UNIVERSITI SAINS MALAYSIA

Second Semester Examination
2010/2011 Academic Session

April/May 2011

EKC 574 – Downstream Processing Of Biochemical and Pharmaceutical Products

Duration : 3 hours

Please ensure that this examination paper contains FIVE printed pages and TWO printed page of Appendix before you begin the examination.

Instruction: Answer **ALL** questions.

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1. [a] Based on the information below, suggest and briefly explain the most likely unit operations that should be used for the isolation and purification of Linomycin:

- *Antibiotic produced by Streptomyces lincolnesis;*
- *Free base,*
- *pKa' 7.6.*
- *More stable in salt form.*
- *Soluble in methanol, ethyl acetate, acetone, chloroform.*
- *Slightly soluble in water.*

[6 marks]

[b] Ultrafiltration process to concentrate protease is to be carried out under continuous operation using modules that consists of tubes of 0.075 cm in diameter and 50 cm long. Knowing that protease has a diffusion coefficient of 8.8 x 10^{-7} cm^2/s. The solution has a viscosity of 0.012 g /cm.s and a density of 1.1 g /cm^3. The bulk stream velocity is 300 cm/s. Based on these information,

[i] the thickness of concentration polarization layer

[ii] the polarization modulus of the fluid for membrane flux of 50 l/(m^2.hr).

[iii] the intrinsic rejection coefficient of the protease for the same membrane flux if the observed rejection coefficient is 95%.

[iv] suggest a method to improve the intrinsic rejection coefficient, justify your suggestion.

[19 marks]

2. [a] Fouling is a major obstacle for the wide spread use of membrane technology. Describe the reversible and irreversible fouling based on the attachment strength of particles to the membrane surface. How membrane fouling can be controlled?

[7 marks]

[b] In a laboratory centrifuge, it takes a minimum of 17 min to clarify the cell lysate at 12000 rpm. After 17 min, the top of the culture being centrifuged was 32 mm and the top of the packed solids was 79 mm from the center of rotation, respectively.

Determine the maximum flow rate for the clarification of a suspension of lysed *Escherichia coli* cells in a plant scale disk centrifuge. Given that the plant centrifuge has a bowl diameter of 25.4 cm, the maximum dimensionless acceleration, G of 14200, θ = 42°, R_1 = 8 cm, R_0 = 20 cm and number of disks = 100.

[18 marks]

Information:

$$G = \frac{\omega^2 R}{g}$$

$$\frac{dr}{dt} = \upsilon_g\left(\frac{r\omega^2}{g}\right)$$

$$Q = \upsilon_g\left[\frac{2\pi l R^2\omega^2}{g}\right]$$

$$Q = \frac{\pi l(R_0^2 - R_1^2)\upsilon_g\omega^2}{g\ln(R_0/R_1)}$$

$$Q = \upsilon_g\left[\frac{2\pi n\omega^2}{3g}\left(R_0^3 - R_1^3\right)\cot\theta\right]$$

$$\upsilon_g = \frac{\rho_p - \rho_f}{18\mu}D_p^{\,2}g$$

g = 9.81 ms^{-2}

3. [a] A 0.6 m length and 7.5 x 10^{-3} m inner diameter high performance liquid chromatography (HPLC) column is used for the separation of two proteins (bovine serum albumin (BSA) and myoglobin (Mb) in a 0.1 x 10^{-6} m^3 solution. Pilot runs of HPLC at flow velocity of 2.17 x 10^{-4} m/s indicate that the capacity factors K_1 and K_2 of two protein are 0.8 and 1.1 respectively. The relationship between the heights equivalent to a theoretical plate (HETP) and the flow velocity is obtained as follows,

$$\text{HETP (m)} = 6.2 \times 10^{-5} \text{ (m)} + 1.13 \text{ (s)}u$$

Where u = the flow velocity (ms^{-1})

[i] Based on the above information, calculate the number of theoretical plates of the HPLC column.

[ii] What resolution R_N of two enzymes may be expected? Comments on the result obtained.

[iii] Calculate the flow rate and sample volume needed to separate the above two enzymes if the column length and inside diameter of a large-scale HPLC are 0.5 m and 0.108 m, respectively

[12 marks]

[b] The equilibrium partitioning of a protein between an organic solvent extract phase and aqueous feed phase has been found to be nonlinear and can be represented by the following equation:

$$y = 1.50 \ln x + 6.0$$

where y and x are concentrations of the protein in the extract and aqueous feed phases, respectively in g/l. It is desired to extract 96% of the protein from a feed stream having a protein concentration of 4.0 g/l. For a feed stream at a flow rate of 5.5 liters/min and an extract stream at a flow rate of 3.0 liters/min

[i] What is the concentration of the peptide in the exit extract stream.

[ii] Graphically estimate how many equilibrium stages will be required for countercurrent flow of the phases

[iii] As the concentration of the peptide in the raffinate decreases, does the partitioning of the protein become more or less favourable?

[13 marks]

4. [a] Crystallization is usually the last step in producing highly purified products such as antibiotics. This process is said to be similar with precipitation but there are some differences between these two processes. Briefly discuss their differences.

[7 marks]

[b] It is desired to filter a cell broth at a rate of 2000 l/h on a rotary vacuum filter at a vacuum pressure of 70 kPa. The cycle time for the drum will be 60 s and the cake formation time (filtering time) will be 15 s. The broth to be filtered has a viscosity of 2.0 cP and a cake solid (dry basis) per volume of filtrate of 10 g/l. From laboratory tests, the specific cake resistance has been determined to be 9 x 10^{10} cm/g. Determine the area of the filter that is required. The resistance of the filter can be neglected.

It is then desired to wash the product (antibiotic) out of the cake so that only 5% of the antibiotic in the cake is left after washing. The washing efficiency is 50%. Estimate the washing time per cycle that would be required. Assume that the filtered cake contains 70% water.

[18 marks]

Information:

$$\frac{At}{V} = \frac{\mu\alpha\rho_0}{2\Delta P}\left(\frac{V}{A}\right) + \frac{\mu R_M}{\Delta P} \qquad \alpha = \alpha'(\Delta P)^s$$

$$R' = \left(1 - \frac{E}{100}\right)^n \qquad \frac{t_w}{t_f} = 2nf \qquad \frac{t_w}{t_f} = \frac{2V_w}{V_f}$$

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Appendix

Graetz-Leveque correlation

$$Sh = 1.62\, Re^{0.33} Sc^{0.33} \left(\frac{d}{l}\right)^{0.33}$$

Dittus-Boelter correlation

$$Sh = 0.023\, Re^{0.8} Sc^{0.33}$$

Column Resolution

$$R_N = \frac{1}{4}\sqrt{N_P}\left(\frac{\delta - 1}{\delta}\right)\left(\frac{k_2}{k_2 + 1}\right)$$

Common Engineering Conversion Factors

Length	**Volume**
1 ft = 12 in = 0.3048 m, 1 yard = 3 ft 1 mi = 5280 ft = 1609.344 m 1 nautical mile (nmi) = 6076 ft	1 ft^3 = 0.028317 m^3 = 7.481 gal , 1 bbl = 42 U.S. gal 1 U.S. gal = 231 in^3 = 3.7853 L = 4qt = 0.833 lmp.gal. 1 L = 0.001 m^3 = 0.035315 ft^3 = 0.2642 U.S. gal
Mass	**Density**
1 slug = 32.174 lb_m = 14.594 kg 1 lb_m = 0.4536 kg = 7000 grains	1 slug/ft^3 = 515.38 kg/m^3 , 1 g/cm^3 = 1000 kg/m^3 1 lb_m/ft^3 = 16.0185 kg/m^3 , 1 lb_m/in^3 = 27.68 g/cm^3
Acceleration & Area	**Velocity**
1 ft/s^2 = 0.3048 m/s^2 1 ft^2 = 0.092903 m^2	1 ft/s = 0.3048 m/s , 1 knot = 1 min/h = 1.6878 ft/s 1 min/h = 1.4666666 ft/s (fps) = 0.44704 m/s
Mass Flow & Mass Flux	**Volume Flow**
1 slug/s = 14.594 kg/s. 1 lb_m/s = 0.4536 kg/s 1 kg/m^2s = 0.2046 lb_m/ft^2s = 0.00636 slug/ft^2s	1 gal/min = 0.00228 ft^3/s = 0.06309 L/s 1 million gal/day = 1.5472 ft^3/s = 0.04381 m^3/s
Pressure	**Force and Surface Tension**
1 lb_f/ft^3 = 47.88 Pa, 1 torr = 1 mm Hg 1 psi = 144 psf, 1 bar = 10^5 Pa 1 atm = 2116.2 psf = 14696 psi = 101,325 Pa = 29.9 in Hg = 33.9 ft H_2O	1 lb_f = 4.448222 N = 16 oz, 1 dyne = 1 g cm/s^2 = 10^{-5}N 1 kg_f = 2.2046 lb_f = 9.80665 N 1 U.S. (short) ton = 2000 lb_f , 1 N = 0.2248 lb_f 1 N/m = 0.0685 lb_f/ft
Power	**Energy and Specific Energy**
1 hp = 550 (ft.lb_f)/s = 745.7 W 1 (ft.lb_f)/s = 1.3558 W 1 Watt = 3.4123 Btu/h = 0.00134 hp	1 ft lb_f = 1.35582 J, 1 hp·h = 2544.5 Btu 1 Btu = 252 cal = 1055.056 J = 778.17 ft lb_f 1 cal = 4.1855 J, 1 ft.lb_f/lb_m = 2.9890 J/kg
Specific Weight	**Heat Flux**
1 lb_f/ft^3 = 157.09 N/m^3	1 W/m^2 = 0.3171 Btu/(h ft^2)
Viscosity	**Kinematic Viscosity**
1 slug/(ft.s) = 47.88 kg/(m.s) = 478.8 poise (p) 1 p=1 g/(cm.s) 0.1 kg/(m.s) = 0.002088 slug/(ft s)	1 ft^2/h = 2.506 .10^{-5} m^2/s, 1 ft^2/s = 0.092903 m^2/s 1 stoke (st) = 1 cm^2/s = 0.0001 m^2/s = 0.001076 ft^2/s
Temperature Scale Readings	
^oF = (9/5)^oC + 32 ^oC = (5/9) (^oF – 32)	^oR = ^oF + 459.69 ^oK = ^oC + 273.16
Thermal Conductivity*	**Gas Constant***
1 cal/(s.cm.^oC) = 242 Btu/(h.ft.^oR) 1 Btu/(h.ft.^oR) = 1.7307 W/(m.K)	R = 82.057 atm.cm^3/(gmol.K) = 62.361 mm Hg.L/(gmol.K) = 1.134 atm.ft^3/(lbmol.K) = 0.083144 bar.L/(gmol.K) = 10.73 psi. ft^3/(lbmol. ^oR) = 555.0 mm Hg.ft^3/(lbmol. ^oR)

- Note that the intervals in absolute (Kelvin) and ^oC are equal. Also, 1 ^oR = 1 ^oF.

Latent heat: 1 J/kg = 4.2995 × 10^{-4} Btu/lb_m = 10.76 lb_f.ft/slug = 0.3345 lb_f.ft/lb_m , 1 Btu/lb_m = 2325.9 J/kg

Heat transfer coefficient: 1 Btu/(h.ft^2.^oF) = 5.6782 W/(m^2.^oC).

Heat generation rate: 1 W/m^3 = 0.09665 Btu/(h ft^3)

Heat transfer per unit length: 1 W/m = 1.0403 Btu/(h ft)

Mass transfer coefficient: 1 m/s = 11.811 ft/h, 1 lb_{mol}/(h.ft^2) = 0.013562 kgmol/(s.m^2)