BIOSEPARATION TECHNOLOGY
(BIOT 4102)

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 gel filtration chromatographic separation, bio-molecules are separated based on what property of bio-molecules?
(a) size (b) charge
(c) hydrophobic interaction (d) metal ion affinity.

(ii) Molecular weight of a protein can be determined by
(a) size exclusion chromatography
(b) ion-exchange chromatography
(c) pseudo-affinity chromatography
(d) affinity chromatography

(iii) What type of protein you can purify by Ni-NTA–Agarose affinity column chromatography?
(a) GST-tagged protein (b) 6 X His-tagged protein
(c) Cys-tagged protein (d) DNA binding protein.

(iv) Basic principle of centrifugation depends on
(a) concentration (b) polarization
(c) centripetal force (d) pressure gradient.

(v) Cell disruption in homogenizer is based on
(a) applied voltage (b) operating pressure
(c) salt concentration (d) osmotic pressure.

(vi) In affinity chromatography, if the reactive group on the matrix is -- OH group then coupling agent is
(a) bisepoxide (b) dichlorotriazine
(c) tricyclic chloride (d) cyanogen bromide.

(vii) Electrophoresis is used for the separation of
(a) charged biomolecule (b) neutral biomolecules
(c) organic molecules (d) inorganic molecules.

(viii) Liquid-liquid extraction depends on
(a) distribution coefficient (b) volatility
(c) solubility (d) partition coefficient.

(ix) Affinity chromatography is based on highly specific interaction between
(a) solute molecules and ligands
(b) among solute molecules
(c) among ligands
(d) solute molecules and ceramic beads.

(x) SDS-PAGE uses
(a) anionic detergent (b) cationic detergent
(c) non-ionic detergent (d) no detergent.

Group - B

2. (a) Define concentration polarization.

(b) What do you mean by polarization modulus?

(c) It is desired to filter a cell broth at a rate of 1000 liters/hr on a rotary vacuum filter at a vacuum pressure of 70 K Pa. The cycle time for the drum will be 60 sec and the cake formation time (filtering time) 15 sec. The broth to be filtered has a viscosity of 2.0 c p and 10 gm cake solids (dry basis) per liter of filtrate.

Data Given:- specific cake resistance 9×10^{10} cm/gm. $R_m = 0$ (filer medium resistance).

2+ 3 + 7 = 12

3. (a) Define distribution coefficient with reference to liquid-liquid extraction.

(b) Yeast cells are recovered from a fermentation broth by using a tubular centrifuge. Sixty percent (60%) of the cells are recovered at a flow rate of 12 L/min with a rotational speed of 4000 rpm. Recovery is inversely proportional to flow rate.
i) To increase the recovery of cells to 95% at the same flow rate, what should be the rpm of the centrifuge?
ii) At a constant rotation of 4000 rpm, what should be the flow rate to result in 95% cell recovery?

(c) A viscous solution contains particles with a density ρ_P = 1200 kg/m^3 and its viscosity is 80 cp. The centrifuge has a bowl with r_2 = 0.02 m and r_1=0.01m and height b = 0.25 m. Calculate the critical particle diameter of the largest particles in the exit stream if N = 15000 rpm and flow rate q = 0.002 m^3/hr?

2+ 5 + 5 = 12

Group - C

4. (a) A laboratory column 10 cm in diameter and 3 m deep, is found to produce good results in COD removal when operated at a flow of 50 L/hr. Calculate the following:

(i) The application rate in m/hr.

(ii)The residence time, t, in the column

(iii)The volumetric flow rate, V_b, in bed volumes per hour, for this residence time

Assume the fractional void volumes for granular carbon columns is 0.5.

(b) What are the characteristic features of solvent precipitation and isoelectric precipitation?

8 + 4 = 12

5.(a) Define operating line and extraction factor

(b) A pilot scale reciprocating-plate extraction column is used for the extraction of an antibiotic from whole fermentation broth using amyl acetate as solvent. The antibiotic has a partition coefficient K = 7.5, The optimal operating conditions are as follows : solvent flow rate of 105 ml/min, flow rate of fermentation broth of 70 ml/min, and ratio of antibiotic in raffinate to antibiotic in feed of 0.07 . The column was 2.54 cm in diameter, and the height of the extractor was 1.83 m. The agitator speed was 280 strokes/min. What column size and agitator speed are required to give a ratio of antibiotic in the raffinate to antibiotic in the feed of 0.03 and to handle fermentation broth at a rate of 150,000 liters every 12 hrs?

3 + 9 = 12

Group - D

6. (a) Discuss about membrane fouling and concentration polarization during membrane based bioseparation.

(b) Equipment is available in your lab for ultra-filtration of a protein solution at constant volume to remove low molecular weight species by the addition of water to the feed in an operation called diafiltration. The flow channels for this system are tubes 0.1 cm in diameter and 100 cm long. The protein has a diffusion coefficient of 9×10^{-7} cm^2 /sec. The solution has a viscosity of 1.2 cp and a density of 1.1 gm/cm^3. The system is capable of operating at a bulk stream velocity of 300 cm/sec. At this velocity, determine the polarization modulus for a transmembrane flux of 45 liters/ m^2 hr.

5 + 7 = 12

7. (a) A column 20 cm long, with an internal diameter of 5 cm gives sufficient purification to merit scale-up. The column produces 3.2 gm of purified protein per cycle, and a cycle takes 6 hr, from equilibration through regeneration. You want a throughput of 10 gm/hr. Find new column's diameter if linear velocity is held constant.

(b) We wish to precipitate protein macroglobulin (mol. wt. 820,000 and diffusion coefficient of 2.41 x 10^{-7} cm^2 /sec at 20^0C) contained in 100 liters of aqueous solution at 20^0C in a tank at a conc. of 0.2 gm/liter. The precipitate particles have a density of 1.3 gm/cm^3. The solution is stirred with a 75 w motor. Calculate the conc. of nuclei at the end of the "initial mixing" period.

3 + 9 = 12

Group - E

8. (a) Discuss nucleation and crystal growth for crystallization operation with the help of most useful relationship (mathematical equations).

(b) It is desired to scale up a batch crystallization of an antibiotic based on experiments with a one liter crystallizer. The use of a 3 cm diameter impeller at a speed of 800 rpm led to good crystallization results. For maintaining power per volume constant upon scale up to 300 liters, what should be the diameter and speed of the large-scale impeller? Solvent has density and viscosity as water.

4 + 8 = 12

9. (a) Give a complete flow diagram of isolation and purification of
(i) Insulin & (ii) Penicillin

(b) Define nucleation with reference to crystallization.

5 + 5 + 2 = 12