

SPECIAL SUPPLE B.TECH/BT/7TH SEM/BIOT 4102/2018

**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, biomolecules are separated on the basis of
- | | |
|-----------------------------|-------------------------|
| (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) Basic principle of centrifugation depends on
- | | |
|-----------------------|------------------------|
| (a) concentration | (b) polarization |
| (c) centripetal force | (d) pressure gradient. |
- (iv) Liquid-liquid extraction depends on
- | | |
|------------------------------|----------------------------|
| (a) distribution coefficient | (b) volatility |
| (c) solubility | (d) partition coefficient. |
- (v) Cell disruption homogenizer is based on
- | | |
|------------------------|------------------------|
| (a) applied voltage | (b) operating pressure |
| (c) salt concentration | (d) osmosis. |
- (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) Affinity chromatography is based on highly specific interaction
- (a) between solute molecules and ligands
 - (b) among solute molecules
 - (c) among ligands
 - (d) between solute molecules and ceramic beads.
- (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) Ultrafiltration process cannot be used for
- (a) fractionation of proteins
 - (b) desalting
 - (c) harvesting of cells
 - (d) selective removal of solvents.
- (x) SDS-PAGE uses
- (a) anionic detergent
 - (b) cationic detergent
 - (c) non-ionic detergent
 - (d) no detergent.

Group - B

2. (a) Calculate the sedimentation rate in gravity separation and centrifugal separation for the particle size limiting to $d_{lim} = 7\mu\text{m}$. The particle density = 1040 kg/m^3 ; liquid density = 1000 kg/m^3 ; viscosity of continuous phase = $1 \times 10^{-3} \text{ N-s/m}^2$.
- (b) What is the applied centrifugal field at a point equivalent to 5 cm from the centre of rotation and an angular velocity of 3000 rad s^{-1} .
- (c) For the pelleting of the microsomal fraction from a liver homogenate, an ultracentrifuge is operated at a speed of 40000 r.p.m. what is the angular velocity, ω , in radians per second?
- 6 + 3 + 3 = 12**
3. (a) Describe the role of distribution coefficient in liquid-liquid extraction.
- (b) Explain the difference between distribution coefficient and partition coefficient.

- (c) What is density gradient centrifugation? Write down the applications of density gradient centrifugation.

$$5 + 4 + 3 = 12$$

Group – C

4. (a) The following data has been taken from a liquid chromatography column:

Column length = 25.7 cm

Flow rate = 0.313 ml/min

A chromatogram of a mixture of A, B, C, D resulted in the following data:

	Retention time	Width of peak base
Nonretained	3.1	--
A	5.4	0.41
B	13.3	1.07
C	14.1	1.16
D	21.6	1.72

Calculate

- (i) the number of plates from each peak.
 (ii) the plate height for the column.
 (iii) the retention factor for each peak.

- (b) What is isoelectric point? Describe the importance of isoelectric point in ion exchange chromatography.

- (c) Write a brief note on gel filtration chromatography.

$$6 + (1 + 2) + 3 = 12$$

5. (a) What is an adsorption isotherm? Give expressions of different adsorption isotherm.

- (b) Define affinity chromatography. Explain the basic operating principles of affinity chromatography.

- (c) What is salting in and salting out of proteins.

$$(1 + 4) + (2 + 3) + 2 = 12$$

Group – D

6. (a) What is reverse osmosis? What are the applications of reverse osmosis?

- (b) 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 60 s, and the cake formation time is 15 s. The broth to be filtered has a viscosity of 2.0 cp and a cake formed per volume of filtrate is 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.

5 + 7 = 12

7. (a) Ultrafiltration of a well-stirred suspension containing 0.1 vol% yeast suspension gives a flux of 378 gal/m²-day under a pressure difference of 130 psi. (i) What is the value of L_p ? (ii) What is the water velocity through the membrane?

(b) Differentiate between Ultrafiltration and Microfiltration. What is concentration polarization?

4 + 6 + 2 = 12

Group - E

8. (a) Briefly discuss about the methods of drying.

(b) What are the applications of drying? Explain in detail.

7 + 5 = 12

9. (a) Discuss briefly about the different techniques for crystallization.

(b) Crystallization process does not violate the second law of thermodynamics – justify the statement.

10 + 2 = 12