SUBJECT: Bioseparation Engineering
COURSE # BT 360

7 May 2023
FULL MARKS: 45

END-SEMESTER EXAMINATION

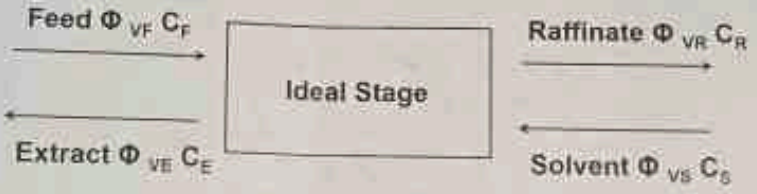
Answer all questions

SECTION-A

For this section, the answers should be comprehensive and with proper justification

1. Consider that **C** is a dissolved species and **S** is the adsorption site for **C** in a resin. (a) Derive the expression, which describes a Langmuir adsorption isotherm for **C**. Explain all the terms used for the derivation. (b) Briefly explain a major advantage associated with a Langmuir adsorption isotherm. **(2.0 + 2.0 = 4.0)**

2. Consider a theoretical ideal single-stage extraction process operated in continuous mode in which after a defined time period, both phases are in thermodynamic equilibrium as shown in the following figure.



Φ_{VF}, Φ_{VR}, Φ_{VS} and Φ_{VE} are the volumetric flow rates of feed, raffinate, solvent and extract stream, respectively. C_F, C_R, C_S and C_E are the concentrations of the solute in feed, raffinate, solvent and extract stream, respectively. Considering ***S*** as the separation factor and ***f*** as the extracted fraction, derive the following:

$$f = \frac{S}{S+1}$$

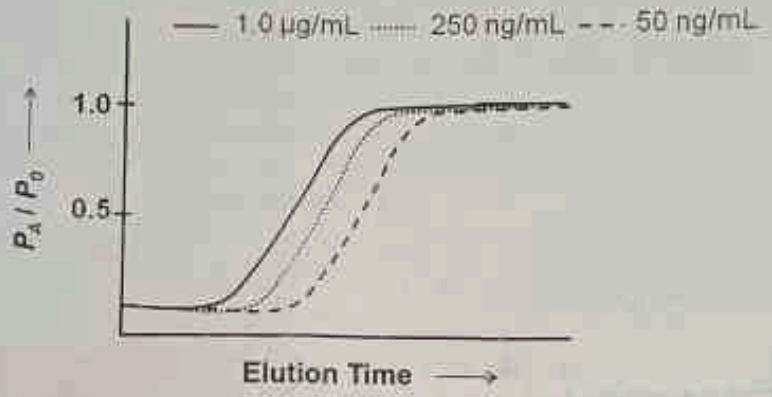
(2.0)

3. You are aiming to purify a lipase enzyme produced in a bioreactor by a natural isolate of *Bacillus* sp. by aqueous two-phase separation (ATPS) using 12% (w/v) polyethylene glycol (PEG) and 15% phosphate buffer (pH 7.0). You decided to use various molecular weights of PEG in ATPS and assessed the purification fold (**PF**) of the enzyme in the bottom phase and partition coefficient (K_e) of the enzyme as indicated in the table shown below. (a) Describe briefly how you had estimated the purification fold (**PF**) of the enzyme in the bottom phase? (b) Provide a brief explanation for the obtained data with justification. **(1.0 + 2.0 = 3.0)**

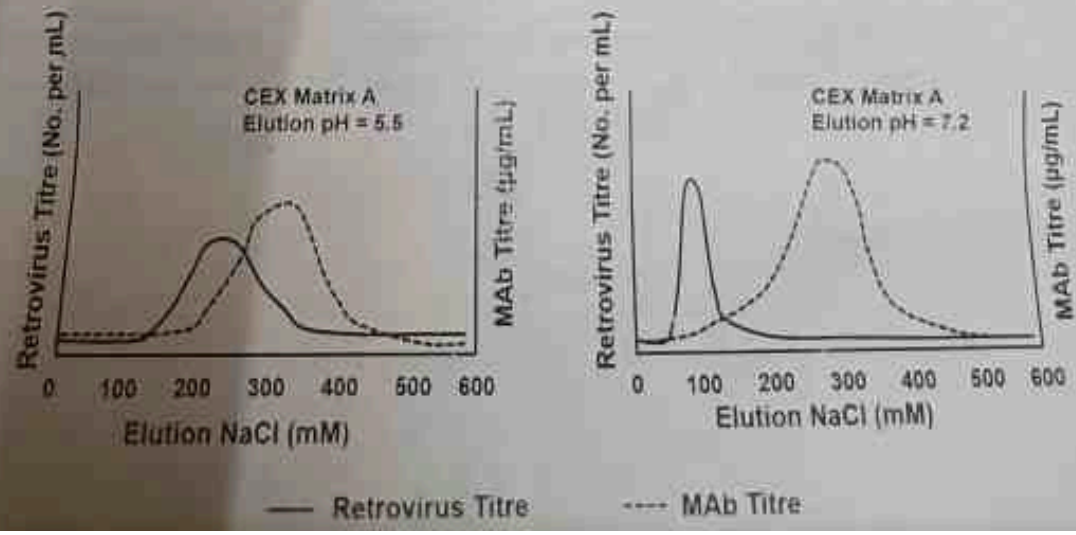
PEG Concentration (%)	Enzyme Purification Fold (PF) in Bottom Phase	Partition Coefficient (K_e)
400	79.2	0.42
1000	85.7	0.37
4000	126.6	0.211
6000	143.4	0.063
8000	197.2	0.052

4. (a) Briefly explain any two physical basis of band broadening commonly encountered in liquid chromatography? (b) State the expression for van Deemter equation and explain the terms. From a van Deemter plot, explain how flow rate effects the height equivalent to theoretical plate (H) in a typical liquid chromatography? **(1.0 + 1.0 + 1.0 = 3.0)**

5. In order to optimize the purification of a recombinant therapeutic protein ***P*** produced in *E. coli*, you subject the protein to adsorption, wherein sufficient sample (a solution of ***P***) is loaded onto the adsorption column to ensure that the elution profile contains a plateau region, in which the protein concentration in the eluted fraction (P_A) equals that in the applied sample (P_0). The concentrations of ***P*** used for loading were 1.0 µg/mL, 250 ng/mL and 50 ng/mL in three separate runs. The advancing elution profile of ***P*** following adsorption (P_A / P_0 versus elution time) is indicated in the figure below. (a) Provide an explanation for the observed profile. (b) From the elution profile, how will you estimate of the amount of non-adsorbed ***P***? **(2.0 + 2.0 = 4.0)**



6. In a bioprocess unit, you are optimizing the separation of retrovirus contamination in a sample of CHO cell (Chinese hamster ovary cell) derived therapeutic monoclonal antibody (MAb). In a pilot experiment aimed at optimizing the separation process, you prepare a MAb sample from CHO cells and spike the same with 3% retrovirus sample. Subsequently, you load equal levels of the virus-spiked MAb samples onto two separate cation exchange (CEX Matrix A) columns. The loading buffers used in the two columns were 50 mM sodium acetate buffer (pH 5.5) and 50 mM sodium phosphate buffer (pH 7.2). You accomplished elution in both the columns using the respective loading buffers in presence of a linear gradient of 0-600 mM NaCl. Following elution, you estimated the retrovirus and MAb titre over elution volume to obtain the chromatogram shown below. Provide a brief explanation for the obtained data and suggest with justification, which of the two elution profiles you would prefer for separation of retrovirus from MAb preparation? **(2.0 + 1.0 = 3.0)**



7. You are involved in the bioseparation of a recombinant therapeutic protein **TP** expressed in an engineered strain of *E. coli*. Your advisor asks you to separate **TP** from contaminating cellular proteins **X1** and **X2** by using an ion exchange column. The isoelectric points of **TP**, **X1** and **X2** are 6.0, 9.7 and 3.2, respectively. After adding the protein mixture (**TP**, **X1** and **X2**) to an ion-exchange column at neutral pH, you add increasing amounts of NaCl, which shields and weakens the ionic interactions. What order will the proteins elute from the column? Provide an explanation for your answer. You may choose to use either a cation exchange or an anion exchange column. ***(3.0)***

8. In a vaccine development project, you are trying to optimize a chromatographic procedure for purification of virus-like particles (VLPs) produced in CHO cells. Following growth of CHO cells and production of VLPs, you subject the clarified and filtered cell culture supernatant to anion exchange chromatography. The loading Buffer A was 20 mM HEPES, 50 mM NaCl, pH 7.2. The elution buffer was Buffer A having 2.0 M NaCl, pH 7.2 with a linear gradient of 0–50% NaCl in 50 bed volumes. The distribution of VLPs and the amount of contaminating double stranded DNA (dsDNA) in various samples is shown in the table below:

Sample	Distribution of VLPs (%)	Contaminating dsDNA (ng/mL)
Load	100	10472
Flow-through	0.2	86
Wash	0.5	Nil
Eluted Fraction 1	35.1	15327
Eluted Fraction 2	64	807

(a) Provide a brief explanation for the data shown in the table. (b) Suggest a viable method with justification, for reduction of the amount of dsDNA in Eluted Fraction 1.
(2.0 + 1.0 = 3.0)

SECTION-B

In this section, all the assumptions, hypothesis or steps required for derivations or solution of the numerical problems and the final answer along with the required units should be clearly indicated. Incomplete steps/answers or answers shown as fraction will not be considered. PART MARKING IS NOT APPLICABLE FOR THESE QUESTIONS.

1. *Following gel filtration, a chromatogram of a mixture containing the proteins A, B and C was obtained. Protein C was not retarded on the column. The column was cylindrical having a length of 40 cm and an internal diameter of 1.2 cm. The flow rate of the sample was 15 mL/min. Calculate (i) Capacity factor **k'** for A, (ii) the mobile phase volume (V_m) and the stationary phase volume (V_s). The total volume of the column is $V_m + V_s$. (iii) Partition coefficient **k** for A. The retention time for proteins A, B and C are 9.5, 12 and 0.7 min, respectively.* **(1.0 + 1.0 + 1.0 + 1.0 = 4.0)**

2. Consider that an HPLC column is used to separate two proteins 1 and 2 in solution. Derive the following expression for resolution (***R***):

$$R = \frac{1}{4}\sqrt{N_2}\left[\frac{k_2(\delta - 1)}{\delta\,(k_2 + 1)}\right]$$

N_2 is the number of theoretical plates obtained for separation of protein 2. k_2 is the capacity factor of the column for protein 2. δ is the ratio of the capacity factors for protein 1 and protein 2 ($\delta = k_2 / k_1$). Assume that the width of the peaks obtained during separation of protein 1 and protein 2 are equal ($W_1 = W_2$). **(2.0)**

3. In a bioprocess plant, you are trying to recover leucine dehydrogenase enzyme from a homogenate of disrupted *Bacillus cereus* cells using an aqueous two-phase polyethylene glycol (PEG)-salt system. The starting sample consisted of 200 litres of homogenate containing 4.0 Units of enzyme per mL. Following addition of a PEG-salt mixture to the homogenate, two phases were formed. The partition coefficient of the enzyme was 3.0. (a) What volume ratios of upper and lower phases must be chosen to achieve 90% extraction of the enzyme in a single extraction step? (b) Following extraction, if the volume of the lower phase is 100 litres, what is the concentration factor for the same 90% recovery? **(2.0 + 2.0 = 4.0)**

4. In a packed-bed adsorption column, dilute solutions of phosphoglycerate kinase enzyme were processed. Following adsorption and attainment of equilibrium, a linear adsorption isotherm was observed. The relationship for the linear adsorption isotherm was: **q (mg/mL) = 32y (mg/mL)**, where q is the amount of adsorbed enzyme per unit volume of the adsorbent. At equilibrium, the non-adsorbed enzyme concentration was 0.80 mg/litre. If 2.0 litre of a feed sample contains a dilute solution of the enzyme at a concentration of 20 mg/mL, how much adsorbent **(in mL)** would be required to achieve 80% recovery of the enzyme? **(3.0)**

5. You are trying to extract an antibiotic produced in a bioreactor by using butyl acetate in a pilot-scale reciprocating-plate extraction column. The antibiotic has a partition coefficient (K) of 3.0. The optimal operating conditions for the pilot-scale column are: (i) solvent flow rate is 100 ml/min, (ii) broth flow rate is 50 ml/min, (iii) ratio of antibiotic in raffinate to feed is 0.010. The diameter of the pilot-scale column was 5.0 cm, the height of the extractor (height of the reciprocating plates) was 6.0 m, the number of theoretical stages was 6.0 and the agitator speed was 300 strokes/min. What should be (a) the diameter **(in cm)** and (b) height equivalent to theoretical stage (HETS) **(in m)** for an industrial-scale extractor in order to achieve a ratio of antibiotic in raffinate to feed of 0.05 and to handle 100,000 litres of feed broth in 12 h? **(2.0 + 2.0 = 4.0)**

6. An anion exchange column 14 cm long with internal diameter 1.5 cm is used for the separation of a therapeutic protein expressed in a recombinant strain of *E. coli*. The column produces 2.4 g of the purified protein/cycle and each cycle takes 6 h from equilibration to regeneration. In a scale-up operation, using a new column of 10 cm diameter, it is desired to achieve a throughput of 50 g protein per hour. What should be the length of the new column **(in cm)** ? **(3.0)**

********** **END OF QUESTION PAPER** **********