Name : .....

Roll No. : .....

Invigilator's Signature : .....

CS/B.Tech/(BT-NEW)/SEM-6/BT-604A/2013

2013

BIOSEPARATION TECHNOLOGY

Time Allotted : 3 Hours *Full Marks :* 70

The figures in the margin indicate full marks.

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

GROUP – A

(Multiple Choice Type Questions)

1. Choose the correct alternatives for any *ten* of the following :

10 × 1 = 10

i) Non-mechanical methods of cell disruption include

a) Osmotic shock

b) Ultrasound

c) Ball Mill

d) Homogenizer.

ii) Liquid-Liquid extraction depends on

a) distribution coefficient

b) volatility

c) solubility

d) partition coefficient.

iii) Dialysis is a membrane separation operation used for the removal of low molecular weight solutes such as organic ions of MW range

a) $10 < MW < 100$

b) $MW > 10$

c) $MW < 10$

d) $MW > 100$.

iv) Microfiltration (MF) is used to separate species that range from

a) 0.1 to 10 μm

b) 1.0 to 10 μm

c) 10 to 20 μm

d) 20 to 50 μm.

v) UF is used for macromolecules with a molecular weight range of

a) 2,000 to 5,00,000

b) 2,000 to 10,000

c) 5,000 to 50,000

d) 1,00,000 to 5,00,000.

vi) Which of the following will help to confirm the molecular weight of the purified protein ?

a) Isoelectric focusing

b) Affinity chromatography

c) Native-PAGE

d) Gel filtration.

vii) Affinity chromatography is based on the highly specific interaction between

a) solute molecules and ligands

b) solute molecules

c) ligands

d) solute molecules and ceramic beads.

viii) In rate-zonal centrifugation separation, which type of particle characteristics is taken ?

a) Size

b) Density

c) Charge

d) Volume.

ix) Extraction factor is determined during

a) Microfiltration

b) Solvent extraction

c) Ultracentrifugation

d) both (a) and (b).

x) Ultrafiltration process cannot be used for

a) Fractionation of proteins

b) Desalting

c) Harvesting of cells

d) Selective removal of solvents.

xi) Proteins are separated in the SDS – PAGE according to their

a) Charge

b) Hydrophobicity

c) Size

d) Affinity.

xii) Which one of the following proteins of different molecular weights migrates faster through a gel filtration chromatography and elutes first ?

a) 66 kDa

b) 100 kDa

c) 200 kDa

d) 30 kDa.

GROUP – B

(Short Answer Type Questions)

Answer any *three* of the following. 3 × 5 = 15

2. What is pervaporation ? How does it differ from distillation and evaporation ?

3. Explain the terms 'salting in' and 'salting out' of proteins. Discuss the principles and practice of salting-out of proteins by ammonium sulphate. 2 + 3

4. What is flocculation and what is the advantage of it during filtration of a fermentation broth ? Discuss the theoretical principles of constant pressure cake filtration. 2 + 3

5. Describe in brief the non-mechanical methods of cell disruption.

6. Define affinity chromatography. Explain basic operating principles of affinity chromatography.

GROUP – C

(Long Answer Type Questions)

Answer any *three* of the following. 3 × 15 = 45

7. a) What is an adsorption isotherm ? Give expressions of different adsorption isotherms.

   b) An enzyme produced from *Aspergillus niger* can be isolated by adsorption on a microcrystalline cellulose powder (MCCP). The adsorption follows a Langmuir Adsorption Isotherm :

   $$Y^* = \frac{Y_{max}.\ X}{K_L + X}$$

   where $Y_{max} = 70$ mg/cm ^{3} of adsorbent

   $K_L = 5 \times 10^{-5}$ g/g solution

   $X = 2$g/g of solution

   It is desired to recover 95% of the product from 1.5 L of cell free culture filtrate of concentration 220 mg/L protein with (MCCP). Estimate the amount of solid required for the purpose ? 1 + 4 + 10

8. a) Discuss the role of distribution coefficient in liquid-liquid extraction.

   b) A slurry with solid concentration of 23.47 kg/m ^{3} was filtered within a plate and frame filtrer press with a filter area of 0.0439 m ^{2} at a constant pressure drop of 330 kNm $^{-2}$. The data obtained are given below :

Time (t) min	4.4	9.5	16.3	24.6	34.7	46.1	59.0	73.6	89.4	107.3
Volume (V) L	0.498	1.000	1.501	2.000	2.489	3.002	3.506	4.004	4.502	5.009

The same slurry is then to be filtered in a plate and frame filter press having 10 frames and 0.873 m^2 area per frame at a constant pressure drop of 330 kNm^{-2} . For the same cake characteristics and 3 layers of filter cloth, calculate the time to recover 3300 L of filtrate. [Assume that filter cloth resistance is directly proportional to the thickness or number of layers of cloth.] 5 + 10

9. What is foam fractionation ? How is it used for the purification purposes in the area of biotechnology ? Explain the concept of spacer arm and its use. What are the advantages of aqueous two phase extraction over the other extraction processes in biotechnology ? The solubility of a protein is 15 g/litre at ammonium sulphate concentration of 2.2 M and 0.25 g/litre at 3.0 M. Calculate the solubility of the protein at 3.8 M of the salt. 2 + 2 + 2 + 5 + 4

10. Give a complete flow diagram of isolation and purification of any hormone or antibiotic in a commercial plant. Briefly describe the major operations involved in this process.

11. Write notes on any *three* of the following : 3 × 5
    a) Ultrafiltration
    b) Microfiltration
    c) Reverse Osmosis
    d) Concentration polarization.