

**BIRLA INSTITUTE OF TECHNOLOGY, MESRA, RANCHI
(MID SEMESTER EXAMINATION)**

**CLASS: BE
BRANCH: BIOTECH**

**SEMESTER: VI
SESSION : SP/2020**

SUBJECT : BT6021 BIOSEPARATION ENGINEERING

TIME: 1.5 HOURS

FULL MARKS: 25

INSTRUCTIONS:

1. The total marks of the questions are 30.
2. Candidates may attempt for all 30 marks.
3. In those cases where the marks obtained exceed 25 marks, the excess will be ignored.
4. Before attempting the question paper, be sure that you have got the correct question paper.
5. The missing data, if any, may be assumed suitably.

- Q1 (a) Prove mathematically that peak resolution increases with column heights in column chromatography. [2]
 (b) Two proteins of MW 2.5×10^5 and 1×10^4 were eluted out of a gel in gel filtration column at 220 mL and 350 mL respectively. Determine the molecular weight of a protein that elutes out at 270 mL under the same condition. [3]
- Q2 (a) Two proteins of MW 330 kDa and 900 kDa are separated using a gel filtration column. Analyse which protein will elute first and why? [2]
 (b) Write the name of the different components of a HPLC system. [3]
- Q3 (a) Define Capacity factor in a column chromatography. [2]
 (b) A protein is to be purified using ion-exchange column chromatography. The relationship between HETP (Height Equivalent to Theoretical Plate) and the linear liquid velocity of mobile phase is given by $H = \frac{A}{u} + Bu + C$; where, H is HETP (m) and u is linear liquid velocity of mobile phase (m/s). Values of A, B and C are $3 \times 10^{-8} \text{ m}^2/\text{s}$, 3 s and $6 \times 10^{-5} \text{ m}$, respectively. What will be the number of theoretical plates based on minimum HETP for a column of 66 cm length? [3]
- Q4 100 L solution contains 10 g/L BSA and a contaminant of 5 g/L. Calculate the salt required to recover 90% of BSA if the value of B and k for BSA are 21.6 and 7.65 and that of contaminant are 20 and 7 respectively. What will be the purity of the lipase at 90% recovery? [2+3]
- Q5 (a) Write the name of one cation exchanger and one anion exchanger. [2]
 (b) Justify the use of ammonium sulphate for salt precipitation. [3]
- Q6 (a) In aqueous two phase extraction with PEG-Dextran system, which form the upper layer? [2]
 (b) Calculate specific activity, purification fold and percentage recovery from the given data: [3]

Steps	Total protein (mg)	Total activity (unit)	Specific activity	Purification fold
Homogenate	2936	27028		
Sediments	1041	22846		