EXERCISE 8C - Postlab

1. Describe any difficulties that you had with the sample dilution, the gel loading, and the gel staining techniques.

2. What is the purpose of SDS-PAGE in this lab exercise?

3. When your gel is placed in the electrophoresis apparatus and the power is turned on, the wire in the upper reservoir serves as a negative electrode (cathode), and the wire in the bottom reservoir serves as a positive electrode (anode). Some of the major components present in the gel, including those drawn into the gel from the wells, are listed below. For each component listed, indicate whether it was drawn toward the anode, the cathode, or neither:

   Hydrogen ion (H^+)

   Tris+ (the buffer)

   Hydroxyl ion (OH^-)

   Water

   Chloride ion (Cl^-)

   Glycerol (polar)

   Bromophenol blue (the tracking dye)

   Sodium dodecyl sulfate (SDS)

   Protein with many SDS molecules bound to it

4. Explain the functions of the following components of the 2X sample treatment buffer that was added to your milk fractions before loading them onto the electrophoresis gel:

   SDS

   Mercaptoethanol

   blue dye

   glycerol

5. Describe and **explain** how each of the following changes would affect the migration distance of α-lactalbumin during SDS-PAGE:

   a. Increasing the ionic strength of the gel buffers.

   b. Decreasing the electrophoresis run time.

   c. Increasing the concentration of bis-acrylamide in the gels.

   d. Increasing the pore size of the gels.

6. Compare gel exclusion chromatography to SDS-PAGE with respect to the following:

   a. What is the basis for protein separation?

   b. Which one maintains protein activity?

   c. Which one destroys protein activity? Explain when, why, and how.