Problem Set #3

Problem 1

You are working with a chromatography column that has a 30 cm diameter and 40 cm length, is packed with 10 μm particles (ε = 0.4), and has a maximum allowable pressure drop of 70 bar. A protein of interest is to be eluted from the column using a mobile phase with a density (ρ) of 1000 kg/m^3 and a viscosity (μ) of 0.002 Pa-sec.

a) Calculate the fluid velocity in the column if the measured pressure drop is 50 bar.

b) A mixture with a viscosity of 0.05 Pa-sec is injected in the column and eluted at the fluid velocity calculated above. Estimate the maximum volume of the mixture that can be safely fed. (*Hint: consider the volume of injected sample as a plug that travels through the column.*)

Problem 2

Immunoglobulin G (IgG) is to be purified from a mixture of proteins. At lab scale, a gel filtration chromatographic column with 1.6 cm diameter and 40 cm length is used with an elution flow rate of 1 cm^3/min. The volume of feed injected was optimized to 4 cm^3. The process is complete after 1.2 hours.

a) Scale-up the column so that you can feed 40 cm^3 of the mixture. Please calculate dimensions and operational parameters.

b) At process scale, 470 L of material are to be processed per batch. The number of cycles should be ≤ 10 per batch. Take into consideration that the maximum available column diameter is 1 meter. Scale up the process by calculating the number of cycles that must be used per batch, the diameter of the columns used, and, if necessary, the number of columns used in parallel per cycle.

c) Qualitatively, what is the distribution of the expense in the chromatographic step?

Problem 3

The chromatogram in Figure 1 shows the purification of an antibody by ion exchange chromatography. At the column outlet, a fraction needs to be collected after 4.4 minutes. You may consider the peaks as Gaussian. For the column that is being used for the separation, the number of theoretical plates (N) is 150. The operating flow rate is 0.5 ml/min.
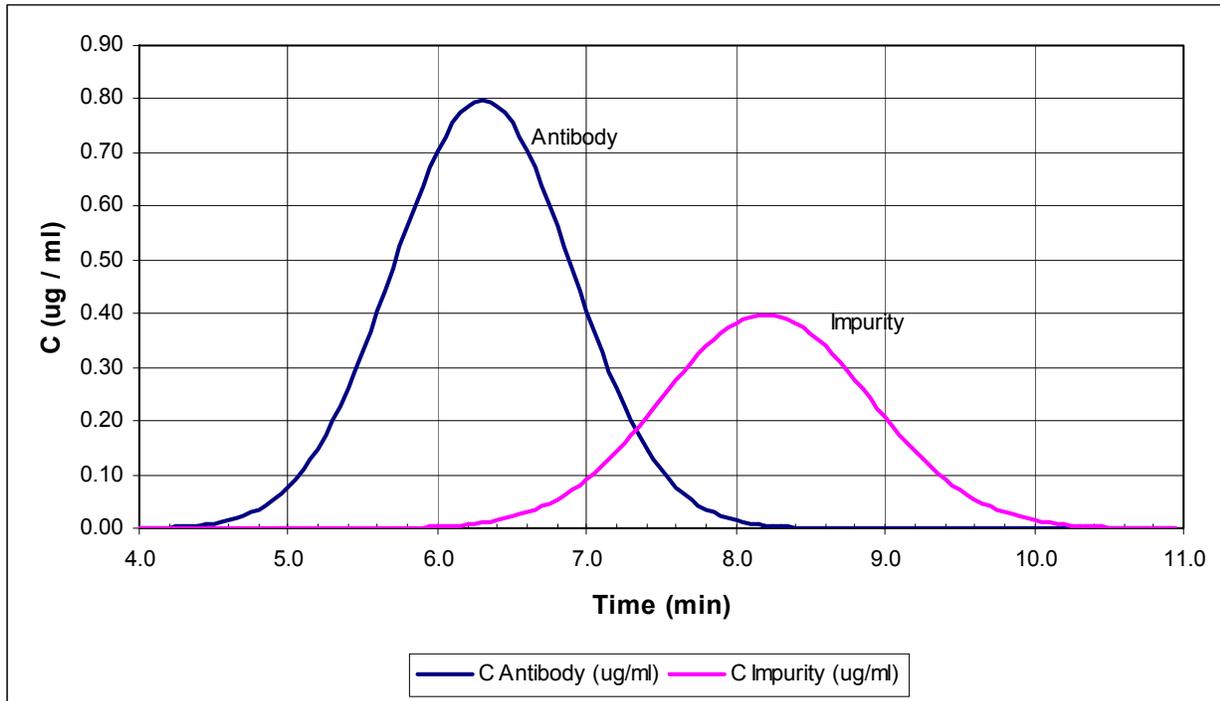

Figure 1: Chromatogram of Antibody and Impurity

a) Plot the yield and purity of the antibody as a function of time.

b) At what time should antibody fraction collection stop if the required purity is > 98%.

Problem 4

In the isolation and purification of recombinant proteins from bacterial cell extracts, ion exchange chromatography is commonly used as a primary capture step. You have been assigned the task of purifying an enzyme from an *E.coli* cell extract. The protein that you are working with has an isoelectric point of 6.0 and is stable in the pH range 5.5 to 10.

a) If your goal for the initial separation is strong binding of the enzyme to the ion exchange resin and subsequent elution via a change in ionic strength, what type of ion exchange resin would you select for the purification and in what pH range would you consider performing the purification (you only need to provide a reasonable pH range)?

b) Suppose that you select an appropriate resin for the purification and begin studies to optimize the separation pH. In your first purification, which is performed at a flow rate of 0.5 ml/min, you find that the flow through during loading of the column appear on the chromatogram after 2.5 minutes. When you elute the enzyme that you are interested in via a change in salt concentration to 150 mM NaCl, you find that the protein elutes from the column after 15 minutes with a peak width of 3 minutes. You decide that it is necessary to increase the concentration of the protein in the collected fraction by using a shorter column with the same number of theoretical plates. If the protein's retention time on the new column is 10 minutes, what is the approximate concentration of the protein in the fraction that is collected?