CS/B.TECH(BT-NEW)/SEM-6/BT-602/2011

2011
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 :

    $10 \times 1 = 10$

    i)   Extraction factor is determined during
         a)   Microfiltration   b)   Solvent extracton
         c)   Ultracentrifugation   d)   both (a) & (b).

    ii) 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.

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

    iv) The optimum length of spacer arm in affinity
         chromatography is
         a)   4-6 carbon atom   b)   6-10 carbon atom
         c)   10-16 carbon atom   d)   12-16 carbon atom.

v) 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.

vi) Proteins were separated in the SDS-PAGE according to their

a) Charge   b) Hydrophobicity

c) Size   d) Affinity.

vii) 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.

viii) Which protein of the following molecular weights will be precipitated first during salt precipitation ?

a) 12 kDa   b) 220 kDa

c) 30 kDa   d) 100 kDa.

ix) Ultrafiltration process cannot be used for

a) Fractionation of proteins

b) Desalting

c) Harvesting of cells

d) Selective removal of solvents.

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

a) Isoelectric focusing

b) Affinity chromatography

c) Microfiltration

d) Gel filtration.

xi) In reverse osmosis, water flows from

a) higher solute concentration to lower solute concentration side

b) lower solute concentration to higher solute concentration side

c) water does not flow

d) nothing can be said.
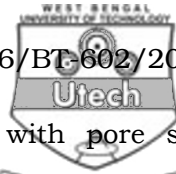
xii) Ultrafiltration is used for separation with pore size range from

a) $0 \cdot 1 - 10\,\mu m$ b) $10 - 100\,\mu m$

c) $100 - 1000\,\mu m$ d) $1 - 10\,pm$.

xiii) SDS-PAGE uses

a)   Anionic detergent   b)   Cationic detergent

c)   Nonionic detergent   d)   No detergent.

GROUP – B

(Short Answer Type Questions)

Answer any *three* of the following.

$3 \times 5 = 15$

2. How can you determine the relative molecular mass of an unknown protein ? Two proteins of molecular weights $2 \cdot 5 \times 10^5$ and 1×10^4 were eluted out of a gel filtration column at 220 ml and 300 ml respectively. Determine the molecular weight of a protein which elutes out at 270 ml from the column under the same conditions.   2 + 3

3. What is Pervaporation ? How does it differ from Distillation and Evaporation ?   2 + 3

4. Define Filter aid, Mesh size and Dialysis. Describe basic principle of Batch Filtration with diagram.

$1 + 1 + 1 + 2$

5. 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

6. Discuss the merit of aqueous two phase extraction over liquid-liquid extraction using an organic extractant. What is foam fractionation ? What are the benefits of this process in protein isolation ?   2 + 1 + 2

GROUP – C

(Long Answer Type Questions)

Answer any *three* of the following. $3 \times 15 = 45$

7. What do you mean by bioseparation and bioseparation technology ? What are the salient features and nature of bioseparation in biotechnology ? Discuss the steps involved in the isolation and purification of ethanol or any organic acid.   2 + 3 + 10

8. Explain the terms and their significances in column chromatography :

(a) Partition coefficient (b) retention time (c) retention volume (d) capacity factor (e) relative retention (f) resolution (g) plate height and number of theoretical plates. (6 × 2) + 3

9. A centrifuge having a radius of bowl of 100 mm is rotating at 2000 r.p.m. Calculate the centrifugal force developed. Discuss about the membrane fouling and concentration polarization during membrane based bioseparation processes. What is flux expression ? What is flocculation ?

3 + 8 + 2 + 2

10. What are the factors that affect the hydrophobic interaction between proteins and the ligands ? Discuss the principle of affinity chromatography. What is pseudo-affinity chromatography ? The solubility of a protein is 15 g/litre at ammonium sulphate concentration of 2·2 M and 0·25 g/litre at 3·0M. Calculate the solubility of the protein at 3·8 M of the salt. 2 + 4 + 5 + 4

11. Streptomycin is extracted from the fermentation broth using an organic solvent in a counter current 5-stage extraction unit. The value of distribution coefficient K_D = 40 and the flow rate of the aqueous phase is 150L/min.

(a) Determine the required flow rate of the organic phase to reduce the streptomycin concentration from 10g/L in feed to 0·2g/L in final raffinate.

(b) Determine the fraction that could be extracted from the same feed when the flow rate of the organic phase is 50L/min.

(c) If ampicillin has a K_D = 50 in the above said system, which antibiotic will be extracted more efficiently and why ? 5 + 5 + 5