

29. It is customary to prepare hemoglobin by the osmotic lysis of red cells. What is the van't Hoff pressure drop across the membrane of a red blood cell that is isotonic (0.30 osM) on the inside and submerged in a 0.01% NaCl solution? [K₆]
30. Human insulin produced by recombinant technology involves fermentation, isolation, purification, refolding and further purification. Develop the conceptual process flow sheet and explain the logic behind the sequence of steps. [K₄]

PART D (2 x 10 = 20 Marks)

31. You are asked to isolate 76 L of alpha-galactosidase enzyme. Initially, it is present in 13.46 g/L along with other contaminated protein of 2.3 g/L. [K₅]
 What will be the purity of the desired protein at 97.5% recovery?
 Calculate the ammonium sulphate concentration required to precipitate 97% of the desired protein using the data given below:

	β	K_s
Alpha-galactosidase	9.33	1.1
Contaminated protein	8.8	0.95

32. i. The data in table were collected for purification of GST-purification. Complete the purification table for this enzyme (5) [K₆]

Purification	Sample volume (mL)	Concentration of protein (mg/mL)	Total protein	GST activity	Total activity	Specific activity
Cell extract	10	1.53		0.12		
Run through	10	0.96		0.12		
Wash	10	0.14		0.04		
GST eluant	4	1.1		0.18		

- ii. One of the reactive dyes is capable of reacting polyhydroxyl matrices and interacts with various proteins. This dye is analogue to the structure of NAD. XRD diffraction data shows that dye binds with liver alcohol dehydrogenase in an NAD site with correspondence of the adenine and ribose rings not to the nicotinamide. Based on the information, develop the chromatography technique to purify the given protein. (5)
