

**BIOSEPARATION TECHNOLOGY
(BIOT 3234)**

Time Allotted : 2½ hrs

Full Marks : 60

Figures out of the right margin indicate full marks.

*Candidates are required to answer Group A and
any 4 (four) from Group B to E, taking one from each group.*

Candidates are required to give answer in their own words as far as practicable.

Group - A

1. Answer any twelve:

12 × 1 = 12

Choose the correct alternative for the following

- (i) What is an ideal fluid?
(a) A fluid which has no viscosity (b) A fluid which is incompressible
(c) A fluid which has no surface tension (d) All of the above.
- (ii) Performance of a centrifuge is measured by
(a) Sigma factor (b) Effectiveness factor
(c) Thiele Modulus (d) None of the above.
- (iii) Reflection coefficient of a membrane separation process should be
(a) less than 100 (b) greater than 1
(c) between 0 and 1 (d) none of the above.
- (iv) At terminal settling velocity
(a) $F_g = F_d + F_B$ (b) $F_d = F_g + F_B$
(c) Infinite net force (d) None of these
- (v) Which of the following is not a non-mechanical method of cell disruption?
(a) Osmotic shock (b) Freeze-drying
(c) Lysozyme (d) Dynamill.
- (vi) The following is/are observed in a membrane separation unit operation
(a) concentration polarization (b) back diffusion
(c) solute rejection (d) all of these.
- (vii) At very low solute concentration, proteins also become less soluble and form crystals. What is this phenomenon known as?
(a) Salt precipitation (b) Salt minimization
(c) Salting out (d) Salting in.
- (viii) Which of the following is not used to promote the formation of proteins crystals?
(a) PEG (b) Ammonium sulfate
(c) Tris-HCL (d) Ammonium nitrate.

- (ix) Which of the following is the distance that the solute moves while undergoing one partition?
 (a) Retention distance (b) Distribution constant
 (c) Plate height (d) Column packing length.
- (x) Which of the following is not true about High pressure liquid chromatography (HPLC)?
 (a) It requires high pressure for the separation of the species
 (b) There is no need to vaporise the samples
 (c) It is performed in columns
 (d) It has high sensitivity.

Fill in the blanks with the correct word

- (xi) This process is employed before centrifugation to form cell aggregates-_____.
- (xii) Wave density of an ultrasonic vibrator used for cell lysis _____.
- (xiii) The final unit operation in separation and purification of intracellular enzymes is _____.
- (xiv) The charge equilibrium in dialysis is _____.
- (xv) Reverse phase chromatography uses stationary phase of _____ type.

Group - B

2. Data for the laboratory filtration of CaCO_3 slurry in water at 298K are reported as follows at a constant pressure drop of 338KN/m^2 . The filter area of the plate and frame press was $A = 0.0439\text{m}^2$ and the slurry concentration was $C_s = 23.74\text{kg/m}^3$. Calculate the constants α and r_m from the experimental data given, where t is time in s and V is filtrate volume collected in m^3 . (Given: viscosity of water at 298K is $8.937 \times 10^{-4} \text{kg/m.s}$.)

t (s)	4.4	9.5	16.3	24.6	34.7	46.1	59.0	73.6	89.4	107.3
$V \times 10^3 (\text{m}^3)$	0.498	1.00	1.501	2.0	2.498	3.002	3.506	4.004	4.502	5.009
$t/V \times 10^{-3} (\text{s/m}^3)$	8.83	9.5	10.86	12.3	13.9	15.35	16.83	18.38	19.85	21.42

[[CO2](Evaluate/HOCQ)]

12

3. (a) Yeast cells are recovered from a fermentation broth by using a tubular centrifuge. Sixty percent of the cells are recovered at a flow rate of 12l/min with a rotational speed of 4000 rpm. Recovery is inversely proportional to flow rate.
 (i) To increase the recovery of the cells to 95% at the same flow rate, what should be the rpm of the centrifuge?
 (ii) At a constant rpm of 4000 rpm, what should be the flow rate to result in 95% cell recovery?
 (b) Write a note on non-mechanical methods of cell disruption mentioning its advantages and disadvantages.

[[CO2](Evaluate/HOCQ)]

[[CO2](Remember/LOCQ)]

(3 + 3) + 6 = 12

Group - C

4. (a) Penicillin is extracted from a fermentation broth using isoamylacetate as the organic solvent in a continuous counter current cascade extraction unit. The flow rates of organic (L) and aqueous (H) phases are $L = 10\text{L}/\text{min}$ and $H = 100\text{L}/\text{min}$ respectively. The distribution coefficient of penicillin between organic and aqueous phases at $\text{pH}=3$ is $K_D=Y_L/X_H= 50$. If the penicillin concentration in the feed stream is $20\text{ g}/\text{L}$, determine the number of stages required to reduce the penicillin concentration to $0.1\text{g}/\text{L}$ in the effluent of the extraction unit. *[[CO3](Calculate/HOCQ)]*
- (b) What are the different factors affecting the selection of a membrane? *[[CO3](Remember/LOCQ)]*
- 8 + 4 = 12**
5. (a) Streptomycin is extracted from the fermentation broth using an organic solvent in a counter current staged extraction unit. The distribution coefficient of streptomycin at $\text{pH} 4$ is $K_D= Y_i/X_i = 40$, and the flow rate of the aqueous phase (H) is $H=150\text{L}/\text{min}$. If only 5 extraction units are available to reduce the streptomycin concentration from $10\text{g}/\text{L}$ in the aqueous phase to $0.2\text{g}/\text{L}$, determine the required flow rate of the organic phase (L) in the extraction unit. *[[CO3](Analyse/HOCQ)]*
- (b) Define dialysis. What is concentration polarization? *[[CO3](Remember/LOCQ)]*
- 8 + (2 + 2) = 12**

Group - D

6. (a) State the principle of HIC chromatography. *[[CO4](Remember/LOCQ)]*
- (b) Define the following with respect to chromatography: (i) stationary phase (ii) mobile phase (iii) retention time. *[[CO4](Remember/LOCQ)]*
- 6 + (2 + 2 + 2) = 12**
7. (a) Describe the principle of affinity chromatography. *[[CO3](Analyse/HOCQ)]*
- (b) Two proteins of molecular weight 2.5×10^5 and 1×10^4 are eluted out of a gel filtration column at 220 ml and 300 ml respectively. Determine the molecular weight of protein which elutes out at 270 ml from the column under the same condition. *[[CO5](Compute/HOCQ)]*
- 6 + 6 = 12**

Group - E

8. (a) Explain the principle behind salting in of protein. *[[CO5](Understand/IOCQ)]*
- (b) NaNO_3 solution at 50°C contains 45% by weight of NaNO_3 . Find out
- (i) % saturation of this solution.
- (ii) Weight of NaNO_3 crystal formed if 100 kg of this solution is cooled to 10°C .
Solubility of NaNO_3 at $50^\circ\text{C} = 104.1\text{ Kg}$ of $\text{NaNO}_3/100\text{ kg}$ of water
Solubility of NaNO_3 at $10^\circ\text{C} = 78\text{ Kg}$ of $\text{NaNO}_3/100\text{ kg}$ of water. *[[CO6](Apply/HOCQ)]*
- 3 + 9 = 12**

9. (a) Explain the principle behind salting out of protein? [[C05](Analyse/HOCQ)]
(b) How does the hydration shell of protein resist protein precipitation? Why addition of ethanol might cause protein precipitation? [[C04](Analyse/IOCQ)]
- 6 + 6 = 12**
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Cognition Level	LOCQ	IOCQ	HOCQ
Percentage distribution	27.08	9.37	63.54

Course Outcome (CO):

After completion of this course, the students will be able to:

1. Acquire basic understanding of different bioseparation processes and design principle for commonly used process equipments.
2. Obtain knowledge about the basic principles and application of sedimentation, centrifugation and filtration.
3. Explain the principles of extraction and membrane based separation of bioproducts and can apply the knowledge for calculations of extraction process.
4. Understand the principle of adsorption, chromatography and relation of adsorption with chromatography.
5. Apply different chromatographic techniques for separation of different Bioproducts.
6. Comprehend the knowledge of precipitation, drying, crystallization and will be able to solve numerical problems related to these processes.

**LOCQ: Lower Order Cognitive Question; IOCQ: Intermediate Order Cognitive Question; HOCQ: Higher Order Cognitive Question.*