

BIRLA INSTITUTE OF TECHNOLOGY, MESRA, RANCHI
(END SEMESTER EXAMINATION)

CLASS: BE
BRANCH: BIOTECHNOLOGY

SEMESTER : III
SESSION : MO/18

SUBJECT: BT3025-BIO-ANALYTICAL TECHNIQUES
TIME: 03:00

FULL MARKS: 60

INSTRUCTIONS:

1. The question paper contains 7 questions each of 12 marks and total 84 marks.
 2. Candidates may attempt any 5 questions maximum of 60 marks.
 3. The missing data, if any, may be assumed suitably.
 4. Before attempting the question paper, be sure that you have got the correct question paper.
 5. Tables/Data hand book/Graph paper etc. to be supplied to the candidates in the examination hall.
-

- Q.1(a) Calculate relative centrifugal force of a fixed angle type rotor with rpm of 160,000 and r_{\min} of 20 mm and r_{\max} of 80 mm. [2]
- (b) Derive Svedberg equation for an un-hydrated molecule in terms of molecular weight of the macromolecule, density of solvent and partial volume particle. Illustrate CsCl_2 and sucrose gradient analytical centrifuge? [4]
- (c) A protein has a sedimentation coefficient of 7.2 S, a diffusion coefficient $D = 8.8 \times 10^{-7} \text{cm}^2 \text{sec}^{-1}$ and $v_p = 0.92 \text{cm}^3 \text{g}^{-1}$ (all measured at 22°C). (a) How long will it require to migrate from $r = 11.0 \text{cm}$ to $r = 11.8 \text{cm}$ in a rotor spinning at 72,000 rpm? (Assume constant velocity throughout this time). (b) What is the molecular weight of the DNA? [6]
- Q.2(a) Compare the Van-Deemeter and Halasz Equation related to chromatography? [2]
- (b) Consider for a LC chromatogram consisting of two peaks with following details, first peak with height of 18,000 au, retention time of 20 mins and FWHM of 2 msec, for second peak height 40,000 au, retention time 30 mins and FWHM 8 msec, calculate (a) number of Theoretical Plates (N), (b) Resolution (RS) and (c) Separation factor. [4]
- (c) Illustrate, sketch, and interpret mechanism of Affinity Chromatography (principle, elution process, ligand immobilization and ligand design) [6]
- Q.3(a) Compare packed columns, WCOT, SCOT and FSOT used for Gas Chromatography. [2]
- (b) In a thin layer chromatography (TLC), if a compound travels 3.4 cm and the solvent front travels 2.6 cm, calculate the retention factor. Calculate distribution Constant (K_c), column's Phase Ratio (B) and retention constant for a HPLC system. [data given: solute concentration in mobile phase = 110 mg/l; solute concentration in stationary phase = 25 mg/l; column radius = 142 micrometer, film thickness = 8 micrometer]. [4]
- (c) Compose and illustrate different components (inlet, column, separation conditions) of Gas Chromatography system with proper diagram including different detectors. [6]
- Q.4(a) Demonstrate electrophoresis and electro-osmosis process. [2]
- (b) Hypothesize and evaluate the mechanism of one-dimensional, two-dimensional gel electrophoresis and isoelectric focusing with proper diagram. [4]
- (c) Design, label and demonstrate SDS-PAGE system for protein separation (different components, constituents of loading and stacking gel, protein identification with marker lane). [6]
- Q.5(a) Illustrate the differences between fluorescence and phosphorescence. [2]
- (b) Sketch and organize the basic system for dual beam UV-VIS spectrometer. [4]
- (c) Write and explain Beers-Lambert law. Design and explain the instrumentation of Spectrofluorimeter. [6]
- Q.6(a) Compare principle of mechanism of Atomic Absorption Spectroscopy (AAS), Flame emission Spectroscopy (FES) and Atomic Fluorescence Spectroscopy (AFS) [2]
- (b) Write the role of a nebulizer in flame spectroscopy? Dramatize and sketch a double beam flame atomic absorption spectrometer with atomizing unit. [4]
- (c) Compose the working principle of MALDI, ESI, Quadra-pole and TOF technique related to Mass spectroscopy. [6]
- Q.7(a) Compare the principle of DTA, DSC and TGA system. [2]
- (b) Sketch and label different components of a Thermal Gravimetric Analysis (TGA) system. Briefly correlate the chemical dynamics of calcium oxalate $[\text{Ca}(\text{C}_2\text{O}_4) \cdot x\text{H}_2\text{O}]$ molecule with TGA graph. [4]
- (c) Illustrate, sketch and explain Differential Scanning Calorimetric (DSC) system with a representative DSC graph of a polymer sample (point different phase transition state). [6]