

**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.
All symbols are of usual significance*

**Group – A
(Multiple Choice Type Questions)**

1. Choose the correct alternative for the following: **10 × 1 = 10**
 - (i) Which one of the following is the best for separating proteins according to their sizes and shapes?

(a) Filtration	(b) membrane separation
(c) centrifugation	(d) gel filtration.
 - (ii) Proteins were separated in the SDS-PAGE according to their

(a) charge	(b) hydrophobicity
(c) size	(d) polarity.
 - (iii) Liquid-liquid extraction depends on

(a) concentration	(b) volatility
(c) solubility	(d) partition coefficient.
 - (iv) Which one of the following is an ion-exchange column?

(a) C M Sepharose	(b) Phenyl agarose
(c) Sephadex G-100	(d) Protein A sepharose.
 - (v) In reverse osmosis, the deposition of solute molecules on membrane surface results in large resistance for solvent flow. This phenomenon is known as

(a) reflection coefficient	(b) rejection coefficient
(c) breakthrough point	(d) concentration polarization.
 - (vi) Which method is commonly used to separate inhibitory fermentation product such as ethanol from fermentation broth?

(a) Aqueous two phase extraction	(b) Liquid-liquid extraction
(c) Adsorption	(d) Ultrafiltration
 - (vii) Filtration rate depends on

(a) pressure difference	(b) area of filter
(c) viscosity of medium	(d) all of these.

- (viii) Which one of the following should be used for the first step of purification of a protein from a complex mixture?
 - (a) Precipitation
 - (b) Ion exchange chromatography
 - (c) Affinity chromatography
 - (d) Hydrophobic interaction chromatography.
- (ix) Which of the following will help to confirm the mol. wt. of the purified protein?

(a) Iso-electric focusing	(b) affinity chromatography
(c) gel-filtration	(d) SDS-PAGE.
- (x) Ultrafiltration is used for separation with molecular weight range from

(a) 0.1-10 μ m	(b) 10-100 μ m
(c) 100-200 μ m	(d) 200-500 μ m.

Group – B

2. (a) A viscous solution contains particles with density = 1500 kg/m³ is to be clarified by centrifugation. Solution density is 1000 kg/m³ and viscosity 20 cp. Centrifuge bowl is with $r_2 = 0.02$ m and $r_1 = 0.008$ m and height, $b = 0.5$ m. Calculate critical diameter of particle in exit stream, if $N = 1000$ rpm and $q_f = 0.05$ m³/h.
 - (b) Define terminal velocity and scour velocity.
 - (c) For a centrifuge of radius 0.05m and rpm of 12000, find out terminal velocity of a particle of diameter 10 μ m and density 1260kg/m³ in an aqueous solution.
 - (d) A solute has a K_D between water and chloroform of 5.00. Suppose we extract a 50.00ml sample of a 0.050M aqueous solution of the solute with 15.00ml of chloroform.
 - (i) What is the extraction efficiency of the separation process?
 - (ii) What volume of chloroform must we use to extract 99.9% of the solute?
- 4 + 1 + 3 + 4 = 12**
3. (a) A municipal waste water plant is to be designed to treat maximum flow rate of 60000 m³/d. Target particle for settling has the following characteristics:

$D_p =$, $K = 0.05$, $F = 0.025$, kg/m³.

For a rectangular classifier having ratio of length to width >6, overflow rate is at least 4 times the settling velocity and horizontal velocity at most one-third of the scour velocity.

- (i) Find the dimensions of rectangular tank.
 (ii) Determine detention time.
- (b) An acidic solute, has a K_a of 1.00×10^{-5} and a K_D between water and hexane of 3.00. Calculate the extraction efficiency if we extract a 50.00ml sample of a 0.025M aqueous solution of HA, buffered to a pH of 3.00, with 50.00ml of hexane. Calculate the extraction efficiency for pH 5.00 and 7.00.

6 + 6 = 12

Group – C

4. (a) “Crystal growth is actually a process that consists of two steps in series” — Explain the two steps.
 (b) What is moments analysis in batch crystallization operation?
 (c) What is diluents? Explain function of diluents in batch crystallization operation.

4 + 4 + 4 = 12

5. (a) Describe the mechanism of precipitate formation.
 (b) The following data were obtained by gas-liquid chromatography on a 40 cm packed column:

Compound	t_R , min	$W_{1/2}$, min
Air	1.9	---
Methylcyclohexane	10.0	0.76
Methylcyclohexene	10.9	0.82
Toluene	11.4	1.06

Calculate the following:

- (i) An average number of plates from the data.
 (ii) An average plate height for the column.
 (iii) Resolution for methylcyclohexene and methylcyclohexane.
 (iv) Resolution for methylcyclohexene and toluene.

4 + 8 = 12

Group – D

6. (a) Define active, passive and facilitated transport.
 (b) What is membrane fouling? What are the different types of fouling?
 (c) It is desired to filter a cell broth at a rate of 2000 L/h on a rotary vacuum filter at a vacuum pressure of 70 kPa. The cycle time for the

drum is 60s, and the cake formation time is 15 s. The broth to be filtered has a viscosity of 2.0 cp and a cake solid per volume of filtrate of 10 g/L. From laboratory tests, the specific cake resistance has been determined to be 9×10^{10} cm/g. Determine the area of the filter that is required.

3 + 6 + 3 = 12

7. (a) What is on-off chromatography? Explain frontal mode and displacement mode with suitable example.
 (b) A unique ultrafiltration (U F) equipment system is available to remove low mol wt species from protein solution. The flow channels for this U F system are tubes 0.1 cm in diameter and 100 cm long. The protein has a diffusion coefficient of 9×10^{-7} cm²/sec. The solution has a viscosity of 1.2 cp and a density of 1.1 gm/cm³. 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 40 liters/ m² hr. (addition of water to the feed is an operation called diafiltration).

(2 + 4) + 6 = 12

Group – E

8. (a) What are the different methods of drying? What is the principle of freeze drying?
 (b) Find out the evaporation rate in a drum dryer for given data: steam temperature = 150°C, vaporisation temperature of milk 103°C, overall heat transfer coefficient 1.3kw/m²-K, drum diameter = 60cm, length of the drum = 100cm, latent heat of vaporisation = 2261kJ/kg. The product is scraped at $\frac{3}{4}$ of a revolution of the drum. Assume that there are no heat losses to the surroundings.

(4 + 4) + 4 = 12

9. (a) What is relative humidity? Calculate R H value at due point.
 (b) Estimate the dimensions of a drying chamber for a spray dryer that has output of 1000 kg/hr of a heat-sensitive biological material at 60°C containing 5% moisture and having a mean particle size of 100 µm. The feed contains 40% solids by weight in an aqueous solution at 4°C. The inlet air has a humidity of 0.01kg/kg dry air and is at 150°C, while the outlet air is at 80 °C. Assume the specific heat of the dry solids is 0.3 K cal/ kg°C. (Humidity of the outlet air is 0.0366 kg water/kg dry air –please calculate and check).

2 + 10 = 12