Total No. of Questions :11]

SEAT No. :

P1128

[Total No. of Pages :4

[4659] - 353
B.E. (Biotechnology)
BIOSEPARATION -II
(2008 Pattern) (Semester-I)

Time : 3 Hours] *[Max. Marks : 100*

Instructions to the candidates:

1) ***Answers to the two sections should be written in separate answer books.***
2) ***Answer any three questions from each section.***
3) ***Neat diagrams must be drawn wherever necessary.***
4) ***Figures to the right side indicate full marks.***
5) ***Use of calculator is allowed.***
6) ***Assume suitable data if necessary.***

SECTION-I

Q1) a) Explain the "Three Phase Strategy" for the application of downstream processing techniques. **[6]**

b) How will you design strategy for purification of any biological product? What are the basic requirements to design strategy? **[10]**

OR

Q2) Following is the Purification profile of α -amylase produced by *Aspergillus flavous*; using information provided in the table find out: **[16]**

a) Purity b) Specific Activity c) %Yield d) Fold Purification

Purification Step	Sample Volume (ml)	Enzyme Activity (units/ml)	Protein Content (mg/ml)	Total Activity (units/ml)	Total Protein (mg)
Cell free filtrate	200	266.07	0.41	53214	82
Ammonium Sulphate Fractionation (60% saturation)	200	660.69	0.18	132138	36
Dialysis against sucrose crystals	15	2213.09	0.55	33196.35	8.25
Sephadex G-200 chromatography	5	3235.8	0.5	16179	2.5

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Q3) A polypeptide has the sequence: **[16]**

Leu-Leu-Trp-Tyr-Ser-Glu

a) Using the data in the table below and assuming a molar absorptivity for Trp and Tyr of 5000 and 1500L/cm.mol, respectively, estimate the extinction coefficient, □ (units: cm^2/mg) for this peptide at a wavelength, □ of 280 nm.

Amino Acid	MW
Leu	131.10
Trp	204.23
Tyr	181.20
Ser	105.04
Glu	147.14

b) A solution of this peptide is placed in a 1cm thick cuvette and its absorbance is found to be 1.3 at 280nm. What is the concentration of the polypeptide in mg/ml?

OR

Q4) What is Spectrofluorometry? Write short note on fluorescence and phosphorescence. Explain instrumentation of Spectrofluorometer along with its application. **[16]**

Q5) a) Write short note on "Linking Chromatography Techniques into a Purification Protocol - General Rules". **[4]**

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b) **[14]**

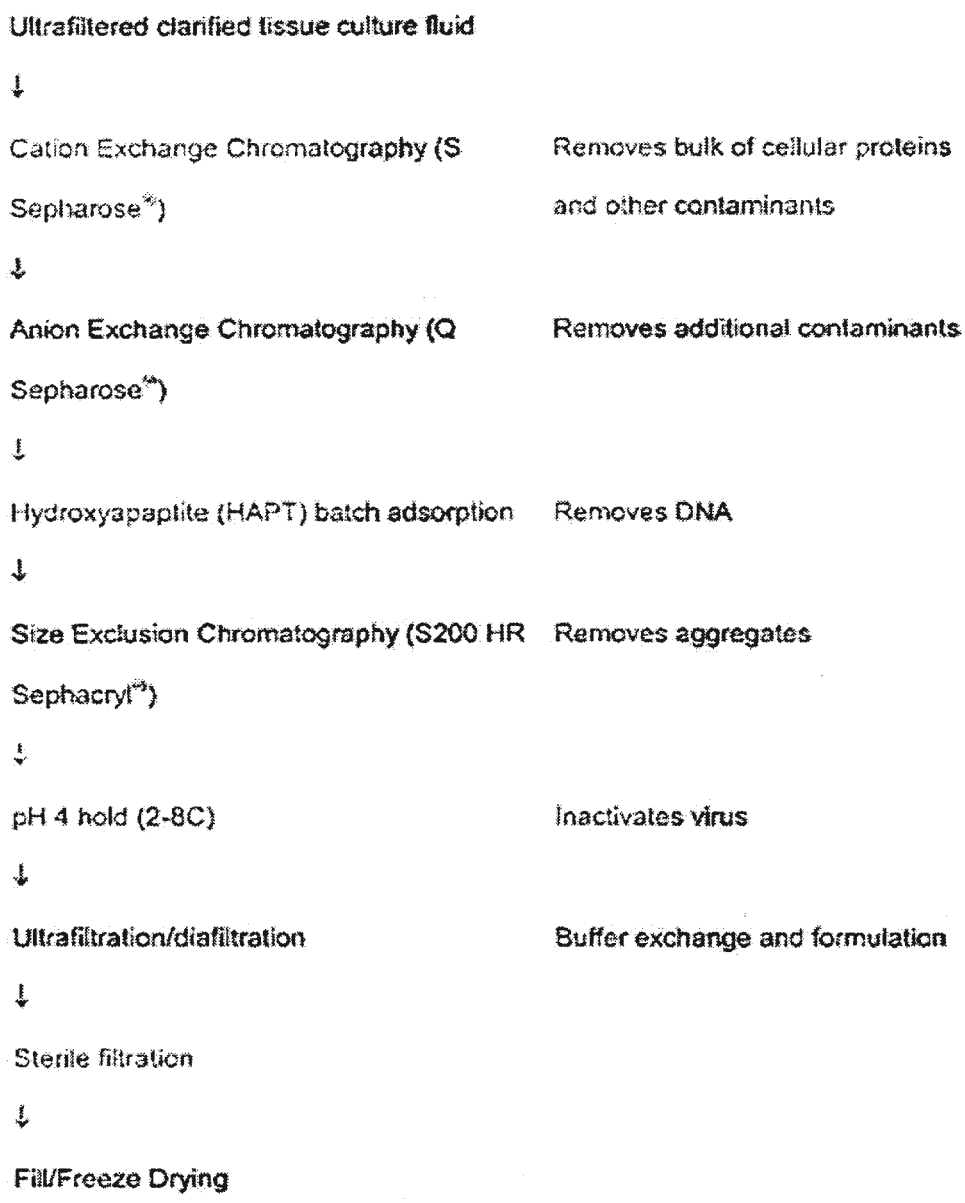

Figure 1 TNF MAb purification flow chart.

Study the provided flow sheet and enlist the bioseparation techniques mentioned in it. Explain any one chromatograpy technique from mentioned in above flow sheet. Design new strategy for purification of the same molecule with proper reasoning.

OR

Q6) Write short note on: **[18]**

a) Reversed Phase HPLC

b) Affinity Chromatography

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SECTION-II

Q7) a) Explain chromatographic process in liquid chromatography with 'Differential Migration' and 'Molecular Spreading'. **[8]**

b) Explain the following in relation with HPLC. **[8]**

i) Sample Reservior

ii) Solvent Pumping System

iii) Sample Injectors

iv) Guard Column

OR

Q8) a) Draw schematic diagram of Gas Chromatography system and explain how chromatography machine works. **[8]**

b) Write requirements of ideal detectors for Gas chromatography. Describe four detectors of Gas chromatography system. **[8]**

Q9) Write principle of Mass spectrometry. How does Mass spectrometer works explain, with different types of MS. **[16]**

OR

Q10) Write short note on: **[16]**

a) Super critical fluid extraction

b) MALDI-TOF

Q11) Explain any two case studies for the application of Downstream Processing from following. Write at least two different protocols for the separation of given biomolecules and discuss the processes in detail. (Any 2, 9M Each) **[18]**

a) Peptide Antibiotics

b) Beer Production

c) Commodity Acids

d) Xanthan or Dextran

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