.(a)   What is the difference between of single molecule methods as opposed to standard spectroscopic methods?

(b)   Briefly describe two characteristics of molecular behaviour that can be studied using single molecule techniques

(c)   Draw a labelled diagram of a confocal microscope and briefly discuss its principle. What are the advantages of confocal microscope over fluorescence microscope?   4+4 +(3+1)= 12

9.(a)   Draw a labelled diagram of an atomic force microscope (AFM).

(b)   Explain the operation of an AFM instrument. What are the different modes of AFM?

(c)   Use an example to highlight how AFM has been used for single molecule studies.   4+(2+2)+4 = 12