1mg/mL BSA = 1 ug/ml BSA
= 1000 ug/mL BSA

Laboratory 2: Quantification of Protein--(Bradford Assay)

General Purpose: The purpose of this procedure is to determine the concentration (weight per volume) of protein contained within an unknown sample.

Background:

Last week, we used polyacrylamide gel electrophoresis to observe proteins from bacteria as bands on a gel. In some instances, it is useful to know just how much protein is present in a sample. There are various methods for quantifying the amount of protein present in a sample. Some methods use titration with acid to determine the concentration, while others depend on a change in color to indicate how much protein present. Each method has its advantages and disadvantages, and each method has a particular degree of sensitivity associated with it.

This lab will utilize the Bradford Assay to determine the concentration of protein in your unknown samples. The Bradford Assay is a very accurate and highly sensitive method for determining protein concentration; it will detect protein concentrations as low as 1 µg/ml. The Bradford Assay uses a dye, Brilliant Blue, that is initially a dark brownish red color. When Brilliant Blue is mixed with proteins, complexes of proteins and dye cause the color of the reagent to change to a deep blue. The more protein that is present, the deeper the blue color will be. The intensity of the blue color can be measured as an absorbance reading in a machine called a spectrophotometer. We will set the spectrophotometer to pass a wavelength of light at 595 nm through a sample in a test tube and then the machine will indicate an absorbance reading on an LCD display.

In order to determine the concentration of protein in your unknown samples, you will first measure the absorbance of known concentrations of Bovine Serum Albumin (BSA) at 595 nm. These samples are called standards and you can construct a standard curve by making a graph using the absorbance values from the standard samples. On the graph, you will plot absorbance (A_{595}) against concentration. Then the absorbance of the unknown samples at 595 nm is measured and compared to the BSA standard curve. Knowing the absorbance of the unknown should allow you to determine the protein concentration of this sample fairly easily.

Materials:

Bovine Serum Albumin (BSA) – Stock solution of 1 mg/ml BSA in water
Unknown Samples of BSA
13x100 mm Test Tubes
Plastic Cuvettes
Disposable plastic pipettes with bulbs
Parafilm/scissors
Bradford Reagent (Sigma #B6916)
Spectrophotometer set at 595 nm
Water

Beer's (Beer-Lambert) (a

Abs(OD) = ε b c → conc.
1 cm