

7.7 Separation of Two Solutes Having Linear Equilibrium

Two solutes have a linear equilibrium with the stationary phase. Their equilibrium constants are 6.5 and 6.6, respectively. The task is to separate 20 ml of the mixture on a column 5 cm in diameter. The flow rate is 10 ml/min. Determine the minimum column length required to just separate the two compounds, with $\varepsilon = 0.3$.

7.8 Calculation of the Shock Wave Velocity

A solute has a Langmuir isotherm characterized by an S_{tot} of 120 $\mu\text{g/ml}$ and a K_{eq} of 60 ml/mg. Calculate the shock wave velocity for an injection of 1 mg/ml and column conditions identical to those presented in Problem 7.7.

7.9 Using the LUB Method to Scale Up Fixed-Bed Adsorption

Breakthrough data (see Table P7.9) have been obtained for a fixed-bed adsorption process with a weakly anionic adsorbent using a feed of filtered fermentation broth containing the antibiotic cephalosporin at a concentration of 4.3 g/liter. The bed is 1 m long $\times$ 3 cm diameter, and the superficial velocity is 2 m/h. It is desired to scale up this process to a bed length of 3 m using the same superficial velocity. Use the LUB method to estimate the break-point time, defined here as occurring when $c_i/c_{i0} = 0.1$, for the large column. (Data from P. A. Belter, E. L. Cussler, W.-S. Hu, *Bioseparations*, p. 174, Wiley-Interscience, New York, 1988.)

7.10 Removal of a Low Level Contaminant

One mode in which adsorbents are used is to strip a low level contaminant like DNA from a product solution. No other solution components are negatively charged at the operating pH for the column.

- (a) If an anion exchanger is used for this purpose, determine the total bed capacity for DNA given these parameters: bed volume = 2 liters, S_{tot} =

2 mg/ml, $K_{\text{eq}} = 2$ ml/mg, and DNA concentration = 5 $\mu\text{g/ml}$.

- (b) Determine the bed capacity for the same conditions except that the DNA concentration is 100 $\mu\text{g/ml}$.

7.11 Chromatography Scaleup

It is desired to scale up the throughput by a factor of 150 for a linear gradient ion exchange chromatography of a product protein from the laboratory to the plant. The conditions for the laboratory chromatography are the following: 1.0 cm bed diameter (ID) $\times$ 20 cm bed height, 20 μm particle size, and 30 cm/h superficial velocity. The particle size of the same type of ion exchange resin available for the plant operation is 40 μm . Two columns are available in the plant: one column 14.0 cm diameter (ID) $\times$ 50 cm high, and another column 18.0 cm diameter (ID) $\times$ 50 cm high. To keep the resolution for the chromatography constant in the plant, which column should be used? For this column, what should be the resin bed height and the superficial velocity and what do you estimate the pressure drop to be? The viscosity of the mobile phase is 1.0 cp, and the void fraction for resin in the plant column is 0.33.

7.12 Design of a Protein Purification Process (Mini-Case Study)

Propose a purification process for the protein described. Assume a bacterial process. Design of the process will involve estimation of protein properties, for which a spreadsheet is provided at the textbook website (<http://www.bioseparations.org>). (Protein adapted from H. Zou, T. J. McGarry, T. Bernal, and M. W. Kirschner, "Identification of a vertebrate sister-chromatid separation inhibitor involved in transformation and tumorigenesis", *Science*, vol. 285, pp. 418–422, 1999.)

Sequence

1 MATLIYVDKENGEPGTRVVAKDGLKLG-
SGPSIKALDGRSQVSTPRFGKTFD

52 APPACLPKATRKALGTVNRATEKSVKT-
|-----S-S-----|
KGPLKQKQPSCFSAKKMTEKTCVKAKS

106 SVPASDDAYPEIEKFFPFNPLD-
FESFDLPEEHQIAHLPLSGVPLMILDEER

157 ELEKLFQLGPPSPVKMPSPWPWESNL-
LQSPSSILSTLDVELPPVCCDIDI

TABLE P7.9

t (h)	c_i (g/liter)
4.7	0.2
6.5	0.4
7.3	1.0
7.8	1.8
8.1	2.7
8.7	3.8
9.3	4.2
10.3	4.3