- Purity specified as [product]/([product] + [contaminants]), specific activity (biological activity/mass), and yield (mass after purification/initial mass) are calculated as quality indicators in purification.

NOMENCLATURE

a	particle radius (μm)
c	solute concentration (M, or g liter^{-1})
C	electrical conductivity (ohm^{-1} cm^{-1})
$\mathscr{D}$	diffusion coefficient (cm^2 s^{-1})
E	electrical potential gradient (V cm^{-1})
J_D	diffusive flux of a solute (mol cm^{-2} s^{-1}, or g cm^{-2} s^{-1})
J_e	electrical current density or flux (A cm^{-2})
J_w	fluid flux (liter cm^{-2} h^{-1})
k	Boltzmann constant (1.3807×10^{-23} J K^{-1})
K	partition coefficient (dimensionless)
K_{eq}	equilibrium constant (units vary)
L_p	permeability coefficient (liter cm^{-2} h^{-1} Pa^{-1})
p	pressure (Pa)
T	absolute temperature (K)
x	linear distance (cm)
x	raffinate phase concentration of a separand in extraction (M, or g liter^{-1})
y	extract phase concentration of a separand in extraction (M, or g liter^{-1})
μ	viscosity (g cm^{-1} s^{-1})

PROBLEMS

The solution to the following problems may require the use of information not in the textbook (which is part of the exercise).

1.1 Product Molecular Weight and Value Rearrange the following five bioproducts approximately according to molecular size (left column) and according to cost per kilogram in the right column: recombinant human growth hormone for injection (hGH), lactic acid (LA), riboflavin for animal feed (B_2), goat antibodies for AIDS testing kits (gAb), and yeast phospholipase C for laundry presoaks (yPLc).

MW	$/kg

1.2 Product Concentrations A 10^{-3} M solution of gamma globulin (antibodies) is called a "concentrated solution." Why is it called a concentrated solution? Support your answer with a calculation.

1.3 pH-Dependent Change of Conformation of Poly-L-glutamic Acid A synthetic polypeptide made up of L-glutamic acid residues is in a random coil configuration at pH 7.0 but changes to α helical when the pH is lowered to 2.0. Explain this pH-dependent conformational transition.

1.4 Properties for Processing Riboflavin (vitamin B_2) is isolated by filtration and polished by spray drying. The efficiency of both processes (higher yield per unit time) increases with increasing temperature. Find the necessary handbook data and determine the maximum temperature at which you could run these two unit operations.

1.5 Process Sequencing Arrange the following products in order of increasing molecular weight (i.e., smallest first), and write after each product the most likely separation process to be useful in its isolation, using the list of processes given in Table P1.5.

1.6 Properties: Diffusivity Estimate the molecular diameter and diffusion coefficient for the proteins ribonuclease (MW 13,700 Da), hemoglobin (MW

TABLE P1.5

Product	Process
Insulin	Distillation
Citric acid	Filtration
B lymphocytes	Adsorption
Vaccine virus	Extraction
Gamma globulin	Centrifugation
Bacitracin	Precipitation
Ethanol	Ultrafiltration
Ribozyme	
Hemoglobin	
Riboflavin	

68,000), and urease (MW 480,000), assuming the molecules are spherical and the density of each protein molecule is 1.3 g/cm^3.

1.7 Preliminary Selection of Purification Steps Based on information in the *Merck Index,* what do you think are the most likely unit operations that should be used for the isolation and purification of the following bioproducts?
(a) Lincomycin
(b) L-Lactic acid
(c) L-Asparaginase

1.8 Calculations for the Purification of a Recombinant Protein The purification of a recombinant protein is carried out starting with 100 liters of a clarified cell lysate (i.e., the cells have been lysed, and the cell debris has been removed to give a clarified solution), which has a total protein concentration of 0.36 mg/ml and a recombinant protein concentration of 2.2 U/ml, where U denotes units of biological activity of the recombinant protein. It is known that the completely pure recombinant protein has a specific activity of 40.0 U/mg. Purification is continued until a chromatography step that yields 2.0 liters of a fraction containing the protein, with a total protein concentration of 1.11 mg/ml and a recombinant protein concentration of 43.2 U/ml.

For the recombinant protein, calculate the starting and ending purity, the starting and ending specific activity, and the percentage yield and fold purification through the chromatography step.

1.9 Process Synthesis A process for isolating an antibody against insulin has, as a unit operation, the reaction of the antibody with the antigen in a continuous stirred tank reactor. The reaction product is a precipitate that is continuously removed from the reactor with 10% of the solution, which is mouse serum. Since insulin is an expensive reagent, only stoichiometric amounts can be added to the mouse serum, which contains 8 mg/liter of anti-insulin. If this particular monoclonal antibody precipitates with its antigen in a 1:1 ratio, how many milligrams of insulin must be added to the reactor per hour to process 100 ml of mouse serum per hour? (Assume that equilibrium is achieved in this reactor.) Sketch a flowchart of this process.

1.10 Product Concentrations Using the *Handbook of Biochemistry and Molecular Biology* (G. Fasman, ed., CRC Press, Cleveland, 1976) or a similar source or a suitable biochemistry textbook (in other words, looking up information), find the necessary information to determine the amount of material required to make 1 ml of each of the following aqueous solutions:
(a) 0.01 M cytochrome c
(b) 1×10^{-7} M β-galactosidase
(c) 0.01 M porcine insulin
(d) 0.01 M human hemoglobin
(e) 0.1 M streptomycin
(f) 1×10^{-6} M oligonucleotide with 10 nucleotides

Also calculate the concentrations in terms of the following additional standard means of expressing bioproduct concentrations: percent (weight per volume) and milligrams per milliliter. Assuming that the solutions are in pure water, also express the concentrations as mole fractions. Discuss the feasibility of making each one of these solutions.

References

1. Consulting Resources Corporation, Newsletter, Spring, 2000 (data reproduced by permission).
2. Bailey, J. E., and Ollis, D. F. (1986). *Biochemical Engineering Fundamentals,* 2nd ed., McGraw-Hill, New York.