Code No: RR412302 **Set No.1**

4 B.Tech. 1 Semester Regular Examinations, November -2005
DOWN STREAM PROCESSING
(Bio-Technology)

Time: 3 hours **Max Marks: 80**

Answer any FIVE Questions
All Questions carry equal marks

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1. (a) Characterize the biotechnology products. [4]
   (b) Explain the various steps involved in Down Stream Processing. [12]
2. Describe the principles of various techniques available for the separation of solids from fermentation broth ad their relative merits and demerits. [16]
3. Discuss the various types of membrane based separations in detail. [16]
4. (a) Describe briefly principles of Gel filtration, affinity and ion-exchange chromatography. [6]
   (b) A protein isolated from tumor biopsy was found to show P^I of 4.8. Which form of anion-exchange chromatography is suitable for the purification of this protein. [4]
   (c) Assume that you are performing Gelfiltration chromatography of sample A on a matrix whose nolecular mass fraction range is 1000 KDa - 5 KDa. Sample A contains Protein 4(2,000,000 Da), Protein U (5,00,000 Da), Peptide T(2500 Da), Protein X (2,25,000 Da), Protein V(1,500, 000 Da), Protein Z(75,000 Da) and Peptide D(5,000 Da). Arrange them in their order of elution and give justification for the order. [6]
5. (a) Describe electrophoresis technique and the principles to estimate the molecular masses of proteins and nucleic acids. [10]
   (b) You have been given a sample containing mixtures of proteins; protein A(Mol. Mass 20 kDa, net charge-2), protein B(Mol. Mass. 20 kDa, net charge -6) and protein C(Mol. Mass 20 kDa, net charge - 15). Theoretically how many protein bands do you expect in the native PAGE ad in SDS-PAGE. Explain the reason. [6]
6. (a) Explain the principle of extraction. Describe single stage and multi stage extraction methods. [10]
   (b) A water solution containing 1% of a certain solute A is to be extracted with a solvent at 20^0C. Water and solvent are immiscible. Determine the percent extraction of the solute when
      i. 100 kg of feed solution is extracted once with 150 kg of solvent.

ii. Three ideal extraction are carried out using 50kg of solvent each time. The equalibrium data is as follows. [6]

x, kg solute/kg water $\times 10^2$:	0.0	1.011	2.46	7.51	9.98	20.40
y, kg solute/ kg solvent $\times 10^2$:	0.0	0.807	2.30	6.86	9.31	18.70

7. (a) Describe biospecific affinity chromatography. [8]

   (b) Write on the criteria used for selection of extraction equipment in Antibiotic Industry. [8]

8. Write short notes on: [4 × 4 = 16]

   (a) Concentration polarization and cross flow filtration

   (b) Principle of super critical fluid extraction

   (c) Cell disruption methods

   (d) Precipitation methods.

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Code No: RR412302 **Set No.2**

4 B.Tech. 1 Semester Regular Examinations, November -2005
DOWN STREAM PROCESSING
(Bio-Technology)

Time: 3 hours **Max Marks: 80**

Answer any FIVE Questions
All Questions carry equal marks

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1. (a) Characterize the Biotechnology products. [4]
   (b) What do you understand by upstream and down stream processing? Discuss with neat flowcharts. [12]
2. When do you Prefer centrifugation over conventional filtration. Explain various types of centrifuges. [16]
3. Describe the operation of super critical fluid extraction. Give a neat sketch of the block diagram. What are the advantages of super critical fluid in the extraction operation. [16]
4. (a) What is liquid liquid extraction and explain partition coefficient? [6]
   (b) Penicillin B to be extracted from the clarified fermentation beer by using pure amyl acetate as solvent at P^H 4.0. The distribution co-effecient, k of the system was found to be 32. The initial concentration of Penicillin in the feed B 400 ml/L. The flowrates of the feed ad solvent streams are 500 L/h ad 30 L/h respectively.
      i. How many ideal stages (counter curret contact)are required to recover 97% of penicillin in the feed.
      ii. If three counter current stages are used, what will be the percent recovery.
      iii. If three cross current stages are used with equal solvent flow rate (10 L/h each), what will be the percet recovery. [10]
5. (a) Explain various methods of carrying out crystallization. [8]
   (b) Discuss the phenomenon of precipitation in detail. [8]
6. (a) Describe briefly principles of Gel filtration, affinity and ion-exchange chromatography. [6]
   (b) A protein isolated from tumor biopsy was found to show P^I of 4.8. Which form of anion-exchange chromatography is suitable for the purification of this protein. [4]
   (c) Assume that you are performing Gelfiltration chromatography of sample A on a matrix whose nolecular mass fractio range is 1000 kDa-5kDa. Sample A contains Protein 4(2,000,000 Da), Protein U (5,00,000 Da), Peptide T(2500 Da), Protein X(2,25,000 Da), Protein V(1,500,000 Da), Protein Z(75,000 Da) and Peptide D(5,000 Da). Arrange them in their order of elutio, and give justification for the order. [6]

7. (a) Describe electrophoresis techniques and the principles to estimate the molecular masses of proteins and nucleic acids. [10]

   (b) You have been given a sample containing mixutres of proteins; protein A(Mol. Mass 20 kDa, net charge-2), Protein B(Mol. Mass. 20 kDa, net charge - 6) and protein C(Mol. Mass 20 kDa, net charge - 15). Theoretically how many protein bands do you expect in the native PAGE and in SDS-PAGE. Explain the reason. [6]

8. Write short notes on: [4 × 4 = 16]

   (a) Chemical cell disruption

   (b) Rotatory vacume filter

   (c) Ultrafiltration

   (d) Dialysis

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Code No: RR412302 **Set No.3**

4 B.Tech. 1 Semester Regular Examinations, November -2005
DOWN STREAM PROCESSING
(Bio-Technology)

Time: 3 hours **Max Marks: 80**

Answer any FIVE Questions
All Questions carry equal marks

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1. Indicate the objectives of upstream and down stream stages in an industrial bio-process. Describe in detail the sequential steps involved in Down Stream processing? [16]

2. (a) Explain how do you classify the memebrane separation methods according to particle size. [5]
   (b) Make a comparision between conventional filtration and cross flow filtration. [5]
   (c) Explain briefly about Reverse Osmosis. [6]

3. (a) What are the criteria for selecting a good solvent for solvent extraction. Explain multistage extraction? [10]
   (b) It is desired to extract 4950 kg/hr of a solution containing 39.4 % of component A and 60.61% solvent B, with a solvent C which is completely immisable with solvent B. How many stages are required to recover 95% of component A in the extract, if 6000 kg/hr of solvent C is fed to the stage of counter current multiple contact process? [6]

kg of A/kg of B:	0.05	0.1	0.2	0.3	0.4	0.5	0.6	0.7
kg of A/kg of C:	0.09	0.14	0.22	0.28	0.34	0.40	0.45	0.50

4. (a) Explain the principle involved in super critical fluid extraction. [8]
   (b) Explain various types of filters used in separation of solids. [8]

5. (a) Describe briefly principles of Gel filtration, affinity and ion-exchange chromatography. [6]
   (b) A protein isolated from tumor biopsy was found to show P^I of 4.8. Which form of anion-exchange chromatography is suitable for the purificatio of this protein. [4]
   (c) Assume that you are performing Gelfiltration chromatography of sample A on a matrix whose nolecular mass fractio range is 1000 kDa-5kDa. Sample A contains Protein 4(2,000,000 Da), Protein U (5,00,000 Da), Peptide T(2500 Da), Protein X(2,25,000 Da), Protein V(1,500,000 Da), Protein Z(75,000 Da) and Peptide D(5,000 Da). Arrange them in their order of elution and give justification for the order. [6]

6. (a) Describe electrophoresis techniques and the principles to estimate the molecular masses of proteins and nucleic acids. [10]

(b) You have been given a sample containing mixutres of proteins; protein A(Mol. Mass 20 kDa, net charge-2), Protein B(Mol. Mass. 20 kDa, net charge - 6) and protein C(Mol. Mass 20 kDa, net charge - 15). Theoretically how many protein bands do you expect in the native PAGE and in SDS-PAGE. Explain the reason. [6]

7. (a) With the help of a neat block diagram describe a typical ion exchange process (Including elution and re-generation) for the recovery of a fermentation product from the broth. [12]

   (b) What are various factors that affect the resolution in a gel filtration chromatography. [4]

8. Write short note on: [4 × 4 = 16]

   (a) Density gradient centrifugation

   (b) Cell disruption methods

   (c) Role of filter aid infiltration

   (d) Crystallization

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Code No: RR412302 **Set No.4**

4 B.Tech. 1 Semester Regular Examinations, November -2005
DOWN STREAM PROCESSING
(Bio-Technology)

Time: 3 hours **Max Marks: 80**

Answer any FIVE Questions
All Questions carry equal marks

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1. (a) Mention at least five bioprocess products and list out the unique characterstics of bioseparation products. [4]
   (b) Explain the various steps involved in Down Stream Processing. [12]
2. (a) Explain the recent development in product isolation. [8]
   (b) What are the various methods used in cell disruption. Explain the advantages and disadvantages of each method. [8]
3. (a) Differentiate between conventional filteration and cross flow filtration. [6]
   (b) What is the use of filter aids infilteration give some examples of filter aids. [4]
   (c) Explain Batch filters used in the seperation of solids. [6]
4. (a) Explain the principle of extraction. Describe single stage and multi stage extraction methods. [10]
   (b) Water containing 6.8 mg/lit of a steriod is extracted wiht pure methylene dischloride. The equalibrium constant (distribution Co-efficient) for the steriod is 770 and the ratio of water to solvent used is 82. What is the concentration in the organic after the extraction. What fraction of the steriod will be removed. [6]
5. (a) Describe electrophoresis techniques and the principles to estimate the molecular masses of proteins and nucleic acids. [10]
   (b) You have been given a sample containing mixutres of proteins; protein A(Mol. Mass 20 kDa, net charge-2), Protein B(Mol. Mass. 20 kDa, net charge - 6) and protein C(Mol. Mass 20 kDa, net charge - 15). Theoretically how many protein bands do you expect in the native PAGE and in SDS-PAGE. Explain the reason. [6]
6. (a) Describe briefly principles of Gel filtration, affinity and ion-exchange chromatography. [6]
   (b) A protein isolated from tumor biopsy was found to show P^I of 4.8. Which form of anion-exchange chromatography is suitable for the purificatio of this protein. [4]
   (c) Assume that you are performing Gelfiltration chromatography of sample A on a matrix whose nolecular mass fractio range is 1000 kDa-5kDa. Sample A contains Protein 4(2,000,000 Da), Protein U (5,00,000 Da), Peptide T(2500

Da), Protein X(2,25,000 Da), Protein V(1,500,000 Da), Protein Z(75,000 Da) and Peptide D(5,000 Da). Arrange them in their order of elution and give justification for the order. [6]

7. (a) Explain various methods of carrying of crystallization. [8]
   (b) Discuss the phenomena of precipitation in detial. [8]

8. Write short note on: [4 × 4=16]
   (a) Tubular Bowl centrifuge
   (b) Membrane separations
   (c) Principle of super critical fluid extraction
   (d) Dialysis

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