JEPPIAAR ENGINEERING COLLEGE
DEPARTMENT OF BIOTECHNOLOGY

BT 6702 Downstream Processing

[Question Bank]

B.TECH. BIOTECHNOLOGY

IV YEAR / VII SEMESTER

REGULATION 2013

Compiled by
[Mr. V. Jeyamanikandan, Asst. Professor, Department of Biotechnology, Jeppiaar Engineering College]

VISION OF THE INSTITUTION

- To build Jeppiaar Engineering College as an institution of academic excellence in technological and management education to become a world class University

MISSION OF THE INSTITUTION

- To excel in teaching and **learning, research and innovation** by promoting the principles of scientific analysis and creative thinking.
- To participate in the production, **development and dissemination of knowledge** and interact with **national and international communities.**
- To equip students with **values, ethics and life skills n**eeded to enrich their lives and enable them to meaningfully contribute to the **progress of society.**
- To prepare students for **higher studies and lifelong learning,** enrich them with the **practical and entrepreneurial skills** necessary to excel as future professionals and contribute to **Nation's economy**

PROGRAM OUTCOMES (PO)

PO 1	**Engineering knowledge**: **Apply** the knowledge of mathematics, science, engineering fundamentals, and an engineering specialization to the solution of complex engineering problems.
PO 2	**Problem analysis**: **Identify, formulate, review** research literature, and **analyze** complex engineering problems reaching substantiated conclusions using first principles of mathematics, natural sciences, and engineering sciences.
PO 3	**Design/development of solutions**: **Design solutions** for complex engineering problems and **design** system components or processes that meet the specified needs with appropriate consideration for the public health and safety, and the cultural, societal, and environmental considerations
PO 4	**Conduct investigations of complex problems**: **Use** research-based knowledge and research methods including **design** of experiments, **analysis** and **interpretation** of data, and **synthesis** of the information to provide valid conclusions.
PO 5	**Modern tool usage**: **Create**, **select**, and **apply** appropriate techniques, resources, and modern engineering and IT tools including prediction and modeling to complex engineering activities with an understanding of the limitations.
PO 6	**The engineer and society**: **Apply** reasoning informed by the contextual knowledge to assess societal, health, safety, legal and cultural issues and the consequent responsibilities relevant to the professional engineering practice.
PO 7	**Environment and sustainability**: **Understand** the impact of the professional engineering solutions in societal and environmental contexts, and **demonstrate** the knowledge of, and need for sustainable development.
PO 8	**Ethics**: **Apply** ethical principles and commit to professional ethics and responsibilities and norms of the engineering practice.
PO 9	**Individual and team work**: **Function effectively** as an individual, and as a member or leader in diverse teams, and in multidisciplinary settings.
PO 10	**Communication**: **Communicate effectively** on complex engineering activities with the engineering community and with society at large, such as, being able **to comprehend** and **write** effective reports and **design** documentation, **make** effective presentations, and **give** and **receive** clear instructions.
PO 11	**Project management and finance**: **Demonstrate** knowledge and understanding of the engineering and management principles and **apply** these to one's own work, as a member and leader in a team, to **manage** projects and in multidisciplinary environments.
PO 12	**Life-long learning**: **Recognize** the need for, and have the preparation and ability to **engage** in independent and life-long learning in the broadest context of technological change.

DEPARTMENT: BIOTECHNOLOGY

VISION OF THE DEPARTMENT

❖ To pursue excellence in producing bioengineers coupled with research attributes

MISSION OF THE DEPARTMENT

M1	To **impart** quality education and **transform** technical knowledge into career opportunities
M2	To **establish** a bridge between the **program and society** by **fostering** technical education
M3	To **generate societal conscious technocrats** towards **community development**
M4	To **facilitate higher studies** and research in order to have an **effective career** / entrepreneurship

PROGRAM EDUCATIONAL OBJECTIVES (PEOS)

PEO - 1	To impart knowledge and produce competent graduates in the field of biotechnology
PEO - 2	To inculcate professional attributes and ability to integrate engineering issues to broader social contexts.
PEO - 3	To connect the program and community by fostering technical education.
PEO - 4	To provide a wide technical exposure to work in an interdisciplinary environment
PEO - 5	To prepare the students to have a professional career and motivation towards higher education.

PROGRAM SPECIFIC OUTCOMES (PSOs)

PSO 1	**Professional Skills:** This programme will provide students with a **solid foundation** in the field of Biological Sciences and Chemical engineering enabling them to work on engineering platforms and applications in Biotechnology as per the requirement of Industries, and facilitating the students to pursue higher studies.
PSO 2	**Problem-solving skills:** This programme will assist the students to **acquire fundamental** and **problem solving knowledge** on subjects relevant to Biotechnology thereby encouraging them to **understand** emerging and advanced concepts in modern biology.
PSO 3	**Successful Career and Entrepreneurship:** Graduates of the program will have a **strong successful career** and **entrepreneurial ability** with the blend of inputs from basic science, engineering and technology, thereby enabling them to **translate the technology and tools** in various industries and/or institutes.

COURSE OUTCOMES (CO)	
C402: BT6702 – DOWNSTREAM PROCESSING	
C402.1	Upon success completion of this course, the students will be **able to Understand** the methods to obtain pure proteins, enzymes and in general about product development R & D
C402.2	**able to Define** the fundamentals of downstream processing for product recovery
C402.3	**able to Understand** the requirements for successful operations of downstream processing
C402.4	**able to Describe** the components of downstream equipment and **explain** the purpose of each
C402.5	**able to Understand** the process of isolation, purification, product formulations and finishing operations involved in bioproduct production

ANNA UNIVERSITY, CHENNAI
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R - 2013
B.TECH. BIOTECHNOLOGY

BT6702 DOWNSTREAM PROCESSING **L T P C**
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OBJECTIVES:

To enable the students to

- Understand the methods to obtain pure proteins, enzymes and in general about product development R & D.
- Have depth knowledge and hands on experience with on Downstream processes.

UNIT I DOWNSTREAM PROCESSING 8

Introduction to downstream processing principles characteristics of biomolecules and bioprocesses. Cell disruption for product release – mechanical, enzymatic and chemical methods. Pretreatment and stabilisation of bioproducts.

UNIT II PHYSICAL METHODS OF SEPERATION 9

Unit operations for solid-liquid separation - filtration and centrifugation.

UNIT III ISOLATION OF PRODUCTS 10

Adsorption, liquid-liquid extraction, aqueous two-phase extraction, membrane separation– ultrafiltration and reverse osmosis, dialysis, precipitation of proteins by different methods.

UNIT IV PRODUCT PURIFICATION 10

Chromatography – principles, instruments and practice, adsorption, reverse phase, ion-exchange, size exclusion, hydrophobic interaction, bioaffinity and pseudo affinity chromatographic techniques.

UNIT V FINAL PRODUCT FORMULATION AND FINISHING OPERATIONS 8

Crystallization, drying and lyophilization in final product formulation.

TOTAL : 45 PERIODS

OUTCOMES:

Upon success completion of this course, the students will be able to:

- Define the fundamentals of downstream processing for product recovery
- Understand the requirements for successful operations of downstream processing
- Describe the components of downstream equipment and explain the purpose of each Apply principles of various unit operations used in downstream processing and

enhance problem solving techniques required in multi-factorial manufacturing environment in a structured and logical fashion.

TEXTBOOKS:

1. Belter, P.A. E.L. Cussler And Wei-Houhu – "Bioseparations – Downstream Processing For Biotechnology, Wiley Interscience Pun. (1988).
2. Sivasankar, B. "Bioseparations : Principles and Techniques". PHI, 2005.

REFERENCES:

1. R.O. Jenkins, (Ed.) – Product Recovery In Bioprocess Technology – Biotechnology By Open Learning Series, Butterworth-Heinemann (1992).
2. J.C. Janson And L. Ryden, (Ed.) – Protein Purification – Principles, High Resolution Methods And Applications, VCH Pub. 1989.
3. R.K. Scopes – Protein Purification – Principles And Practice, Narosa Pub. (1994).

S. No.	Title	Reference Book	Page No.
UNIT I DOWNSTREAM PROCESSING (8)			
1.	Introduction to downstream processing	TB2	1-3
2.	Principles, characteristics of biomolecules and bioprocesses.	TB2	3-5
3.	Cell disruption for product release – mechanical, enzymatic and chemical methods.	TB2	13-25
4.	Pretreatment and stabilisation of bioproducts.	TB2	9-13
UNIT II PHYSICAL METHODS OF SEPERATION (9)			
5.	Unit operations for solid-liquid separation	TB2	26-27
6.	Filtration	TB2	26-41
7.	Centrifugation.	TB2	42-53
UNIT III ISOLATION OF PRODUCTS (10)			
8.	Adsorption	TB2	54-66
9.	Liquid-liquid extraction	TB2	67-80
10.	Aqueous two-phase extraction	TB2	81-99
11.	Membrane separation	TB2	100-111
12.	Ultrafiltration	TB2	112-113
13.	Reverse osmosis	TB2	114-115
14.	Dialysis	TB2	116-118
15.	Precipitation of proteins by different methods.	TB2	119-142
UNIT IV PRODUCT PURIFICATION (10)			
16.	Chromatography – principles, instruments and practice.	TB2	143-144
17.	Adsorption	TB2	143-145
18.	Reverse phase	TB2	201-202
19.	Ion-exchange	TB2	188-190
20.	Size exclusion	TB2	175-199
21.	Hydrophobic interaction	TB2	200-214
22.	Bioaffinity , pseudo affinity chromatographic techniques.	TB2	215, 227
UNIT V FINAL PRODUCT FORMULATION AND FINISHING OPERATIONS (8)			
23.	Crystallization	TB2	255-258
24.	Drying	TB2	259-263
25.	Lyophilization in final product formulation.	TB2	264-265

TB1: Belter, P.A. E.L. Cussler And Wei-Houhu – "Bioseparations – Downstream Processing For Biotechnology, Wiley Interscience Pun. (1988).

TB2: Sivasankar, B. "Bioseparations : Principles and Techniques". PHI, 2005.

RB1: R.O. Jenkins, (Ed.) – Product Recovery In Bioprocess Technology – Biotechnology By Open Learning Series, Butterworth-Heinemann (1992).

RB2: J.C. Janson And L. Ryden, (Ed.) – Protein Purification – Principles, High Resolution Methods And Applications, VCH Pub. 1989.

RB3: R.K. Scopes – Protein Purification – Principles And Practice, Narosa Pub. (1994).

Reg. No. : | | | | | | | | | | | | |

QUESTION PAPER CODE : 50203

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2017

Seventh Semester

Biotechnology

BT 6702 – DOWNSTREAM PROCESSING

(Regulation 2013)

Time : Three hours **Maximum : 100 marks**

Answer ALL questions.

PART A – (10 X 2 = 20 marks)

1. Name one cationic and anionic detergent used for cell disruption.

Anionic Detergent:

- Sodium dodecyl sulfate
- Sodium deoxycholate
- Sarkosyl – (sarcosyl or sodium lauroyl sarcosinate)

Cationic Detergent:

- CTAB (Cetyl trimethylammonium bromide)
- ETAB (ethyl trimethylammonium bromide)

2. What is filter aid?

- Filter aid is a group of inert materials consisting of solid particles (as of diatomite) that improves filtering efficiency (as by increasing the permeability of the filter cake) and that is either added to the suspension to be filtered or placed on the filter as a layer through which the liquid must pass.
- The common filter aids are diatomaceous earth (DE), perlite, cellulose and others.
- There are several other special materials used as filter aids, including asbestos, cellulose, agricultural fibers, saw dust, rice hull ash, paper fibers etc.

3. Define buoyant force.

Buoyancy refers to a force that arises from the pressure exerted on an object by a fluid (a liquid or a gas). Since it's a force, we call it the buoyant force.

Buoyant Force, $F_{buoyant} = F_{up} - F_{down}$

F_{up} - Fluid on the top of the particle is pushing down

F_{down} - Fluid on the bottom of the particle is pushing up

4. Define isopycnic sedimentation.

Upon centrifugation, particles of a specific density sediment until they reach the point where their density is the same as the gradient media (i.e., the equilibrium position). The gradient is then said to be isopycnic and the particles are separated according to their buoyancy.

In isopycnic separation, also called buoyant or equilibrium separation, particles are separated solely on the basis of their density. Particle size only affects the rate at which particles move until their density is the same as the surrounding gradient medium. The density of the gradient medium must be greater than the density of the particles to be separated. By this method, the particles will never sediment to the bottom of the tube, no matter how long the centrifugation time

5. Define adsorption.

Adsorption is the phenomenon of accumulation of large number of molecular species at the surface of liquid or solid phase in comparison to the bulk.

On the basis of type of forces of attraction existing between adsorbate and adsorbent, adsorption can be classified into two types: Physical Adsorption or Chemical Adsorption.

- Charcoal is used as a decoloriser as it adsorbs the coloring matter from the coloured solution of sugar.
- Silica and alumina gels are used as adsorbents for removing moisture and for controlling humidity of rooms.
- Activated charcoal is used in gas masks as it adsorbs all the toxic gases and vapours and purifies the air for breathing. Adsorption processes are useful in carrying out heterogeneous catalysis.

6. Give the Freundlich equation.

An adsorption isotherm, is an empirical relation between the concentration of a solute on the surface of an adsorbent to the concentration of the solute in the liquid with which it is in contact.

$$\frac{x}{m} = kP^{\frac{1}{n}}$$

Where x is the mass of the gas adsorbed on mass m of the adsorbent at pressure p and k, n are constants whose values depend upon adsorbent and gas at particular temperature.

7. Define retention ratio.

Ratio of the distance that a compound moves to the distance that the eluent front moves.

In chromatography, it is the fraction of an analyte in the mobile phase of a chromatographic system.

$$R = \frac{\text{quantity of substance in the mobile phase}}{\text{total quantity of substance in the system}}$$

In planar chromatography,

$$R_f = \frac{\text{migration distance of substance}}{\text{migration distance of solvent front}}$$

8. Define Partition co-efficient.

The ratio of the concentrations of a solute in two immiscible or slightly miscible liquids, or in two solids, when it is in equilibrium across the interface between them.

The partition coefficient is defined as the ratio of unionized drug distributed between organic phase and aqueous phase at equilibrium.

9. What is lyophilisation?

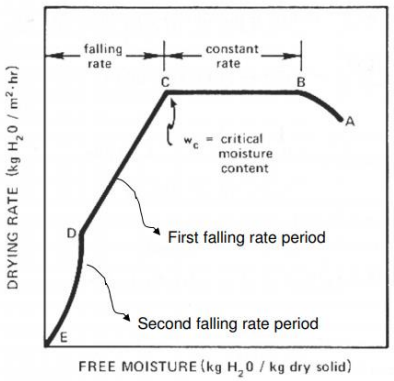
Free drying - A method of drying food or blood plasma or pharmaceuticals or tissue without destroying their physical structure; material is frozen and then warmed in a vacuum so that the ice sublimes.

It is a dehydration process typically used to preserve a perishable material or make the material more convenient for transport. Freeze-drying works by freezing the material and then reducing the surrounding pressure to allow the frozen water in the material to sublime directly from the solid phase to the gas phase.

10. Define first falling-rate period.

In the final stages of drying, known as the falling-rate period, the temperature of the product increases, causing water to move from the interior to the surface for evaporation.

A first falling drying rate occurs when wetted spots in the surface continually diminish until the surface is dried (Point D).



PART B – (5 X 16 = 80 marks)

11. (a) What is cell disruption? Explain in detail the various mechanical and chemical methods employed for cell disruption. (16) [Ans: TB2: 13-25]

Or

11. (b) What are the various steps in downstream processing? Explain them in detail with a neat schematic diagram. (16) [Ans: TB2: 1-3]

12. (a) Explain in detail :

(i) Plate and Frame filter press. (8) [TB2: 31-33]

(ii) Continuous rotary vacuum filter press. (8) [TB2: 31-33]

Or

12. (b) i) A bowl centrifuge is used to concentrate a suspension of Escherichia coli prior to cell disruption. The bowl of this unit has an inside radius of 12.7 cm and a length of 73.0 cm. the speed of the bowl is 16,000 rpm and the volumetric capacity is 200 L/h. under these conditions, this centrifuge works well. Calculate the settling velocity V_g for the cells. (12) [Class notes]

ii) A tubular bowl centrifuge is used to recover yeast cells from a fermentation broth. At a flow rate of 10 L/min and 5000 rpm, 50% of the cells can be recovered. You are

now asked by your manager to increase the recovery to 90% using the same equipment. What flow rate should you use? (4) [Class notes]

13. (a) Explain in detail :

(i) Ultrafiltration. (6) [TB2: 112-113]

(ii) Reverse osmosis (6) [TB2: 114-115]

(iii) Dialysis (4) [TB2: 116-118]

Or

13.(b) Explain in detail the different methods employed for protein precipitation. (16) [TB2: 119-142]

14. (a) Explain in detail :

(i) Ion exchange chromatography (8) [TB2: 188-194]

(ii) Affinity Chromatography (8) [TB2: 215-230]

Or

14. (b) Explain in detail :

(i) Size exclusion chromatography. (8) [TB2: 175-187]

(ii) Reverse phase chromatography (8) [TB2: 201-202]

15. (a) Give short notes on the following : [TB2: 255-257]

(i) Saturation (4)

(ii) Supersaturation (4)

(iii) Nucleation (4)

(iv) Single crystal growth (4)

Or

15. (b) Describe in detail the various dryers used in downstream processing of biomolecules. (16) [TB2: 260-263]

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B.TECH. BIOTECHNOLOGY
IV YEAR / VII SEMESTER
BT6702 DOWNSTREAM PROCESSING
IMPROTANT QUESTIONS

UNIT I

DOWNSTREAM PROCESSING

PART A

1. Name any three molecules manufactured by fermentation.
2. Write the spectrum of separations used in biotechnology.
3. Write any two design questions to improve the sepcoution process.
4. Write the four steps in the bioseparation process.
5. What is the principle used in Osmotic shock and enzyme digestion?
6. Name any two chemical methods for cell disruption.
7. Name any two mechanical methods for cell disruption.
8. Give two examples for solubilization and liquid dissolution.
9. Name the layers of gram negative all.
10. Write short notes on solubilization.
11. Explain Schmidl number.
12. Explain Sherwood number.
13. Define Reynold's number.
14. What is principle of alkali treatment?
15. What is Cell disruption?
16. What are the solvents used in lipid dissolution?
17. List out the four familiar steps in most bio-separation. NOV/DEC 2012
18. Explain about lipid dissolution. NOV/DEC 2012
19. What are the different steps in downstream processing? NOV/DEC 2013
20. What are the physical methods of cell disruption? NOV/DEC 2013
21. Write the principles of sonication for disruption of bacterial cells. NOV/DEC 2014
22. How does lysozyme act on bacterial cells? NOV/DEC 2014
23. List out mechanical methods for cell disruption.
24. List out chemical methods for cell disruption.
25. Define stabilization.
26. Short notes on pretreatment
27. Define high pressure homogenizer

28. Explain Cell disruption using French press

29. Notes on Disadvantages of bead mill

30. Write down the Disadvantages of enzymatic method in cell disruption technique.

PART B

1. Write short notes on the characteristics and principles of bioseparation in detail. NOV/DEC 2012.2014,2015

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 1

2. Explain the characteristics and principles of biomolecules in detail. NOV/DEC 2012

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 3

3. Describe the cell disruption for product release by chemical methods with suitable examples. NOV/DEC 2012,2014

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 13

4. Describe in detail the pretreatment and stabilization of bioproducts. NOV/DEC 2014

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 09

5. Detail notes on the cell disruption for product release by mechanical methods. NOV/DEC 2010,2015

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 20

6. Write note on the cell disruption for product release by enzymatic methods. NOV/DEC 2010

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 17

PART C

1. (i) The diffusivity of a protein having a Stokes-Einstein radius of 2 nanometers in a particular liquid is known to be 4.5×10^{-11} m^2s^{-1}. Predict the diffusivity of another protein having twice the Stokes-Einstein radius in the same liquid at the same temperature. (8) (notes) (Nov./Dec., 2016)

(ii) Discuss the design and working principle of a ball mill. (8) [TB2: 20-22] (Apr./May, 2017) (Nov./Dec., 2016)

2. (i) What is the criterian for selection of electrolyte for flocculation. (8) (Nov./Dec., 2016)

(ii) Discuss the construction and working principles of a microfluidizer. (8) (Nov./Dec., 2016)

3. (i) Explain in detail the construction and working principles of a high pressure mechanical homogenizer. (8) [TB2: 22-23] (Apr./May, 2017)

(ii) Discuss the use of ultrasound for cell disruption. (8) [TB2: 16] (Apr./May, 2017)

UNIT II

PHYSICAL METHODS OF SEPARATION

PART A

1. What is depth filtration?
2. Write the Poiseuille's equation.
3. Write the formula for Poiseuille's equation.
4. What is cake compressibility?
5. Short note on Diaphragm pumps.
6. Short note on centrifugal pumps.
7. Short notes on Positive displacement pumps.
8. What is porosity?
9. Short notes on filter aid.
10. Filter media.
11. Short notes on equipment selection.
12. How will you use continuous and Batch filtration together.
13. Short notes on Rotary vacuum filter.
14. Short notes on Nutsches.
15. Short notes on cross flow filtration.
16. Short notes on factors affecting the characteristics of filters.
17. What is continuous mode?
18. What is Dia filtration?
19. What are the parameters which affect for filter performance?
20. Explain trans membrane pressure.
21. Define Cohn equation NOV/DEC 2010
22. Write down the need for filter aid.
23. Identify four basic types of centrifuge and explain. NOV/DEC 2012
24. What are filter aids?Give some examples? NOV/DEC 2013
25. What is isopycnic centrifugation. NOV/DEC 2014
26. Define zonal centrifugation
27. Explain the density gradient centrifugation
28. Define differential centrifugation
29. Define high speed centrifuges
30. Define low speed centrifuge

PART B

1. Fermented broth is filtered under constant pressure of recovery of protease. A pilot scale filter is used to measure filtration properties. The filter area is 0.25 m^2, the pressure drop is 360mmHg and the filtrate viscosity is 4cP. The cell suspension deposits 22g cake/Lit of filtrate. The following data are obtained.

Time (Mins)	2	3	6	10	15	20
Filtrate volume(Lit)	10.8	12.1	18.0	21.8	28.4	32.0

i. Determine the specific cake resistance and filtrate medium resistance.
ii. What size filter is required to process 4000lit cell suspension in 30min at a pressure of 360 mmHg? NOV/DEC 2015

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 40

2. Centrifugation of yeast: yeast cells are to be separated from a fermentation broth. Assume that the cells are spherical with diameter 5µm and density 1.06g/cm3. Viscosity of culture broth is $1.36*10^{-3}$Ns/m^2. During separation, the density of suspending fluid is 0.997g/cm^3.500lit broth must be treated every 1hr.

i) Specify £ for a suitably sized tubular bowl centrifuge.
ii) If instead of yeast, quartz particles of diameter 0.1mm and specific gravity 2.0 are used to separate from the culture liquid, how much is £ reduced? NOV/DEC 2008

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 41

3. Determine the time required for carrying out the process of filtration. NOV/DEC 2008
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 39

4. Determine the position of particular particles as a function of time and volumetric flow rate of feed in a disc type centrifuge with the diagram and explain the different types of centrifuge. NOV/DEC 2014,2012,2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 44

5. Describe filtration types in detail. NOV/DEC 2014,2013
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 38

6. Explain in detail solid liquid separations NOV/DEC 2008
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 26

PART C

1. (i) Describe the design and working principles of disc stack centrifuges. (8) [TB2: 47-49] (Nov./Dec., 2016)

(ii) Describe the design and working principle of rotary drum vacuum filtration. (8) [TB2: 34-37] (Nov./Dec., 2016)

2. A 30-ml sample of broth from a rifamycin B fermentation is filtered in the laboratory on a 3 cm^2 filter at a pressure drop of 3.45 x 10^4 N/m^2. The filtration time is 4.5 min. Previous studies have shown that filter cake of *Streptomyces mediterranensis* is significantly

compressible with s = 0.5. If 500 litres broth from a pilot-scale fermenter must be filtered in 1hour, what size is required if the pressure drop is:

(i) 6.86×10^{-4} N/m^2

(ii) 3.45×10^{4} N/m^2

Resistance due to filter medium is negligible. (notes) (Nov./Dec., 2016)

3. (i) Describe the general theory of filtration. (8) [TB2: 27-28] (Apr./May, 2017)

(ii) Explain detail the design and working of a tubular bowl centrifuge. (8) [TB2: 45-47] (Apr./May, 2017)

UNIT III

ISOLATION OF PRODUCTS

PART A

1. What is Isolation?
2. What is partition coefficient?
3. Write the logarithmic equation for the partition coefficient.
4. Write the partition coefficient equation in terms of partial molar volumes.
5. Name any four solvents used for extraction process.
6. Name any three counter ions for Ion paired extractions.
7. Write the PKa values for acetic acid and propionic acid.
8. Write the PKa values for any two antibiotics.
9. Write short notes on Equilibrium constraint.
10. Write the formula for extraction factors.
11. Write short notes on Chemistry of adsorption.
12. Name any four adsorbents used in extraction process.
13. Name any three adsorption isotherm.
14. Write the empirical equation for freundlich isotherm.
15. Write the empirical equation for the Langmuir isotherm.
16. Write the equation explaining the theory of langmuir isotherm.
17. What is break point? How it can be used to design the adsorption unit. NOV/DEC 2010
18. Types of adsorption:
19. Factors affecting adsorption:
20. Explain the term extraction.
21. Define partition coefficient in extraction. NOV/DEC 2013
22. Define Salting in of proteins NOV/DEC 2013
23. Define Salting out of proteins NOV/DEC 2013
24. How does ammonium sulphate helps in precipitation of proteins? NOV/DEC 2014
25. What is adsorption? NOV/DEC 2014
26. Write down the operational approaches of Ammonium Sulphate Precipitation
28. How the Biological macromolecules can be precipitated?
29. Explain the Applications of dialysis.
30. Explain the flux equation

PART B

1. Explain in detail ultra filtration, and Reverse osmosis. NOV/DEC 2011,2012,2013,2014,2015

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 112,114

2. Discuss protein precipitation NOV/DEC 2014,2015
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 119

3. Write short notes on dialysis. NOV/DEC 2008
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 116

4. Explain in detail aqueous two phase extraction. NOV/DEC 2011,2013,2014,2015
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 81

5. Describe in detail liquid- liquid extraction. NOV/DEC 2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 67

6. Explain in detail adsorption. NOV/DEC 2008
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 54

PART C

1. (i) Clarified fermentation broth contains a polysaccharide product with a gelation concentration of 25 g/l. The fluid density is 1020 kg m^{-3}, the viscosity is 1.8 cP, and the polysaccharide diffusivity is 5.63 x 10^{-11} m^2 s^{-1}. The product is harvested from the broth using ultrafiltration at a fluid velocity of 0.34 ms-1 in open membrane tubes of diameter 2.4 cm and length 2.0 m. Estimate the permeate flux if the filter is operated under gel polarization conditions and the polysaccharide concentration in the feed is 12 $g.l^{-1}$. (8) (Nov./Dec., 2016) (notes)

(ii) Describe the various models describing the ultrafiltration process.(8) (Nov./Dec., 2016; Apr./May, 2017) [TB2: 112-114]

2. (i) An adsorptive membrane has a thickness of 2 mm and a diameter of 5 cm. The porosity of the membrane is 0.75 and the tortuosity is 1.5. The pore diameter was estimated to be 2 x 10^{-6} m. If we are to use this membrane for adsorption of a DNA fragment with diffusivity of 9.5 x 10^{-12} m^2 s^{-1} from an aqueous solution of viscosity 0.001 kg/m.s at 25°C, what is the maximum solution solution flow rate that can be used? Assume that the flow through the pores is laminar. (8) (Nov./Dec., 2016) (notes)

(ii) Discuss the principles of aqueous two-phase partitioning of proteins. Add a note on affinity partitioning.(8) (Nov./Dec., 2016; Apr./May, 2017) [TB2: 81-90]

3. (i) Describe the various stages involved in precipitate formation (8) (Apr./May, 2017) [TB2: 119-139]

(ii) What is adsorption isothern? Describe the types of adsorption isotherms. (8) (Apr./May, 2017) [TB2: 54-60]

UNIT IV

PRODUCT PURIFICATION

PART A

1. Write short notes on elution chromatography.
2. Name some adsorbents used in elution chromatography.
3. Write short notes on yield and purity.
4. Write the material balance equation for elution chromatography.
5. Explain NTU.
6. What is nucleation?
7. What is diffusion limited growth?
8. Write the second order equation for the diffusion limited growth.
9. What is flow influenced growth?
10. Write the equation to calculate the yields.
11. Write the equation to find the purity of solute.
12. Write the yield equation in teams of eluted volume 'v'.
13. Write any three typical stationary phases.
14. Write any two advantages of ION exchange chromatography.
15. What are the Ionic groups in the Ion exchange resins?
16. What is bonded stationary phase? NOV/DEC 2010
17. What is pre-column derivatisation? NOV/DEC 2010
18. Define yield.
19. Define purity.
20. Differentiate between yield and purity. NOV/DEC 2012
21. Give the solute mass balance in symbolic terms for discrete stage analysis of
22. Define retention time NOV/DEC 2013
23. Define retention volume NOV/DEC 2013
24. Define total ionic capacity. NOV/DEC 2013 NOV/DEC 2014
25. What is the purpose of using sephadex G50. NOV/DEC 2014
26. Write notes on Protein purification:
27. Define Amphiphiles:
28. Principles of Size exclusion chromatography
29. Write down the Application of ion exchange chromatography.
30. What are the material can be used as Ion exchangers

PART B

1. Explain the general description of chromatography. NOV/DEC 2008
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 143

2. Ion exchange chromatography, size exclusion chromatography –discuss NOV/DEC 2015,2014,2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 188

3. Write notes on Affinity chromatography NOV/DEC 2015,2014,2013,2011
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 215

4. Describe Adsorption chromatography NOV/DEC 2014,2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 143

5. Explain in detail hydrophobic interaction. NOV/DEC 2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 200

6. Detail notes on Reverse phase chromatography NOV/DEC 2015,2013
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 201

PART C

1. (i) A pilot-scale gel-chromatography column packed with Sephacryl resin is used to separate two hormones A and B. The column is 5 cm in diameter and 0.3 m high; the void volume is 1.9 x 10^{-4} m^3. The water regain value of the gel is 3 x 10^{-3} kg m^{-3} dry Sephacryl; the density of wet gel is 1.25 x 103 kg m^{-3}. The partition coefficient for hormone A is 0.38; the partition coefficient for hormone B is 0.15. If the eluant flow rate is 0.71 h^{-1}, what is the retention time for each hormone? (10) (notes)

(ii) Describe the principles of reversed phase adsorption chromatography. (6) [TB2: 203-207]

2. (i) Describe the principles of hydrophobic interaction chromatography. (8) [TB2: 209-213]

(ii) Discuss the chromatographic theory of retention. (8) [TB2: 202-203]

3. Discuss the solid phases used for elution chromatography of different scales and types. (8) (Nov./Dec/, 2014) [TB1: 288-293]

(ii) Describe the scaling up chromatograph and scaling the separation. (8) (Nov./Dec/, 2014) [TB1: 306-308]

UNIT V

FINAL PRODUCT FORMULATION AND FINISHING OPERATIONS

PART A

1. What is crystallization?
2. What is saturation?
3. What is purity?
4. What is nucleation?
5. What is single crystal growth?
6. What is crystal size distributions?
8. What is batch crystallization?
9. What is Batch scale – up?
10. What is drying?
11. What is water content?
12. What is significance of relative humidity?
13. What is evaporation rate?
14. What is conduction dryers?
15. What is adiabatic dryers?
16. Write the equation for constant rate drying.
17. What is physical extraction?
18. What is Dissociation extraction?
19. What is reactive extraction?
20. What is membrane adsorbers?
21. Define concentration polarization NOV/DEC 2010
22. Define relative humidity. NOV/DEC 2012
23. Write the industrially important dryers. NOV/DEC 2013
24. Write the stages of crystallization process. NOV/DEC 2012,2013
25. How are nucleic acids and proteins stored in cold storage? NOV/DEC 2014
26. Disadvantages of Lyophilization
27. Advantages of Lyophilization
28. Explain the Characteristics of Freeze Dry Product
29. Explain Freeze Drying NOV/DEC 2014
30. What are the Steps involved in lyophilization

PART B

1. Explain in detail crystallization with suitable diagram. NOV/DEC 2013,2014,2015
Refer: Sivasankar, B. “Bioseparations : Principles and Techniques” –Page no 255

2. Write notes on Lyophilization. NOV/DEC 2015
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 259

3. Discuss in detail the manufacturing process of drying. NOV/DEC 2010,2013,2015
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 259

4. What is formulation? How are final products formulated after lyophilisation? NOV/DEC 2010,2014
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 264

5. Explain the following in detail with suitable plots. NOV/DEC 2012
   (i) Adiabatic drying
   (ii) Constant rate drying and time required to reach a critical moisture content.
   (iii)Falling rate period.
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 259

6. Write notes on the following. NOV/DEC 2012
   (i) Crystal size distributions
   (ii) Dominant crystal size
   (iii)Batch crystallization
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 255

PART C

1. (i) Explain in detail the design and working of a lyophilizer. (8) [TB2: 263-264]

(ii) What are the molecular alterations that lead to loss of a proteins biological activity? Explain the methods of improving protein stability. (8) [TB2: 258-259]

2. (i) Discuss the crystallization equipment used in biotechnology industry. (10) [TB2: 258-259]

(ii) Describe the classes of precipitants used in biomolecular crystallization. (6) [TB1: 373-375]

3. A commercial drier needed 7 hrs to dry a material from a moisture content of 33% to 9% on dry basis. The critical moisture content of the material was 16% and the equilibrium moisture content was 5%. Determine the time needed to dry this material from a moisture content of 37% to 7% if the drying condition remains unchanged. (16) (Nov./Dec., 2015) (notes)

UNIT I
DOWNSTREAM PROCESSING

PART A

1. Name any three molecules manufactured by fermentation.

- Food products: from milk (yogurt, kefir, fresh and ripened cheeses), fruits (wine, vinegar), vegetables (pickles, sauerkraut, soy sauce), meat (fermented sausages: salami)
- Industrial chemicals (solvents: acetone, butanol, ethanol; enzymes; amino acids)
- Organic acids: (Lactic Acid, Fumaric Acid, Tartaric Acid, Vinegar, Citric Acid)
- Specialty chemicals (vitamins, pharmaceuticals)
- Flavor Modifiers (Monosodium Glutamate (MSG), Maltol and ethyl Maltol)
- Biopolymers
- Antibiotics

2.Write the spectrum of separations used in biotechnology.

1. Physical separtions.
2. Equilibrium controlled separations
3. Rate controlled seperations.

3. Write any two design questions to improve the sepcoution process.

1. What is the value of the product?
2. What is an acceptable product quality?

4. Write the four steps in the bioseparation process.

1. Removal of insolubles
2. Isolation of products.
3. Purification
4. Polishing

5. What is the principle used in Osmotic shock and enzyme digestion?

Osmotic shock – Osmotic repture of membrane. Osmotic shock or osmotic stress is physiologic dysfunction caused by a sudden change in the solute concentration around a cell, which causes a rapid change in the movement of water across its cell membrane. Under conditions of high concentrations of either salts, substrates or any solute in the supernatant, water is drawn out of the cells through osmosis. This also inhibits the transport of substrates and cofactors into the cell thus "shocking" the cell. Alternatively, at low concentrations of solutes, water enters the cell in large amounts, causing it to swell and either burst or undergo apoptosis.

enzyme digestion -Cell wall digisted providing disruption

Different cell types and strains have different kind of cell walls and membranes, and thus the used enzyme depends on microbe. For example, lysozyme is commonly used enzyme to digest cell wall of gram positive bacteria. Lysozyme hydrolyzes β-1-4-glucosidic bonds in the peptidoglycan

6. Name any two chemical methods for cell disruption.

1. Osmoticshock
2. Antibodies
3. Chelating agent
4. Solvents
5. Chaotropes
6. Detergents.

7. Name any two mechanical methods for cell disruption.

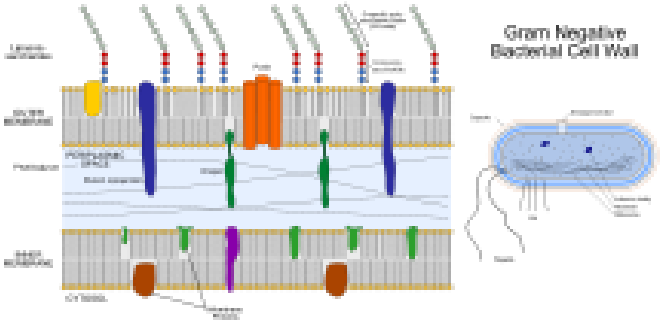
1. Homogenization
2. Grinding
3. Ultrasound
4. Bead mill
5. Microfluidizer
6. French press

8. Give two examples for solubilization and liquid dissolution.

1. Bile salts acting on E-coli
2. Toluenc disruption of yeast

9. Name the layers of gram negative all.

1. Outer layer
2. peptidoglycan
3. Plasmamembrane



10. Write short notes on solubilization.

A concentrated detergent solution is added to about half the solution's volume of cells. The detergent disrupts the cell membrane. The resulting suspension can be centrifuged to remove cell fragments.

11. Explain Schmidl number.

Schmidt Number, Sc, is a dimensionless parameter representing the ratio of diffusion of momentum to the diffusion of mass in a fluid. It is defined as

$$\mathrm{Sc} = \frac{\nu}{D} = \frac{\mu}{\rho D} = \frac{\text{viscous diffusion rate}}{\text{molecular (mass) diffusion rate}}$$

Schmidt number is the mass transfer equivalent of Prandtl Number. For gases, Sc and Pr have similar values (≈0.7)

OR

$$N_{sc} = \mu / \delta D$$

It gives the relative speed of momentum transport to diffusive transport.

12. Explain Sherwood number.

The Sherwood number (Sh) (also called the mass transfer Nusselt number) is a dimensionless number used in mass-transfer operation. It represents the ratio of the convective mass transfer to the rate of diffusive mass transport, and is named in honor of Thomas Kilgore Sherwood.

$$\mathrm{Sh} = \frac{K}{D/L} = \frac{\text{Convective mass transfer rate}}{\text{Diffusion rate}}$$

L is a characteristic length (m)
D is mass diffusivity (m2.s−1)
K is the convective mass transfer film coefficient (m.s−1)

OR

$$N_{sh} = \frac{kd}{D}$$ It is used to correlate results in extraction and chromotagraphy

13. Define Reynold's number.

$$R_e = \frac{\rho V L}{\mu}$$

Where,
ρ is the density of the fluid,
V is the velocity of the fluid,
μ is the viscosity of fluid,
L is the length or diameter of the fluid.
The Reynolds number (Re) is an important dimensionless quantity in fluid mechanics used to help predict flow patterns in different fluid flow situations.
The Reynolds number is the ratio of inertial forces to viscous forces within a fluid which is subjected to relative internal movement due to different fluid velocities, in what is known as a boundary layer in the case of a bounding surface such as the interior of a pipe.
It describes the nature of flow, whether it is laminar or turbulent.

14. What is principle of alkali treatment?

Saponification of lipids solubilizers membrane.

The alkali solution mostly contains sodium hydroxide (NaOH) and the detergent Sodium Dodecyl (lauryl) Sulfate (SDS). SDS is there to solubilize the cell membrane. NaOH helps to break down the cell wall, but more importantly it disrupts the hydrogen bonding between the DNA bases, converting the double-stranded DNA (dsDNA) in the cell, including the genomic DNA (gDNA) and the plasmid, to single stranded DNA (ssDNA). This process is called denaturation and is central part of the procedure, which is why it's called alkaline lysis. SDS also denatures most of the proteins in the cells, which helps with the separation of the proteins from the plasmid.

15. What is Cell disruption?

Cell Disruption is the method or process fordisrupting or lysing the cell inorder to release the contents out of the cell.

Bioseperations usually begin with the separation of biomass from the broth and the trapped material is released by rupturing the cell wall which is also called as cell disruption.

16. What are the solvents used in lipid dissolution?

1. Chloroform
2. Chlorobenzene
3. Cumene.
4. alcohol (methanol and ethanol)
5. Diethyl ether, dioxan, isopropyl ether and tetrahydrofuran

17. List out the four familiar steps in most bio-separation. NOV/DEC 2012

- Product isolation
- Product purification
- Product polishing
- Product recovery

18. Explain about lipid dissolution. NOV/DEC 2012

The maximum concentration of a chemical that will dissolve in fatty substances. Lipid soluble substances are insoluble in water. They will very selectively disperse through the environment via uptake in living tissue.

19. What are the different steps in downstream processing? NOV/DEC 2013

1. Separation of Particles
Filtration
Centrifugation
Flocculation and Floatation
2.Disintegration of Cells:
Mechanical Cell Disruption
Drying:
Lysis:
3.Extraction:
Liquid-Liquid Extraction
Whole Broth (Medium + Cells) Extraction
Aqueous Multiphase Extraction
4.Concentration:
Evaporation
Membrane Filtration
Ion Exchange Resins
Adsorption Resins
5.Purification:
Crystallization
Chromatographic Methods
6. Drying.

20. What are the physical methods of cell disruption?

Thermolysis
Osmotic shock
Ultrasonication
French press

21. Write the principles of sonication for disruption of bacterial cells. NOV/DEC 2014

- The cells are subjected to ultrasonic vibrations by introducing an ultrasonic vibration emitting tip into the cell suspension. The duration of ultrasound needed depends on the cell type, the sample size and the cell concentration. These high frequency vibrations cause cavitations, i.e. the formation of tiny bubbles within the liquid medium.
- When these bubbles reach resonance size, they collapse releasing mechanical energy in the form of shock waves equivalent to several thousand atmospheres of pressure.
- The shock waves disrupts cells present in suspension.

22. How does lysozyme act on bacterial cells? NOV/DEC 2014

Lysozymes, also known as muramidase or N-acetylmuramide glycanhydrolase, are glycoside hydrolases. These are enzymes that damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues inchitodextrins. Lysozyme is abundant in a number of secretions, such as tears, saliva, human milk, and mucus. It is also present in cytoplasmic granules of the macrophages and the polymorphonuclear neutrophils (PMNs). Large amounts of lysozyme can be found in egg white.

23. List out mechanical methods for cell disruption.

Bead mill disruption

High pressure homogenizer
Sonication
Grinding
Blenders
Freezing
Microwave

24. List out chemical methods for cell disruption.

Alkali treatment
Detergent solubilization
Cell wall permeabilization
Enzyme digestion

25.Define stabilization.

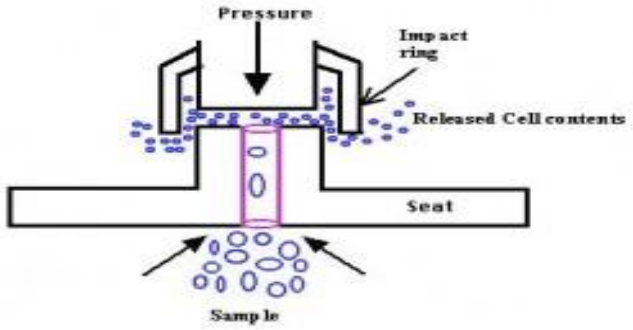
The act of stabilizing something or making it more stable; "he worked for price stabilization for farm products"; "wage stabilization is necessary for industrial peace"; "stabilization means that the product can be handled under atmospheric conditions" stabilization.

26. Short notes on pretreatment

- The separation of suspended solids in fermentation broths is facilitated by subjecting the broths to pretreatment to enhance their filterability.
- Any one of the following three general methods may be adopted depending on requirements.

1. Heating
2. Coagulation and flocculation and
3. Adsorption on filter aids

27. Define high pressure homogenizer



- High pressure homogenizer consists of a high pressure positive displacement pump coupled to an adjustable discharge valve with a restricted orifice.

The cell suspension is pumped through the homogenizing valve at 200-1000 atmospheric pressure(depending on the type of microorganism and concentration of the cell suspension) Cell disruption occurs due to various stresses developed in the fluid.

28. Explain Cell disruption using French press

- It is a device commonly used for small-scale recovery of intracellular proteins and DNA from bacterial and plant cells.
- The device consists of a cylinder fitted with a plunger which is connected to a hydraulic press.
- The cell suspension is placed within the cylinder and pressurized using the plunger.

The cylinder is provided with an orifice through which the suspension emerges at very high velocity in the form of a fine jet.

29. Notes on Disadvantages of bead mill

- Foaming and sample heating - especially for larger samples.

- Tough tissue samples such as skin or seeds are difficult to disrupt unless the sample is very small or has been pre-chopped into small pieces.

30. Write down the Disadvantages of enzymatic method in cell disruption technique.

1. Not always reproducible.
2. Not usually applicable to large scale.
3. Large scale applications of enzymatic methods tend to be costly and irreproducible.
4. The enzyme must be removed (or inactivated) to allow cell growth or permit isolation of the desired material.

PART B

1. **Write short notes on the characteristics and principles of bioseparation in detail. NOV/DEC 2012.2014,2015**

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 1

2. **Explain the characteristics and principles of biomolecules in detail. NOV/DEC 2012**

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 3

3. **Describe the cell disruption for product release by chemical methods with suitable examples. NOV/DEC 2012,2014**

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 13

4. **Describe in detail the pretreatment and stabilization of bioproducts. NOV/DEC 2014**

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 09

5. **Detail notes on the cell disruption for product release by mechanical methods. NOV/DEC 2010,2015**

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 20

6. **Write note on the cell disruption for product release by enzymatic methods. NOV/DEC 2010**

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 17

PART C

1. (i) The diffusivity of a protein having a Stokes-Einstein radius of 2 nanometers in a particular liquid is known to be 4.5×10^{-11} m^2s^{-1}. Predict the diffusivity of another protein having twice the Stokes-Einstein radius in the same liquid at the same temperature. (8) (notes) (Nov./Dec., 2016)

(ii) Discuss the design and working principle of a ball mill. (8) [TB2: 20-22] (Apr./May, 2017) (Nov./Dec., 2016)

2. (i) What is the criterian for selection of electrolyte for flocculation. (8) (Nov./Dec., 2016)

(ii) Discuss the construction and working principles of a microfluidizer. (8) (Nov./Dec., 2016)

3. (i) Explain in detail the construction and working principles of a high pressure mechanical homogenizer. (8) [TB2: 22-23] (Apr./May, 2017)

(ii) Discuss the use of ultrasound for cell disruption. (8) [TB2: 16] (Apr./May, 2017)

UNIT II

PHYSICAL METHODS OF SEPARATION

PART A:

1. What is depth filtration?

Solids are trapped in the interstices of the medium. As solids accumulate, flow approaches zero and the pressure drop across the bed increases (Eg) Sand and cartridge filtration.

2. Write the Poiseuille's equation.

$$\frac{dv}{A.d\theta} = \frac{P}{\mu\left[\alpha\left(\frac{W}{A}\right)+r\right]}$$

V= volume of filtrate
A= filter are a surface
θ=time
P= pressure across filter medium
α= average specific cake resistance
w= weight of cake
r= resistance of filter medium
u= viscosity

3. Write the formula for Poiseuille's equation.

$$\frac{\text{Flow Rate}}{\text{Unit Rate}} = \frac{\text{Force}}{\text{Viscosity}\left[\begin{array}{l}\text{Cake Resistance +}\\ \text{filter medium resistance}\end{array}\right]}$$

4. What is cake compressibility?

The specific cake resistance is a function of compressibility of the cake.

$$\boxed{\alpha = \alpha' P^{s}}$$

$$\alpha' = \text{constant}$$

5. Short note on Diaphragm pumps.

1. Diaphragm pumps offer very gentle handling of slurries and are in expensive and mobile.
2. The pulsating flow can cause fading and distribution problem in some filtration systems.

6. Short note on centrifugal pumps.

- Most common source of particle attrition problems is the centrifugal pump.
- The high shear forces inherent to these pumps, Particularly in the eye of the impeller, make some crystal damage inevitable in all but the toughest crystals.

7. Short notes on Positive displacement pumps.
The minimal shear operation of progressive cavity or lobe pumps make them ideal for slurries.

8. What is porosity?

$$\text{Porosity} = \frac{\text{Volume of voids}}{\text{Volume of filter cake}}$$

9. Short notes on filter aid.
Slurry additives such as diatomaceous silica or perlite (pulverized oock) are employed to aid filtration. Diatomite is a scdimentary oock containing skeletons of unicellular plant organisms (diatoms).

10. Filter media.
Filter media are required in both cake filtration and depth filtration.
Essential to selection of a filter medium is the solvent composition of the slurry and washes, and the particles size retention required of the solids.

11. Short notes on equipment selection.
Every – increasing environmental concerns may make it necessary to evaluate the existing process to reduce emissions, operator exposure, limit waster disposal of filter aid, or reduce wash quantities requiring solvent recovery or wash treatment.

12. How will you use continuous and Batch filtration together.
Continuous and batch equipment can be used in the same process by incorporating holdup tanks, vessels or hoppers between them.

13. Short notes on Rotary vacuum filter.
Raw fermentation booth is an example of large volume production Rotary drum vacuum filters hare traditionally ban found in this service. Slow – setting materials or more difficult filtrations with larges cake production requirements are typical application.

14. Short notes on Nutsches.
The nutsche filter is increasingly prevalent in post crystallization filtrations. Relatively fast filtrations with predictable crystal structures often found in the intermediate and final step purifications of antibiotic drugs.

15. Short notes on cross flow filtration.
Cross flow filtration also known as tangential flow filtration. The technique was applied to the concentration and fractionation of macromolecules commonly recognized as ultra filtration.

16. Short notes on factors affecting the characteristics of filters.
1. The nature of the membrane material
2. Pore dimensions.
3. Pore size distributions
4. Porosity

17. What is continuous mode?
When large volumes processed the batch feed and bleed system is replaced with a continuous system. The size of the feed tank is much smaller compared to that of batch system.

18. What is Dia filtration?
The product purification or recovery objective in most ultra filtration operations is achievable by concentrating the suspended particles or micro solutes retained by the membrane while allowing almost quantitative permeation of soluble products into the permeate.

19. What are the parameters which affect for filter performance?

1. Membrane Pore Diameter or Molecular weight cut off
2. Cross flow velocity
3. Concentration of solute or particle loading
4. Membrane fouling

20. Explain trans membrane pressure.

It is the average driving force for permeation across the membrane. Neglecting osmotic pressure effects for most MF/UF applications, it is defined as the difference between the average pressure on the feed side and that on the permeate.

21. Define Cohn equation NOV/DEC 2010

$$\ln S = \beta - K'_s I$$

K_s' is a salting-out constant characteristic of the specific protein and salt that is independent of temperature and pH above the isoelectric point. The constant β, the hypothetical solubility of the protein at zero ionic strength, depends only on temperature and pH for a given protein and is a minimum at the isoelectric point

22. Write down the need for filter aid.

Filter Aids" is a group of inert materials that can be used in filtration pretreatment. There are two objectives related to the addition of filter aids. One is to form a layer of second medium which protects the basic medium of the system. This is commonly referred to as "precoat". The second objective of filter aids is to improve the flow rate by decreasing cake compressibility and increasing cake permeability. This type of usage is termed as "admix" or "body feed".

23. Identify four basic types of centrifuge and explain. NOV/DEC 2012

1. Small Benchtop – with or without refrigeration – slow speed (eg up to 4000 RPM) – common in clinical labs (blood/plasma/serum separation) – can take approx (up to) 100 tubes, depending on diameter

2. Microcentrifuges ("microfuge", "Eppendorf") – take tubes of small vols (up to 2 mL) – very common in biochemistry/molecular biology/ biological labs – can generate forces up to ~15,000 x g – with or without refrigeration

3. High Speed centrifuges – 15,000 – 20,000 RPM – large sample capacity depending on rotor – normally refrigerated – research applications

4. Ultracentrifuges → 65,000 RPM (100,000's x g) – limited lifetime – expensive – require special rotors – care in use – balance critical! – research applications

24. What are filter aids?Give some examples? NOV/DEC 2013

Filter Aids" is a group of inert materials that can be used in filtration pretreatment. The common filter aids are diatomaceous earth (DE), perlite, cellulose and others.

25. What is isopycnic centrifugation. NOV/DEC 2014

Isopycnic Centrifugation. In isopycnic separation, also called buoyant or equilibrium separation, particles are separated solely on the basis of their density. Particle size only affects the rate at which particles move until their density is the same as the surrounding gradient medium.

26. Define zonal centrifugation

a) Zonal centrifugation is also known as band or gradient centrifugation

b) It relies on the concept of sedimentation coefficient (ie movement of sediment through liquid medium)
c) In this technique a density gradient is created in a test tube with sucrose and high density at the bottom.
d) The sample of protein is placed on the top of the gradient and then centrifuged.
e) The proteins sediment according to their sedimentation coefficient and the fractions are collected by creating a hole at the bottom of tube.

27. Explain the density gradient centrifugation

a) This type of centrifugation is mainly used to purify viruses, ribosome's, membranes etc.
b) A sucrose density gradient is created by gently overlaying lower concentrations of sucrose on higher concentrations in centrifuge tubes
c) The particles of interest are placed on top of the gradient and centrifuge in ultra centrifuges.
d) The particles travel through the gradient until they reach a point at which their density matches with the density of surrounding sucrose the fraction is removed and analyzed.

28. Define differential centrifugation

a) Differential centrifugation is a technique commonly used by biochemists.
b) Tissue such as liver is homogenised at 32 degree in a sucrose solution that contains buffer
c) The homogenate is then placed in a centrifuge and spun at constant centrifugal force at constant temperature.
d) After sometime a sediment forms at the bottom of centrifuge called pellet and overlying solution called supernatant.
e) The overlying solution is then placed in another centrifuge tube which is then rotated at higher speeds.

29. Define high speed centrifuges

1) High speed centrifuges are used in more sophisticated biochemical applications, higher speeds and temperature control of the rotor chamber are essential.
2) The operator of this instrument can carefully control speed and temperature which is required for sensitive biological samples.
3) Three types of rotors are available for high speed centrifugation-fixed angle, swinging bucket, vertical rotors

30. Define low speed centrifuge

1) Most laboratories have a standard low-speed centrifuge used for routine sedimentation of heavy particles
2) The low speed centrifuge has a maximum speed of 4000-5000rpm
3) These instruments usually operate at room temperatures with no means of temperature control.
4) Two types of rotors are used in it, Fixed angle and swinging bucket.
5) It is used for sedimentation of red blood cells until the particles are tightly packed into a pellet and supernatant is separated by decantation.

PART B

1. Fermented broth is filtered under constant pressure of recovery of protease. A pilot scale filter is used to measure filtration properties. The filter area is 0.25 m^2, the

pressure drop is 360mmHg and the filtrate viscosity is 4cP. The cell suspension deposits 22g cake/Lit of filtrate. The following data are obtained.

Time (Mins)	2	3	6	10	15	20
Filtrate volume(Lit)	10.8	12.1	18.0	21.8	28.4	32.0

iii. Determine the specific cake resistance and filtrate medium resistance.

iv. What size filter is required to process 4000lit cell suspension in 30min at a pressure of 360 mmHg? NOV/DEC 2015

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 40

2. Centrifugation of yeast: yeast cells are to be separated from a fermentation broth. Assume that the cells are spherical with diameter 5µm and density 1.06g/cm3. Viscosity of culture broth is $1.36*10^{-3}$Ns/m^2. During separation, the density of suspending fluid is 0.997g/cm^3.500lit broth must be treated every 1hr.

iii) Specify £ for a suitably sized tubular bowl centrifuge.

iv) If instead of yeast, quartz particles of diameter 0.1mm and specific gravity 2.0 are used to separate from the culture liquid, how much is £ reduced? NOV/DEC 2008

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 41

3. Determine the time required for carrying out the process of filtration. NOV/DEC 2008

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 39

4. Determine the position of particular particles as a function of time and volumetric flow rate of feed in a disc type centrifuge with the diagram and explain the different types of centrifuge. NOV/DEC 2014,2012,2012

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 44

5. Describe filtration types in detail. NOV/DEC 2014,2013

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 38

6. Explain in detail solid liquid separations NOV/DEC 2008

Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 26

PART C

1. (i) Describe the design and working principles of disc stack centrifuges. (8) [TB2: 47-49] (Nov./Dec., 2016)

(ii) Describe the design and working principle of rotary drum vacuum filtration. (8) [TB2: 34-37] (Nov./Dec., 2016)

2. A 30-ml sample of broth from a rifamycin B fermentation is filtered in the laboratory on a 3 cm^2 filter at a pressure drop of 3.45 x 10^4 N/m^2. The filtration time is 4.5 min. Previous studies have shown that filter cake of *Streptomyces mediterranensis* is significantly compressible with s = 0.5. If 500 litres broth from a pilot-scale fermenter must be filtered in 1hour, what size is required if the pressure drop is:

(i) 6.86 x 10^{-4} N/m^2

(ii) 3.45 x 10^4 N/m^2

Resistance due to filter medium is negligible. (notes) (Nov./Dec., 2016)

3. (i) Describe the general theory of filtration. (8) [TB2: 27-28] (Apr./May, 2017)

(ii) Explain detail the design and working of a tubular bowl centrifuge. (8) [TB2: 45-47] (Apr./May, 2017)

UNIT III

ISOLATION OF PRODUCTS

PART A:

1. What is Isolation?

Isolation is one of the aspects which make bio-separations unlike commodity chemical separations. Isolation involves taking a highly dilute aqueous feed and removing most of water. The resulting concentrate can be purified by a variety of methods.

2. What is partition coefficient?

The solute concentration increases in the first liquid phase as a result of depletion from the second liquid phase. This partitioning is conveniently summarized as a partition coefficient 'K'

$$\boxed{K = \frac{x}{y}}$$

3. Write the logarithmic equation for the partition coefficient.

$$\frac{x}{y} = K = \exp\left(\frac{\mu^{\circ}(H) - \mu^{\circ}(L)}{RT}\right)$$

4. Write the partition coefficient equation in terms of partial molar volumes.

$$\ln K = \frac{\overline{V}_H (\delta_A - \delta_H)^2 - \overline{V}_L (\delta_A - \delta_L)^2}{\left(RT\ \overline{V}_A\right)}$$

$\overline{V}_i$ = Partial molar volumes

H = Heavy solvent

L = Light solvent

A = Solute

δ_i = Solubility parameter

5. Name any four solvents used for extraction process.

Amyl acetate, Benzene, Butanol, Butyl acetate

6. Name any three counter ions for Ion paired extractions.

Acetate, Butytrate and cholate

7. Write the PKa values for acetic acid and propionic acid.

Acetic acid = 4.76

Propionic acid = 4.87

8. Write the PKa values for any two antibiotics.

Celesticetins = 7.7
Novobiocin = 4.3

9. Write short notes on Equilibrium constraint.

The equilibrium constraint for dilute solution,

$$\begin{pmatrix}\text{Solute Concentration}\\ \text{in light phase L}\end{pmatrix}\times\begin{pmatrix}\text{Solute concentration}\\ \text{in heavy phase H}\end{pmatrix}$$

$$\boxed{x = Ky}$$

10. Write the formula for extraction factors.

$$\boxed{E = \frac{KL}{H}}$$

11. Write short notes on Chemistry of adsorption.

Adsorption requires adsorbents, solid materials to which solutes of interests bind reversibly.

12. Name any four adsorbents used in extraction process.

Styrene, divinyl benzene, hydrogels and synthetic polymers

13. Name any three adsorption isotherm.

1. Freundlich isotherm
2. Langmuir isotherm
3. Linear isotherm

14. Write the empirical equation for freundlich isotherm.

$$\boxed{q = K\ y^n}$$

n – constant
k – Partition coefficient
q – amount of solute adsorbed per amount of adsorbent.

15. Write the empirical equation for the Langmuir isotherm.

$$\boxed{q = \frac{q_o y}{K + y}}$$

k, q_o – constants determined experimentally.

16. Write the equation explaining the theory of langmuir isotherm.

Solute + Vacant Site = Filled site

17. What is break point? How it can be used to design the adsorption unit. NOV/DEC 2010

The *break point* occurs when the concentration of the fluid leaving the bed spikes as unadsorbed solute begins to emerge. The bed has become ineffective. Usually, a *breakpoint composition* is set to be the maximum amount of solute that can be acceptably lost, typically something between 1 and 5 percent.

18. Types of adsorption:

Depending upon the nature of forces existing between adsorbate molecules and adsorbent, the adsorption can be classified into two types:

1. **Physical adsorption (physisorption):** If the force of attraction existing between adsorbate and adsorbent are Vander Waal's forces, the adsorption is called physical adsorption. It is also known as Vander Waal's adsorption. In physical adsorption the force of attraction between the adsorbate and adsorbent are very weak, therefore this type of adsorption can be easily reversed by heating or by decreasing the pressure.
2. **Chemical adsorption (chemisorption):** If the force of attraction existing between adsorbate and adsorbent are almost same strength as chemical bonds, the adsorption is called chemical adsorption. It is also known as Langmuir adsorption. In chemisorption the force of attraction is very strong, therefore adsorption cannot be easily reversed.

19. Factors affecting adsorption:

The extent of adsorption depends upon the following factors:

1. Nature of adsorbate and adsorbent.
2. The surface area of adsorbent.
3. Activation of adsorbent.
4. Experimental conditions. E.g., temperature, pressure, etc.

20. Explain the term extraction.

Extraction is a very common laboratory procedure used when isolating or purifying a product. Organic chemistry employs solid-liquid, liquid-liquid, and acid-base extractions.

21. Define partition coefficient in extraction. NOV/DEC 2013

A partition-coefficient (*P*) or distribution-coefficient (*D*) is the ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium. Hence these coefficients are a measure of the difference in solubility of the compound in these two phases.

22. Define Salting in of proteins NOV/DEC 2013

At low salt concentrations, the solubility of the protein increases with increasing salt concentration (i.e. increasing ionic strength), an effect termed salting in.

23. Define Salting out of proteins NOV/DEC 2013

As the salt concentration (ionic strength) is increased further, the solubility of the protein begins to decrease. At sufficiently high ionic strength, the protein will be almost completely precipitated from the solution (salting out).

24. How does ammonium sulphate helps in precipitation of proteins? NOV/DEC 2014

proteins differ markedly in their solubilities at high ionic strength, salting-out is a very useful procedure to assist in the purification of a given protein. The commonly used salt is ammonium sulfate, as it is very water-soluble, forms two ions high in the Hofmeister series. It is generally used as a saturated aqueous solution which is diluted to the required concentration, expressed as a percentage concentration of the saturated solution (a 100% solution).

25.What is adsorption? NOV/DEC 2014

Adsorption is the adhesion of atoms, ions, or molecules from a gas, liquid, or dissolved solid to a surface. This process creates a film of the adsorbate on the surface of the adsorbent.

26. Write down the operational approaches of Ammonium Sulphate Precipitation

Two operational approaches could be used:

1. Direct addition of solid ammonium sulphate crystals to the sample
2. Addition of a saturated ammonium sulphate solution to sample

27. Explain the Use of anti-chaotropic salts

- Anti-chaotropic salts such as ammonium sulphate and sodium sulphate expose hydrophobic patches on proteins by removing the highly structured water layer which usually covers these patches in solution.
- As a result hydrophobic residues on a protein molecule can interact with those on another and this eventually leads to aggregation and precipitate formation.
- Salts can also reduce the solubility of proteins by shielding charged groups which normally keep proteins apart in solution.
- When the electrostatic charge on protein molecules are shielded, the molecules can easily interact, form aggregates and eventually precipitate.

28. How the Biological macromolecules can be precipitated?

- Cooling
- pH adjustment
- Addition of solvents such as acetone and ethanol
- Addition of anti-chaotropic salts such as ammonium sulphate and sodium sulfate
- Addition of chaotropic salts such as urea and guanidine hydrochloride
- Addition of biospecific reagents as in immunoprecipitation

29. Explain the Applications of dialysis.

- Removal of acid or alkali from products
- Removal of salts and low molecular weight compounds from solutions of macromolecules
- Concentration of macromolecules
- Haemodialysis, i.e. purification of blood

30. Explain the flux equation

$Jc = K_M(C1 – C2)$

Where

C1 = feed concentration (kg/m3)

C2 = dialysate concentrations (kg/m3)

PART B

1. Explain in detail ultra filtration, and Reverse osmosis. NOV/DEC 2011,2012,2013,2014,2015

Refer: Sivasankar, B. “Bioseparations : Principles and Techniques” –Page no 112,114

2. Discuss protein precipitation NOV/DEC 2014,2015

Refer: Sivasankar, B. “Bioseparations : Principles and Techniques” –Page no 119

3. Write short notes on dialysis. NOV/DEC 2008

Refer: Sivasankar, B. “Bioseparations : Principles and Techniques” –Page no 116

4. Explain in detail aqueous two phase extraction. NOV/DEC 2011,2013,2014,2015

Refer: Sivasankar, B. “Bioseparations : Principles and Techniques” –Page no 81

5. Describe in detail liquid- liquid extraction. NOV/DEC 2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 67

6. Explain in detail adsorption. NOV/DEC 2008
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 54

PART C

1. (i) Clarified fermentation broth contains a polysaccharide product with a gelation concentration of 25 g/l. The fluid density is 1020 kg m^{-3}, the viscosity is 1.8 cP, and the polysaccharide diffusivity is 5.63 x 10^{-11} m^2 s^{-1}. The product is harvested from the broth using ultrafiltration at a fluid velocity of 0.34 ms-1 in open membrane tubes of diameter 2.4 cm and length 2.0 m. Estimate the permeate flux if the filter is operated under gel polarization conditions and the polysaccharide concentration in the feed is 12 $g.l^{-1}$. (8) (Nov./Dec., 2016) (notes)

(ii) Describe the various models describing the ultrafiltration process.(8) (Nov./Dec., 2016; Apr./May, 2017) [TB2: 112-114]

2. (i) An adsorptive membrane has a thickness of 2 mm and a diameter of 5 cm. The porosity of the membrane is 0.75 and the tortuosity is 1.5. The pore diameter was estimated to be 2 x 10^{-6} m. If we are to use this membrane for adsorption of a DNA fragment with diffusivity of 9.5 x 10^{-12} m^2 s^{-1} from an aqueous solution of viscosity 0.001 kg/m.s at 25°C, what is the maximum solution solution flow rate that can be used? Assume that the flow through the pores is laminar. (8) (Nov./Dec., 2016) (notes)

(ii) Discuss the principles of aqueous two-phase partitioning of proteins. Add a note on affinity partitioning.(8) (Nov./Dec., 2016; Apr./May, 2017) [TB2: 81-90]

3. (i) Describe the various stages involved in precipitate formation (8) (Apr./May, 2017) [TB2: 119-139]

(ii) What is adsorption isothern? Describe the types of adsorption isotherms. (8) (Apr./May, 2017) [TB2: 54-60]

UNIT IV

PRODUCT PURIFICATION

TWO MARKS

1. Write short notes on elution chromatography.

Elution chromatography commonly uses a packed column of adsorben particles, which can be solid, porous solid or get. As a result, elution chromatography is similar to fixed bed adsorption.

2. Name some adsorbents used in elution chromatography.

In organic materials like alumina, activated carbon and Diat amaceous carth.

3. Write short notes on yield and purity.

Yield and purity involves infecting a pulse of mixed solutes in to a packed column and then washing these solutes out of the column. The elute solutes are collected as fractions.

4. Write the material balance equation for elution chromatography.

Solute accumulation in liquid and packing = Solute dispeosion in – out + solute convection in-out

5. Explain NTU.

By analogy with differential extraction, we call the quantity [v/ka] the height of a transfer unit and the integral the number of transfer units NTU.

$$l = HTU.NTU$$

6. What is nucleation?

Nucleation is that part of the process where small particles appear and being to grow. In inorganic systems, nucleation can be slow and super saturation can pesist for long periods.

7. What is diffusion limited growth?

After nucleation, the precipitated particles grow as dissolved solute diffuse to them from the surrounding solution.

8. Write the second order equation for the diffusion limited growth.

$$\left(\frac{dy_i}{dt}\right) = -xy_1^2$$

9. What is flow influenced growth?

Diffusion limits the growth of small particles, but it is less important for the growth of larger particles. For those larger particles, growth usually involves collisions between species which are swept together by the mixing.

10. Write the equation to calculate the yields.

$$y = yo\ \exp\left(-\frac{\left(t/t_0 - 1\right)^2}{2\sigma^2}\right)$$

y_0 = maximum concentration
t_0 = time at which this concentration exists.
$t_{0\sigma}$ = standard deviation of the peak

11. Write the equation to find the purity of solute.

$$\text{Purity of solute i} = \frac{y_o(i)\,\text{yield}(i)}{\sum \text{jyo}(i)\,\text{yield}(i)}$$

12. Write the yield equation in teams of eluted volume 'v'.

$$y = y0\ \exp\left(-\frac{\left[v/v_0 - 1\right]^2}{2\sigma^2}\right)$$

V_0 = volume require to clute the maximum concentration
$V_0\sigma$ = Standard deviation

13. Write any three typical stationary phases.

1. Polymers of dextran
2. Enzymes bound to polymer supports
3. Alumina
4. Silical gel

14. Write any two advantages of ION exchange chromatography.

1. Good selectivity
2. In expensive.

15. What are the Ionic groups in the Ion exchange resins?

1) $-SO_3^-$
2) $-NH_2$
3) $-NH_3^+$
4) $-COO^-$
5) $-PO_3^=$

16. What is bonded stationary phase? NOV/DEC 2010

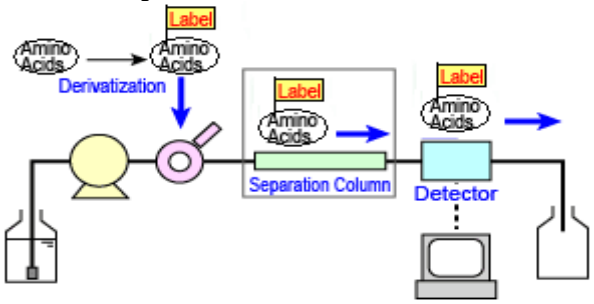
A bonded phase is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing.

17. What is pre-column derivatisation? NOV/DEC 2010

Pre-column derivatization, the amino acids are derivertized before injection, and then the reaction products are separated and detected.

The advantages of this method are as follows:

- In general, reagent consumption rates can be minimized by specifying a small reaction system.
- Allows increasing sensitivity by using more expensive reagents that provide lower background levels (than post-column derivatization).
- Even if unreacted derivatizing reagent is detected, as long as it is separated in the column, it does not cause a problem.



18.Define yield.

The result, product, or amount yielded

The formula for effective yield is:

$$(1+(i/n)^n-1$$

Where,i=the nominal rate

N=the number of payment periods

19. Define purity.

- The quality or condition of being pure.
- The process of extracting something from a substance is called purification. example: filtration, centrification, affinity purification, etc.

20. Differentiate between yield and purity. NOV/DEC 2012

The yield is how much actual physical product you get out of reation. If you combine 5 grams of A with 5 grams of B and get 7 grams of product, and yield is 7 grams. Purity deals with how much of your yield is the intended product, and how much is contaminants or byproducts or unreacted starting products etc.

21. Give the solute mass balance in symbolic terms for discrete stage analysis of chromatography. NOV/DEC 2012

(dc/dt)x+F(dn/dt)x=-u0(dc/dx)t+D(d2c/dx2)t

C=solute concentration in the mobile phase

N=solute concentration on the stationary phase

F=column phase ratio

D=diffusion coefficient of the solute

X=axial column coordinate

T=time

22. Define retention time NOV/DEC 2013

Retention time is the time it takes a solute to travel through the column.The retention time is assigned to the corresponding solute peak. The retention time is a measure of the amount of time a solute spends in a column. It is the sum of the time spent in the stationary phase and the mobile phase.

23. Define retention volume NOV/DEC 2013

Retention volume is proportional to retention time,based on the constant flow rate of a chromatography system.

24. Define total ionic capacity. NOV/DEC 2014

The factor which defines the ability of an ion exchange resin to remove ions from solution in the capacity.the total ionic capacity is defined as the theoretical number of exchangeable ions per unit volume or weight of resin.

25. What is the purpose of using sephadex G50. NOV/DEC 2014

It is used to separate low and high molecular weight molecules. Sephadex is a faster alternative to dialysis,requiring a low dilution factor with high activity recoveries. It is also used for buffer for buffer exchange and the removal of small molecules during the preparation of large molecules.

26. Write notes on Protein purification:

In biochemistry, the hydrophobic effect can be used to separate mixtures of proteins based on their hydrophobicity. Column chromatography with a hydrophobic stationary phase such as phenyl-sepharose will cause more hydrophobic proteins to travel more slowly, while less hydrophobic ones elute from the column sooner. To achieve better separation, a salt may be added (higher concentrations of salt increase the hydrophobic effect) and its concentration decreased as the separation progresses.

27. Define Amphiphiles:

Amphiphiles are molecules that have both hydrophobic and hydrophilic domains. Detergents are composed of amphiphiles that allow hydrophobic molecules to be solubilized in water by forming micelles and bilayers (as in soap bubbles). They are also important to cell membranes composed of amphiphilic phospholipids that prevent the internal aqueous environment of a cell from mixing with external water.

28. Principles of Size exclusion chromatography

- Gel filtration or gel permeation chromatography
- Separation - Molecular size of its components.
- Larger molecules are rapidly washed through the column, smaller molecules penetrate inside the porous of the packing particles and elute later.

29. Write down the Application of ion exchange chromatography.

- Conversion from one salt to other e.g we can prepare tetra propyl ammonium hydroxide from a tetra propyl salt of some other anion.
- household (laundry detergents and water filters) to produce soft water
- Ion exchange is used to prepare de-ionized water
- separate and purify metals
- Dealkalization
- analysis and purification of immunoglobulins
- Separation of inorganic ions

30. What are the material can be used as Ion exchangers

There are three classes of ion exchangers , these include

1. Resins
2. Gels
3. Inorganic exchangers

PART B

1. Explain the general description of chromatography. NOV/DEC 2008

Refer: Sivasankar, B. “Bioseparations : Principles and Techniques” –Page no 143

2. Ion exchange chromatography, size exclusion chromatography –discuss NOV/DEC 2015,2014,2012

Refer: Sivasankar, B. “Bioseparations : Principles and Techniques” –Page no 188

3. Write notes on Affinity chromatography NOV/DEC 2015,2014,2013,2011
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 215

4. Describe Adsorption chromatography NOV/DEC 2014,2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 143

5. Explain in detail hydrophobic interaction. NOV/DEC 2012
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 200

6. Detail notes on Reverse phase chromatography NOV/DEC 2015,2013
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 201

PART C

1. (i) A pilot-scale gel-chromatography column packed with Sephacryl resin is used to separate two hormones A and B. The column is 5 cm in diameter and 0.3 m high; the void volume is 1.9 x 10^{-4} m^3. The water regain value of the gel is 3 x 10^{-3} kg m^{-3} dry Sephacryl; the density of wet gel is 1.25 x 103 kg m^{-3}. The partition coefficient for hormone A is 0.38; the partition coefficient for hormone B is 0.15. If the eluant flow rate is 0.71 h^{-1}, what is the retention time for each hormone? (10) (notes)

(ii) Describe the principles of reversed phase adsorption chromatography. (6) [TB2: 203-207]

2. (i) Describe the principles of hydrophobic interaction chromatography. (8) [TB2: 209-213]

(ii) Discuss the chromatographic theory of retention. (8) [TB2: 202-203]

3. Discuss the solid phases used for elution chromatography of different scales and types. (8) (Nov./Dec/, 2014) [TB1: 288-293]

(ii) Describe the scaling up chromatograph and scaling the separation. (8) (Nov./Dec/, 2014) [TB1: 306-308]

UNIT V

FINAL PRODUCT FORMULATION AND FINISHING OPERATIONS

TWO MARKS

1. What is crystallization?

Crystallization, the formation of solid particles of defined shape and size from a homogeneous liquid phase, is the oldest and most common purification.

2. What is saturation?

Saturation is the maximum concentration of solute which is thcomodynamically stable in solution. Saturation is the result of phase equilibria.

3. What is purity?

The second concept basic to crystallization is that of purity can be quantified by using the factor (E)

$$E_A = \frac{\text{Weight of solute A in crystalcake}}{\text{Weight of solute A in filtrate}}$$

4. What is nucleation?

Nucleation is the formation of crystals from the liquid phase as result of sup co saturation only which concerns the genesis of new crystals.

5. What is single crystal growth?

The growth rate depends on the growth mechanism in nonagitated systems, the growth rate is limited by diffusion and the growth is described as "diffusion controlled growth".

6. What is crystal size distributions?

Crystal size distribution extends the mass and energy balances basic. The extension is necessary because crystallization produces not a single homogeneous lump of solid.

7. What is population density?

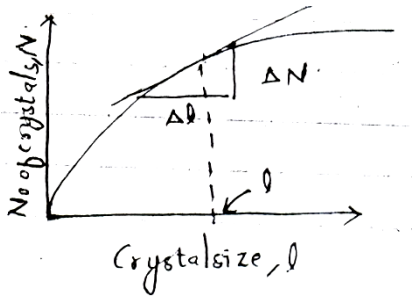


Figure:

A typical crystal size distribution is described by a population density. The abscissa in this plot is the characteristic size L. The ordinate N is the number of crystals per volume between size zero and l.

8. What is batch crystallization?

Batch crystallization is the most common method of polishing antibiotics and other bio molecules of moderate molecular weight.

9. What is Batch scale – up?

The scale – up of crystallization requires an estimate of the overall nucleation rate. This nucleation rate is affected by cooling, agitation and vessel geometry.

10. What is drying?

Drying biological materials can serve several purposes it can stabilize the bulk product until it con be formulated.

11. What is water content?

Water in most biological materials is present in two forms, free water and bound water free water is that held in spaces between cells or in capillaries of porous precipitates.

12. What is relative humidity?

$$\text{Relative humidity} = \frac{\text{Actual partial pressure of water}}{\text{Equilibrium vapor pressure of water}}$$

13. What is evaporation rate?

Evaporation rate per area j_i by means of the equation,

$$\boxed{j_i = k\left(C_{1i} - C_1\right)}$$

where C_{1i} and C_1 are water concentrations at the water – air interface and in the bulk air.

14. What is conduction dryers?

The simplest conductive dryer is a try. The tray is filled with wet solids and placed on an over shelf. Heat conducted from the shelf through the tray and into the solids evaporates the water to dry, the solids.

15. What is adiabatic dryers?

The second major type of dryer does not depend on conduction, but on a circulating gas. This gas enters warm and dry it evaporates liquid and so exits cold and wet.

16. Write the equation for constant rate drying.

$$\boxed{\frac{d}{dt}\left(\frac{\pi}{6}d^3\delta_s.w\right) = -\left(\pi d^2\right)j1}$$

17. What is physical extraction?

The compound gets itself distributed between two liquid phases based on the physical properties. This technique is used for extraction of non – ionizing compounds.

18. What is Dissociation extraction?

This technique is suitable for the extraction of lonisable compounds certain antibiotics can be extracted by thus procedure.

19. What is reactive extraction?

In this case, the desired product is made to react with a carrier molecule (e.g., phosphorous, compound, aliphatic amine) and extracted into organic solvent. Reactive extraction proud or is quite useful for the extraction of certain compounds that are highly soluble in water e.g., Organic acids.

20. What is membrane adsorbers?

They are micro or macro porous membranes with lon exchange groups and / or affinity ligands membrane adsorbcos can bind to proteins and retain them.

21. Define concentration polarization NOV/DEC 2010

Concentration polarization is the polarization component that is caused by concentration changes in the environment adjacent to the surface.

22. Define relative humidity. NOV/DEC 2012

The amount of water vapour present in air expressed as a percentage of the amount needed for saturation at the same temperature**.**

23. Write the industrially important dryers. NOV/DEC 2013

a. Tray and Pharmaceutical Freeze Dryers
b. Multibatch Freeze Dryers
c. Continuous Freeze Dryers
d. Tunnel Freeze Dryers
e. Vacuum-Spray Freeze Dryers

24. Write the stages of crystallization process. NOV/DEC 2012,2013

1. Super saturation of the solution : It can be done by three ways.
Heating the solution
Cooling the solution
Salting out

2.Nucleation : This takes place in several steps.
During their random motion, the atoms/ molecules/ ions will come closer to one another and forms aggregates called as CLUSTERS.
These clusters will combine to form an EMBRYO. In this stage only the lattice formation begins.
This embryo's combine to form NUCLEI.
From nuclei crystals are formed

3.Crystal Growth : Once the crystals are formed, nuclei formation stops and crystal growth begins.

25. How are nucleic acids and proteins stored in cold storage? NOV/DEC 2014

The storage medium is based on the natural principles of anhydrobiosis (meaning life without water) a biological mechanism is employed by some multi cellular organisms that enables their survival while dry for>100yrs.

26. Disadvantages of Lyophilization

- Many biological molecules are damaged by the stress associated with freezing, freeze-drying, or both.
- The product is prone to oxidation, due to high porosity and large surface area. Therefore the product should be packed in vacuum or using inert gas or in a container impervious to gases
- Cost may be an issue, depending on the product
- Long time process

27. Advantages of Lyophilization

- Removal of water at low temperature
- Thermolabile materials can be dried.
- Compatible with aseptic operations
- More precise fill weight control
- Sterility can be maintained.
- Reconstitution is easy

28. Explain the Characteristics of Freeze Dry Product

- Sufficient strength
- Uniform color
- Sufficiently dry
- Sufficiently porous
- Sterile
- Free of pyrogens and particulates
- Chemically stable both in dry state and reconstitution

29.Explain Freeze Drying

- Freezing the product solution to a temperature below its eutectic temperature.
- Decrease the shelf temperature to -50^{o}c.
- Low temperature and low atmospheric pressure are maintained.
- Freons are used as refrigerant.
- Formation of ice crystals occurs.
- The rate of ice crystallization define the freezing process and efficiency of primary drying.

30. What are the Steps involved in lyophilization

- Freezing stage

- Primary drying stage
- Secondary drying stage
- Packing

PART B

1. Explain in detail crystallization with suitable diagram. NOV/DEC 2013,2014,2015
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 255

2. Write notes on Lyophilization. NOV/DEC 2015
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 259

3. Discuss in detail the manufacturing process of drying. NOV/DEC 2010,2013,2015
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 259

4. What is formulation? How are final products formulated after lyophilisation? NOV/DEC 2010,2014
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 264

5. Explain the following in detail with suitable plots. NOV/DEC 2012
(iv) Adiabatic drying
(v) Constant rate drying and time required to reach a critical moisture content.
(vi) Falling rate period.
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 259

6. Write notes on the following. NOV/DEC 2012
(iv) Crystal size distributions
(v) Dominant crystal size
(vi) **Batch crystallization**
Refer: Sivasankar, B. "Bioseparations : Principles and Techniques" –Page no 255

PART C

1. (i) Explain in detail the design and working of a lyophilizer. (8) [TB2: 263-264]

(ii) What are the molecular alterations that lead to loss of a proteins biological activity? Explain the methods of improving protein stability. (8) [TB2: 258-259]

2. (i) Discuss the crystallization equipment used in biotechnology industry. (10) [TB2: 258-259]

(ii) Describe the classes of precipitants used in biomolecular crystallization. (6) [TB1: 373-375]

3. A commercial drier needed 7 hrs to dry a material from a moisture content of 33% to 9% on dry basis. The critical moisture content of the material was 16% and the equilibrium moisture content was 5%. Determine the time needed to dry this material from a moisture content of 37% to 7% if the drying condition remains unchanged. (16) (Nov./Dec., 2015) (notes)

Reg. No. : | | | | | | | | | | | | |

Question Paper Code : 50203

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2017
Seventh Semester
Biotechnology
BT 6702 – DOWNSTREAM PROCESSING
(Regulations 2013)

Time : Three Hours | Maximum : 100 Marks

Answer ALL questions

PART – A **(10×2=20 Marks)**

1. Name one cationic and anionic detergent used for cell disruption.
2. What is filter aid ?
3. Define buoyant force.
4. Define isopycnic sedimentation.
5. Define adsorption.
6. Give the Freundlich equation.
7. Define retention ratio.
8. Define Partition co-efficient.
9. What is lyophilisation ?
10. Define first falling-rate period.

PART – B **(5×16=80 Marks)**

11. a) What is cell disruption ? Explain in detail the various mechanical and chemical methods employed for cell disruption. **(16)**

(OR)

b) What are the various steps in downstream processing ? Explain them in detail with a neat schematic diagram. **(16)**

50203

12. a) Explain in detail :
    i) Plate and Frame filter press. (8)
    ii) Continuous rotary vacuum filter press. (8)

(OR)

b) i) A bowl centrifuge is used to concentrate a suspension of Escherichia coli prior to cell disruption. The bowl of this unit has an inside radius of 12.7 cm and a length of 73.0 cm. The speed of the bowl is 16,000 rpm and the volumetric capacity is 200 L/h. Under these conditions, this centrifuge works well. Calculate the settling velocity v_g for the cells. (12)

ii) A tubular bowl centrifuge is used to recover yeast cells from a fermentation broth. At a flow rate of 10 L/min and 5000 rpm, 50% of the cells can be recovered. You are now asked by your manager to increase the recovery to 90% using the same equipment. What flow rate should you use ? (4)

13. a) Explain in detail :
    i) Ultrafiltration. (6)
    ii) Reverse osmosis. (6)
    iii) Dialysis. (4)

(OR)

b) Explain in detail the different methods employed for protein precipitation. (16)

14. a) Explain in detail :
    i) Ion exchange chromatography. (8)
    ii) Affinity chromatography. (8)

(OR)

b) Explain in detail :
    i) Size exclusion chromatography. (8)
    ii) Reverse phase chromatography. (8)

15. a) Give short notes on the following :
    i) Saturation. (4)
    ii) Supersaturation. (4)
    iii) Nucleation. (4)
    iv) Single crystal growth. (4)

(OR)

b) Describe in detail the various dryers used in downstream processing of biomolecules. (16)

Reg. No.: | | | | | | | | | | | | |

QUESTION PAPER CODE : 71504

B.E./B.Tech DEGREE EXAMINATION, APRIL/MAY 2017.

Seventh Semester

Biotechnology

BT 6702 – DOWNSTREAM PROCESSING

(Regulation 2013)

Time : Three hours **Maximum : 100 marks**

Answer ALL questions.

PART A – (10 X 2 = 20 marks)

1. Write a short note on enzymes used in cell lysis.

2. What are the performance indicators of a typical bioseparation step?

3. What is Sedimentation coefficient?

4. Write a short note on the scale-up of centrifugation.

5. What is concentration polarization?

6. What are the basic characteristics of ion exchange resins?

7. Give an example of a column used for reversed-phase chromatography.

8. What are the terms in Van Decmtor equation?

9. Give two methods for minimizing proteolytic degradation.

10. Give two examples for stabilizers used in bioproduct formulation.

PART B – (5 X 16 = 80 marks)

11. a. (i) Describe the important physicochemical characteristics of biomolecules with respect to downstream processing. (8)

(ii) Discuss the use of ultrasound for cell disruption. (8)

Or

11. b. (i) Explain in detail the construction and working principles of a high pressure mechanical homogenizer. (8)

(ii) Discuss the design and working principle of a ball mill. (8)

12. a. (i) Describe the general theory of filtration. (8)

(ii) Explain detail the design and working of a tubular bowl centrifuge. (8)

Or

12. b. (i) Describe the design and working principles of disc stack centrifuges. (8)

(ii) Describe the design and working principle of rotary drum vacuum filtration. (8)

13. a. (i) Describe the various stages involved in precipitate formation (8)

(ii) Describe the various models describing the ultrafiltration process. (8)

Or

13. b. (i) What is adsorption isothern? Describe the types of adsorption isotherms. (8)

(ii) Discuss the principles of aqueous two-phase partitioning of proteins. Add a note on affinity partitioning. (8)

14. a. (i) Describe the principles of hydrophobic interaction chromatography. (8)

(ii) Discuss the chromatographic theory of retention. (8)

Or

14. b. (i) A pilot-scale gel-chromatography column packed with Sephacryl resin is used to separate two hormones A and B. The column is 5 cm in diameter and 0.3 m high; the void volume is 1.9×10^{-4} m^3. The water regain value of the gel is 3×10^{-3} kg m^{-3} dry Sephacryl; the density of wet gel is 1.25×10^3 kg m^{-3}. The partition coefficient for hormone A is 0.38; the partition coefficient for hormone B is 0.15. If the eluant flow rate is 0.71 h^{-1}, what is the retention time for each hormone? (10)

(ii) Describe the principles of reversed phase adsorption chromatography. (6)

15. a. (i) Discuss the crystallization equipment used in biotechnology industry. (10)

(ii) Describe the classes of precipitants used in biomolecular crystallization. (6)

Or

15. b. (i) Explain in detail the design and working of a lyophilizer. (8)

(ii) What are the various molecular alterations that usually result in loss of a protein's biological activity? Explain the methods of improving protein stability. (8)

Reg. No.: | | | | | | | | | | | | |

QUESTION PAPER CODE : 80178

B.E./B.Tech DEGREE EXAMINATION, NOVEMBER/DECEMBER 2016

Seventh Semester

Biotechnology

BT 6702 – DOWNSTREAM PROCESSING

(Regulation 2013)

Time : Three hours **Maximum : 100 marks**

Answer ALL questions.

PART A – (10 X 2 = 20 marks)

1. What is the mechanism of cell lysis mediated by ultrasound waves?

2. What is sedimentation coefficient?

3. Define equivalence time.

4. What are filter aids? Give examples.

5. Define selectivity parameter in ultrafiltration.

6. Write examples of ion exchange resins.

7. What is the importance of resolution in chromatography?

8. Write the van Deemter equation.

9. What are the inhibitors used for minimizing proteolytic degradation?

10. Give examples of stabilizers used in bioproduct formulation.

PART B – (5 X 16 = 80 marks)

11. a. (i) What is the criterian for selection of electrolyte for flocculation. (8)

(ii) Discuss the construction and working principles of a microfluidizer. (8)

Or

11. b. (i) The diffusivity of a protein having a Stokes-Einstein radius of 2 nanometers in a particular liquid is known to be 4.5 x 10^{-11} m^2s^{-1}. Predict the diffusivity of another protein having twice the Stokes-Einstein radius in the same liquid at the same temperature. (8)

(ii) Discuss the design and working principle of a ball mill. (8)

12. a. A 30-ml sample of broth from a rifamycin B fermentation is filtered in the laboratory on a 3 cm^2 filter at a pressure drop of 3.45 x 10^4 N/m^2. The filtration time is 4.5 min. Previous studies have shown that filter cake of *Streptomyces mediterranensis* is significantly compressible with s = 0.5. If 500 litres broth from a pilot-scale fermenter must be filtered in 1hour, what size is required if the pressure drop is:

(i) 6.86 x 10^{-4} N/m^2

(ii) 3.45 x 10^4 N/m^2

Resistance due to filter medium is negligible. (16)

Or

12. b. (i) Describe the design and working principles of disc stack centrifuges. (8)

(ii) Describe the design and working principle of rotary drum vacuum filtration. (8)

13. a. (i) Clarified fermentation broth contains a polysaccharide product with a gelation concentration of 25 g/l. The fluid density is 1020 kg m^{-3}, the viscosity is 1.8 cP, and the polysaccharide diffusivity is 5.63 x 10^{-11} m^2 s^{-1}. The product is harvested from the broth using ultrafiltration at a fluid velocity of 0.34 ms-1 in open membrane tubes of diameter 2.4 cm and length 2.0 m. Estimate the permeate flux if the filter is operated under gel polarization conditions and the polysaccharide concentration in the feed is 12 $g.l^{-1}$. (8) (Nov./Dec., 2016)

(ii) Describe the various models describing the ultrafiltration process.(8) (Nov./Dec., 2016)

Or

13. b. (i) An adsorptive membrane has a thickness of 2 mm and a diameter of 5 cm. The porosity of the membrane is 0.75 and the tortuosity is 1.5. The pore diameter was estimated to be 2 x 10^{-6} m. If we are to use this membrane for adsorption of a DNA fragment with diffusivity of 9.5 x 10^{-12} m^2 s^{-1} from an aqueous solution of viscosity 0.001 kg/m.s at 25°C, what is the maximum solution solution flow rate that can be used? Assume that the flow through the pores is laminar. (8)

(ii) Discuss the principles of aqueous two-phase partitioning of proteins. Add a note on affinity partitioning. (8)

14. a. (i) Describe the principles of hydrophobic interaction chromatography. (8)

(ii) Discuss the chromatographic theory of retention. (8)

Or

14. b. (i) A pilot-scale gel-chromatography column packed with Sephacryl resin is used to separate two hormones A and B. The column is 5 cm in diameter and 0.3 m high; the

void volume is 1.9 x 10^{-4} m^3. The water regain value of the gel is 3 x 10^{-3} kg m^{-3} dry Sephacryl; the density of wet gel is 1.25 x 103 kg m^{-3}. The partition coefficient for hormone A is 0.38; the partition coefficient for hormone B is 0.15. If the eluant flow rate is 0.71 h^{-1}, what is the retention time for each hormone? (10)

(ii) Describe the principles of reversed phase adsorption chromatography. (6)

15. a. (i) Discuss the crystallization equipment used in biotechnology industry. (10)

(ii) Describe the classes of precipitants used in biomolecular crystallization. (6)

Or

15. b. (i) Explain in detail the design and working of a lyophilizer. (8)

(ii) What are the molecular alterations that lead to loss of a proteins biological activity? Explain the methods of improving protein stability. (8)

Reg. No. : | | | | | | | | | | | | |

Question Paper Code : 21211

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2015.

Seventh Semester

Biotechnology

BT 2401/BT 71/10155 BT 702 — DOWNSTREAM PROCESSING

(Regulations 2008/2010)

Time : Three hours Maximum : 100 marks

Provide diagrams and equations where necessary.

Use of Calculators is permitted.

Graph sheets shall be provided.

Answer ALL questions.

PART A — (10 × 2 = 20 marks)

1. What is critical micelle concentration?
2. Give two examples each for intracellular and extracellular bioproducts.
3. What is coagulation and flocculation?
4. Differentiate between simple washing and through washing.
5. Draw the graphical representation showing the different types of adsorption isotherm.
6. How is reverse micellar extraction of a biopolymer carried out?
7. Classify chromatographic techniques based on its mechanisms of separation.
8. What is selectivity curve? Give its significance.
9. Give the difference between crystallization and precipitation.
10. Define secondary nucleation.

PART B — (5 × 16 = 80 marks)

11. (a) Explain the mechanical and non-mechanical methods of cell disruption with neat diagram. (16)

Or

(b) (i) Discuss the ranges and characteristics of bio products that are utilized in downstream processing. (8)

(ii) Discuss the characteristics of fermentation broth that influences the downstream processing. (8)

12. (a) The following data was obtained during a filtration process of a test filter.

Volume of filtrate, V (liters) :	0.040	0.055	0.080	0.095
Filtration time, t (sec) :	5	10	20	30

The filter leaf has a total area of 0.1 ft^2 and the filtrate has a viscosity of 1.1 cP. The pressure drop is 20 inch of mercury and the feed contains 0.015 kg dry cake per liter. Determine the specific cake resistance and filter medium resistance. (16)

Or

(b) (i) Explain the principle and operation of a plate and frame filter press and continuous rotary drum filter with neat diagram. (10)

(ii) Discuss the principle of basket centrifuge with neat diagram. (6)

13. (a) (i) If 1000 kg/hr of Nicotin (C) — H_2O (A) solution containing 1% of Nicotin is to be counter currently extracted with Kerosene (B) at 20°C to reduce the Nicotin content to 0.1%. Determine

(1) Minimum Kerosene rate

(2) Number of theoretical stages required if 1150 kg of solvent is used per hour. Assume pure solvent is used. The equilibrium data are as follows. (10)

Kg C/Kg A :	0.002	0.004	0.006	0.008	0.01
Kg C/Kg B :	0.0017	0.0036	0.0054	0.0073	0.0092

(ii) Discuss the principle of reverse osmosis. (6)

Or

(b) (i) Explain the theoretical principles and steps involved in the aqueous two-phase extraction of an enzyme. (10)

(ii) Discuss the protein precipitation by addition of salts. (6)

21211

14. (a) Explain the principle, operation and applications of Size exclusion chromatography and Affinity chromatography. (16)

Or

(b) Describe the principle, practice and applications of Reverse phase chromatography and Ion exchange chromatography. (16)

15. (a) A commercial drier needed 7 hrs to dry a material from a moisture content of 33% to 9% on dry basis. The critical moisture content of the material was 16% and the equilibrium moisture content was 5%. Determine the time needed to dry this material from a moisture content of 37% to 7% if the drying conditions remains unchanged. (16)

Or

(b) (i) Explain the principle and operation of any two types of equipments for crystallization of proteins with neat diagram. (10)

(ii) Discuss the drying rate curve and bring out its salient features. (6)

Reg. No. : | | | | | | | | | | | | |

Question Paper Code : 91174

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2014.

Seventh Semester

Biotechnology

BT 2401/BT 71/10155 BT 702 — DOWNSTREAM PROCESSING

(Regulation 2008/2010)

Time : Three hours Maximum : 100 marks

Answer ALL questions.

PART A — (10 × 2 = 20 marks)

1. Write the principles of sonication for disruption of bacterial cells.
2. How does lysozyme act on bacterial cells?
3. How does negative pressure facilitates filtration?
4. What is isopycnic centrifugation?
5. How does ammonium sulphate helps in precipitation of proteins?
6. What is adsorption?
7. Write the principle of paper chromatography.
8. What is the purpose of using sephadex G50?
9. What is meant by freeze drying?
10. How are nucleic acids and proteins stored in cold storage?

PART B — (5 × 16 = 80 marks)

11. (a) Write the principles of downstream processing and briefly describe the characteristics of any ONE biomolecule.

Or

(b) Write the principles and applications of cell disruption. Also describe the methods of stabilization of byproducts.

12. (a) What are principles and applications of different methods of centrifugation? How are mitochondria separated using density gradient centrifugation?

Or

(b) Filtration is elegant method for separation of thermolabile biomolecules and removal of microbes. Discuss with suitable examples.

13. (a) Write in detail different methods of precipitation of proteins with the underlying principles.

Or

(b) Write short note on:

(i) Ultrafiltraction (8)

(ii) Aqueous two phase extraction. (8)

14. (a) Write about the following:

(i) Adsorption chromatography (8)

(ii) Ion exchange chromatography. (8)

Or

(b) Describe affinity chromatography. Where and how it is used?

15. (a) Write the principles and applications of crystallization.

Or

(b) What is formulation? How are final products formulated after lyophilisation?

Reg. No. : | | | | | | | | | | | | |

Question Paper Code : 31172

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2013.

Seventh Semester

Biotechnology

BT 2401/ BT 71/ 10155 BT 702 — DOWNSTREAM PROCESSING

(Regulation 2008/2010)

Time : Three hours Maximum : 100 marks

Answer ALL questions.

PART A — (10 × 2 = 20 marks)

1. What are the different steps in downstream processing?
2. What are the physio-mechanical methods of Cell disruption?
3. What are filter aids? Give some examples?
4. Which type of centrifuge is used to separate starch from gluten and cream from milk?
5. Define partition coefficient(K) in extraction.
6. Define the terms 'Salting in ' & 'Salting out' of proteins
7. Define the terms 'Retention time' & 'Retention volume' in chromatography
8. Define the terms 'available capacity' & 'total ionic capacity' of ion exchangers?
9. Write the industrially important dryers.
10. Write the stages of crystallization process.

PART B — (5 × 16 = 80 marks)

11. (a) Draw the generalized block diagram of downstream processing of bioproducts and explain briefly the unit operations involved in primary, intermediate and final purification stages. (16)

Or

(b) Give a detailed note on the Non-mechanical methods of cell disruption. (16)

12. (a) With neat diagram, explain the working principle of rotary vacuum filters. (16)

Or

(b) Explain with a neat diagram, the principle and working of the Tubular Bowl Centrifuge. (16)

13. (a) Write notes on:

(i) Aqueous two-phase extractions. (8)

(ii) Reverse osmosis. (8)

Or

(b) (i) Discuss the theoretical models for membrane separation processes. (8)

(ii) Discuss the various factors that affect the membrane separation processes. (8)

14. (a) Explain the basic principles and applications of:

(i) Affinity chromatography (8)

(ii) Reverse phase chromatography. (8)

Or

(b) Explain the principle involved in gel permeation chromatography applicable to bio molecules and write a note on applications of this technique. (16)

15. (a) With suitable diagram explain the principle, theory and application of crystallization. (16)

Or

(b) With suitable diagram explain the drying process. (16)

Reg. No. : 3 0 6 0 9 2 1 4 0 0 8

Question Paper Code : 11159

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2012.

Seventh Semester

Biotechnology

BT 2401/BT 71 – DOWNSTREAM PROCESSING

(Regulation 2008)

Time : Three hours Maximum : 100 marks

Answer ALL questions.

PART A — (10 × 2 = 20 marks)

1. List out the four familiar steps in most bio-separations and explain.
2. Explain about lipid dissolution.
3. With a plot explain the effect on filtrate volume of pH and filter aid.
4. Identify four basic types of centrifuge and explain.
5. Distinguish between equilibrium line and operating line in batch adsorption with a suitable plot.
6. Explain the chemistry of extraction.
7. Differentiate between yield and purity.
8. Give the solute mass balance in symbolic terms for discrete stage analysis of chromatography.
9. The third basic facet of crystallization is nucleation - explain.
10. Define relative humidity.

PART B — (5 × 16 = 80 marks)

11. (a) (i) Explain the characteristics of bio-separations in detail. (8)

(ii) Explain the characteristics of bio-molecules in detail. (8)

Or

(b) Explain the cell disruption for product release by chemical methods with suitable examples. (16)

12. (a) With neat sketches explain the equipments for conventional filtration. (16)

Or

(b) (i) Determine the position of a particular particle as a function of time and volumetric flow rate of feed in a disc type centrifuge with the diagram. (8)

(ii) Explain about the settling of solids in centrifugation. (8)

13. (a) Explain the following membrane separations with suitable diagrams.

(i) Ultrafiltration (8)

(ii) Reverse Osmosis. (8)

Or

(b) (i) Derive the equation for calculating the length of a differential extractor. (8)

(ii) Explain in detail about fractional extractions with a stationary phase. (8)

14. (a) (i) Discuss the solid phases used for elution chromatography of different scales and types. (8)

(ii) Describe the scaling up chromatography and scaling the separation. (8)

Or

(b) Explain the following product purification using chromatography.

(i) Adsorption (4)

(ii) Ion-exchange (4)

(iii) Size exclusion (4)

(iv) Hydrophobic interaction. (4)

15. (a) Explain the following in detail with suitable plots.

(i) Adiabatic drying (4)

(ii) Constant rate drying and time required to reach a critical moisture content. (8)

(iii) Falling rate period. (4)

Or

(b) Write notes on the following.

(i) Crystal size distributions (6)

(ii) Dominant crystal size (5)

(iii) Batch crystallization. (5)

Reg. No. : | | | | | | | | | | | |

Question Paper Code : 42433

B.E./B.Tech. DEGREE EXAMINATION, NOVEMBER/DECEMBER 2010.

Seventh Semester

Biotechnology

BT 1401 — DOWNSTREAM PROCESSING

(Regulation 2004)

Time : Three hours Maximum : 100 marks

Answer ALL questions.

PART A — (10 × 2 = 20 marks)

1. List the factors influencing the performance of bead mills.

2. Distinguish three market sectors based on market volume and selling price with suitable examples.

3. Why pretreatment is needed for the fermentation broth during the filtration process?

4. Interpret Hofmeister series and Cohn equation.

5. What is break point time? How it can be used to design the adsorption unit?

6. Explain the use of small support particles in improving chromatography resolution based on Van Deemter equation.

7. What is (a) Bonded stationary phase (b) pre-column derivatisation?

8. Imagine a protein molecule of charge (+6) and diffusion coefficient 0.71×10^{-6} cm^2/sec in excess small electrolyte at 25°C. If this protein is placed under an electric field of 0.13 V/cm, how fast will it move?

9. Define concentation polarization.

10. Mention the process variables and its effects on the drying rate.

PART B — (5 × 16 = 80 marks)

11. (a) (i) Give an account of the action of enzymes and chemicals in cell disruption. (8)

(ii) Outline the downstream processing steps in lactic acid production. (8)

Or

(b) (i) Compare and contrast the mechanical and enzymatic disruption. (8)

(ii) What is the effect of viscosity, cell morphology and size in the downstream processing efficiency? (8)

12. (a) You are filtering a beer containing 6% citric acid on a continuous rotary vacuum filter. The filter has an area of 18.1 m^2, a negligible medium resistance, a cycle time of 75 sec and a pressure difference of close to 1 atm. The cake which forms has a washing efficiency of only 60%, but it is incompressible and permeable :

$\rho\alpha\mu / 2\Delta p = 86 \text{ sec/cm}^2$

The cake retains 7% of filtrate leaving and should be washed until the cake contains only 10% citric acid originally entrained. Calculate the filtration time and washing time required to process 3000 litres /hr of beer. (16)

Or

(b) (i) Classify the different types of centrifuges. Explain the working principle of disc nozzle centrifuge with a neat sketch. (8)

(ii) A tubular bowl centrifuge is used to recover yeast cells from a fermentation broth. At a flow rate of 10 *l* /min and 5000 r/min, 50% of the cells can be recovered. You are now asked by your manager to increase the recovery to 90% using the same equipment.

What flow rate should you use?

If you double the rotation speed, how much does the centrifugation velocity change? (8)

13. (a) (i) A broth of 100 litres contain the desired protein at 14.8 g/l as well as a contaminant protein at 1.8 g/l. Calculate the ammonium sulphate concentration required to recover 98% of the desired protein if the precipitation constants and k of the desired protein are 9.33 and 1.1 respectively and that of the contaminant protein are 8.8 and 0.95 respectively. What will be the purity of the desired protein at 99% recovery? (8)

(ii) Outline the mechanism of reverse osmosis and its applications. (8)

Or

(b) (i) What are aqueous biphasic systems? Explain the steps involved in the aqueous two-phase extraction of an enzyme. (8)

(ii) Zymase isolated from yeast can be adsorbed on activated carbon. The adsorption can be assumed to follow a Langmuir isotherm. The maximum uptake is 60 mg/cm^3 : half of this maximum occurs when the solution contains 50 mg/liter of the enzyme. We have to recover atleast 95% of this enzyme from 2.5 liters of broth containing 200 mg/liter enzyme. How much activated carbon will be required? (8)

14. (a) (i) How is the molecular weight of a protein determined by gel filtration? (8)

(ii) Fumarate and Diacetyl fumarate were found to have a retention time of 10.42 and 10.96 minutes respectively on a 25 cm column. An unretained species passed through the column in 1.2 min. The peak base width was 0.78 and 0.83 minutes respectively. From this data calculate, (1) Column resolution (2) Plate height (3) Length of column required to achieve a resolution of 1.5 (4) Plate height required for a resolution of 1.5 on the original 25 cm column in the original time. (8)

Or

(b) (i) Compare the gradient elution and displacement chromatography. (8)

(ii) Describe the affinity chromatographic techniques. (8)

15. (a) (i) Explain, using suitable examples, the importance of formulation especially relating to safety ,efficacy and shelf life. (8)

(ii) What is meant by Homogeneous and Heterogeneous nucleation in crystallization? (8)

Or

(b) (i) With a neat sketch, explain the working principle of Spray dryer and Tray dryer. Give any two applications for each. (8)

(ii) Explain the theoretical considerations in the drying process. How can we predict the drying? (8)