

## EXERCISE 8A - Postlab

1. How does size exclusion chromatography separate a mixture of molecules?
2. Describe some of the factors that affect the resolution of a size exclusion chromatography column.
3. When preparing the standard curve for your size exclusion chromatography column, why did you plot the log of the molecular weight of each protein rather than the molecular weight itself? Why did you plot the  $V_e/V_0$  of each protein rather than  $V_e$ ?
4. Use your standard curve to determine the expected  $V_e$ 's for the 6 major groups of proteins found in milk. (Note: The 6 major groups of milk proteins are listed in a table in the Prelab along with their molecular weights.)
5. During size exclusion chromatography you collected 14 one-mL fractions. At this point in the purification, do you know which proteins are in each fraction? Explain why or why not.

**Answer the next 2 questions only if you measured the  $A_{280}$  values for your column fractions using the Scanning Spectrophotometer.**

6. On a sheet of graph paper, graph the elution profile for your run by plotting the fraction number for all 14 fractions on the x-axis and the corresponding  $A_{280}$  values on the y-axis.. Make sure your graph is big enough so that it fills an entire 8.5" x 11" sheet of graph paper.
7. Examine the elution profile for your run. How many protein peaks did you obtain? Do any of the peaks correspond to the expected  $V_e$ 's for the major milk proteins, which you calculated in question 4? If so, match each of these peaks with the corresponding milk protein.