



ENGINEERING &amp; MANAGEMENT EXAMINATIONS, JUNE – 2009

**BIOSEPARATION TECHNOLOGY****SEMESTER - 6**

Time : 3 Hours ]

[ Full Marks : 70

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

1. Choose the correct alternatives for any *ten* of the following : 10 × 1 = 10
- i) In gel filtration chromatographic separation, bio-molecules are separated based on what property of bio-molecules ?
- a) Size b) Charge
- c) Hydrophobic interaction d) Metal ion affinity.
- ii) To purify Lac-repressor from *E. coli* cell extract by affinity column chromatography, what type of ligand molecule will be immobilized to the gel matrix ?
- a) Lac promoter RNA b) Lac operator DNA
- c) Lac Z mRNA d) Lac I mRNA.
- iii) By Ni-NTA-Agarose affinity column chromatography what type of protein can you purify ?
- a) GST-tagged protein b) 6 X His-tagged protein
- c) Cys-tagged protein d) DNA binding protein.
- iv) Molecular weight of a protein can be determined by
- a) Size exclusion chromatography
- b) Ion-exchange chromatography
- c) Pseudo-affinity chromatography
- d) Affinity chromatography.



v) Cell disruption in homogenizer is based on

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|-----------------------|-----------------------|
| a) applied voltage    | b) operating pressure |
| c) salt concentration | d) osmosis.           |




vi) Pervaporation involves change of

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|--------------------|-------------------|
| a) mass volume     | b) isoelectric pH |
| c) applied voltage | d) Phase.         |

vii) Basic principle of centrifugation depends on

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|----------------------|-----------------------|
| a) concentration     | b) polarization       |
| c) centripetal force | d) pressure gradient. |

viii) In affinity chromatography, if the reactive group on the matrix is – OH group, then coupling agent is

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|-----------------------|----------------------|
| a) Bisepoxide         | b) Dichlorotriazine  |
| c) Tricyclic chloride | d) Cyanogen bromide. |

ix) Ultrafiltration is used for separation with molecular weight range from

- |                            |                              |
|----------------------------|------------------------------|
| a) 0.1 – 10 $\mu\text{m}$  | b) 10 – 100 $\mu\text{m}$    |
| c) 100 – 200 $\mu\text{m}$ | d) 200 – 500 $\mu\text{m}$ . |

x) Which method is commonly used to separate inhibitory fermentation product such as ethanol from fermentation broth ?

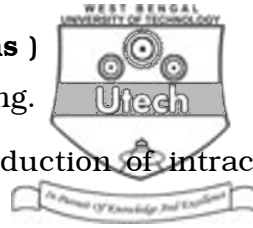
- |                                 |                             |
|---------------------------------|-----------------------------|
| a) Aqueous two phase extraction | b) Liquid-Liquid extraction |
| c) Adsorption                   | d) Ultrafiltration.         |

xi) In reverse osmosis, the deposition of solute molecules on membrane surface results in large resistance for solvent flow. This phenomenon is known as

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|---------------------------|--------------------------------|
| a) Reflection coefficient | b) Rejection coefficient       |
| c) Break through point    | d) Concentration polarization. |



5

**GROUP – B****( Short Answer Type Questions )**Answer any *three* of the following.

3 × 5 = 15

2. Discuss the downstream processing steps in the production of intracellular enzyme from fermentation broth. 5
3. Write a short note on Pseudo-affinity chromatography. 5
4. Discuss the theoretical principles and practice of salting out of proteins by ammonium sulphate. 5
5. A centrifuge having a radius of bowl of 100 mm is rotating at 1000 r.p.m. Calculate the centrifugal force developed. 5
6. A mixture of two proteins A and B have to be separated by gel chromatography. The partition coefficient ( $K_D$ ) for A is 0.5 and for B is 0.15. The void volume ( $V_o$ ) in the column is  $20 \text{ cm}^3$  and the included volume ( $V_i$ ) within the gel particle is  $30 \text{ cm}^3$ . The total volume of the column is  $60 \text{ cm}^3$ . The flow rate of eluent is  $100 \text{ cm}^3 / \text{h}$ . Ignoring dispersion and other effects, how long will it take for A to exit the column? How long is for B? 5

**GROUP – C****( Long Answer Type Questions )**Answer any *three* questions.

3 × 15 = 45

7. Write short notes on any *three* of the following : 3 × 5
  - a) Affinity chromatography
  - b) Non-mechanical methods of cell disruption
  - c) Hyper-filtration
  - d) Pervaporation.
8. a) What do you mean by membrane separation? Name the different types of membrane separation processes. 1 + 2
  - b) Write short notes on any *four* of the following : 4 × 3
    - i) Reverse osmosis.
    - ii) Ultra-filtration.
    - iii) Membrane fouling.
    - iv) Concentration polarization.
    - v) Hollow fibre module.



9. a) Describe different types of Chromatographic separation technique available for the separation of metabolic products present in fermentation broth. 10
- b) What are the basic principles exploited for the separation of biomolecules by chromatographic process ? 5
10. a) Aqueous two-phase extraction is used to recover alpha-amylase from solution. A polyethylene glycol-dextran mixture is added and the solution separates into two phases. The partition coefficient is 4.2. Calculate the maximum possible enzyme recovery when :
- i) the volume ratio of upper to lower phases is 5.0 and
- ii) the volume ratio of upper to lower phases is 0.5. 7
- b) Cell free fermentation liquor contains  $8 \times 10^{-5}$  mol / lit. immunoglobulin G. It is proposed to recover at least 90% of this antibody by adsorption on synthetic, non-polar resin. Experimental equilibrium data are correlated as follows :
- $$C_{AS}^* = 5.0 \times 10^{-5} C_A^{*0.30}$$
- where  $C_{AS}^*$  is mol solute adsorbed per  $\text{cm}^3$  adsorbent and  $C_A^*$  is liquid phase conc. in mol/lit. What minimum quantity of resin is required to treat  $2 \text{ m}^3$  fermentation liquor in a single-stage mixed tank ? 8
11. Describe in brief the operations involved for the isolation and purification of ethanol or any organic acid from commercial plant. ( Complete flowchart is a must ) 15

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END