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Total number of printed pages – 3

B. Tech
PCBT 4306

Sixth Semester Regular Examination – 2014

DOWNSTREAM PROCESS ENGINEERING

BRANCH : BIOTECH

QUESTION CODE : F 249

Full Marks – 70

Time : 3 Hours

Answer Question No. 1 which is compulsory and any **five** from the rest.
The figures in the right-hand margin indicate marks.



1. Answer the following questions : 2 × 10
- What do you mean Ostwald ripening ?
 - What are the different types of rotors used in centrifugation ?
 - What is triple point of water ? Discuss its importance in drying.
 - What do you mean IMAC ?
 - Write the different types of chromatography depending upon principles involved.
 - Find the g-number of a centrifuge with radius of 20 cm and rotating at 40 rps.
 - Write the principle of aqueous two phase extraction.
 - A protein has sedimentation coefficient value of 3.12×10^{-13} sec. Its diffusion coefficient in water is found to be 8.2×10^{-7} cm²/sec. Both the above values have been corrected for 20°C in water. The partial specific volume of the protein is 0.735, and the density of water at 20°C is 0.9982. Determine the molecular weight of the protein.
 - Differentiate between dialysis and electro dialysis.
 - Distinguish between salting out and salting in.
2. (a) Write the theory of centrifugation. Add a note on continuous centrifugation. 5
- (b) You have a crude lysate sample (CL) containing a mixture of six proteins (1, 2, 3, 4, 5, and β -galactosidase), and your goal is to obtain purified

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β -galactosidase. Some characteristics of these proteins are shown in the table below :

Protein	Concentration of ammonium sulfate (AS) required for precipitation	Molecular Weight (kDa)	Isoelectric point (pI)
1	45%	38	3.7
2	80%	22	4.8
3	65%	4	5.3
4	20%	75	6.8
5	30%	55	9.5
β -galactosidase	45%	115	5.3

You begin your purification by performing an ammonium sulfate (AS) precipitation. You add the appropriate concentration of AS to your CL sample, incubate overnight at 4°C, then centrifuge to generate a supernatant (AS-S) and pellet (AS-P).

One way to purify β -galactosidase away from any contaminating proteins in the AS-P sample would be to separate the proteins based on their molecular weight.

- (i) What type of column separates on this basis ?
- (ii) Which protein (from your AS-P) would elute first from this type of column ?

Instead, you decide to use ion exchange chromatography to further purify β -galactosidase away from other proteins in your AS-P sample. You first run an anion exchange column equilibrated using column buffer with a pH of 5.0.

- (iii) What charge does the matrix of an anion exchange column have ?
- (iv) At pH 5.0, which protein(s) from the AS-P will stick to the column ?
Explain your answer to part in one or two sentences. 5

3. (a) Cells of fall army worm *S. frugiperda* are cultured in a fermenter to produce viral particles for insecticide. Viral particles are released into the culture broth after lysis of the host cells. The initial culture volume is 5 lit. An aqueous two-phase polymer solution of volume 2lit is added to this liquid; the volume of the bottom phase is 1 lit. The virus partition coefficient is 0.01. Find the yield of virus at equilibrium. 5
- (b) Explain the process of ion exchange chromatography. 5

4. Write the principle behind HPLC. Discuss in details about the instrumentation and working of HPLC. Write a note on various detectors used in HPLC. 10
5. (a) What are the different cell disruption methods ? Discuss in details about the enzymatic method of cell disruption with suitable examples. 5
 (b) What is ultrafiltration? How it is different from microfiltration ? Write any two applications of ultrafiltration in biotechnology. 5
6. (a) Write the principle of drying. What is drying curve ? Explain the working of freeze dryer. 5
 (b) Write the principle of crystallization. Describe the working of draft tube crystallizer. 5
7. (a) What is liquid-liquid extraction ? How it is different from aqueous two phase extraction ? Write in details about the mode of operation of liquid-liquid extraction. 5
 (b) Discuss in details about the process of electrodialysis. 5
8. Answer the following : 5×2
 (a) Gel filtration chromatography.
 (b) Differentiate between hydrophobic and reverse phase chromatography.

