

References:

Bradford, M. M. *Anal. Biochem.* **1976**, 72, p 248.

Robyt, J.F.; White, B.J. *Biochemical Techniques Theory and Practice*; Waveland: Prospect Heights, IL, 1990.

Bollag, D.M.; Edelman, S.J. *Protein Methods*; Wiley-Liss: New York, 1991.

The solubility of proteins is a function of the nature of the protein structure and form as well as the solution environment in which it exists. Factors such as pH, salt concentration, presence of organics, and presence of surfactants will affect the solubility. The procedure outlined here will illustrate the effect of ammonium sulfate concentration on the solubilities of different proteins in a mixture. Proteins extracted from mung bean sprouts will be precipitated in a 40% saturated ammonium sulfate solution. The precipitate is collected by centrifugation, redissolved, and the total protein concentration determined quantitatively by the Bradford protein assay.

Solutions:

- 1) 0.05 M TRIS buffer, pH 7.2, 0.005 M NaCl.
- 2) Saturated solution of ammonium sulfate [528 g $(\text{NH}_4)_2\text{SO}_4$ /L solution or 75 g + 100 mL water] with the pH adjusted to 7.
- 3) **Standard protein** solution (1.25 mg/mL **bovine serum albumin** in buffer #1)
- 4) Bradford Reagent Solution - 100 mg Coomassie Brilliant Blue G (65 -75% dye) in 50 mL of 95% ethanol. To this solution, add 100 mL of 85% phosphoric acid. Dilute the concentrate to one liter with distilled water. **Reagent must be filtered before use.**

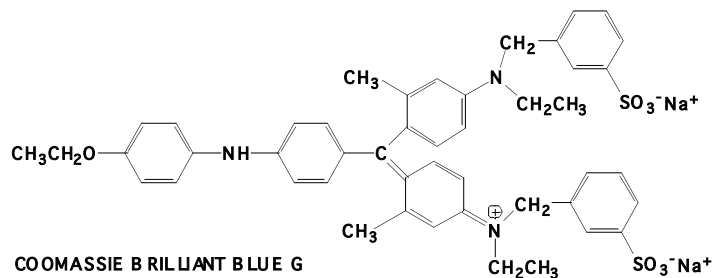
Laboratory:

A. Protein Precipitation

- 1) Your instructor will prepare a crude protein extract from mung bean sprouts. (20 g of sprouts blended in 75 mL of buffer #1, strained through cheesecloth, and centrifuged at 2000Xg for 8 minutes. The supernatant is the crude protein extract.)
- 2) You will perform an ammonium sulfate precipitation of the proteins in the crude extract. Take 20.0 mL of the crude extract and put in a labeled plastic centrifuge tube. Keep the tube on ice. Next **slowly, dropwise** add 13.3 mL of the saturated ammonium sulfate solution while gently swirling the protein solution. This addition should take about 10-15 minutes.
- 3) Give the tube to your instructor. The solution will be centrifuged for 20 minutes at 9000Xg.
- 4) Decant the supernatant into a test tube. To the centrifuge tube containing the precipitated protein, add 20 mL of buffer #1 and use a glass rod to free the precipitate and redissolve it in the buffer.
- 5) Determine the total protein concentration of the crude protein extract and the redissolved protein precipitate by the Bradford Method.

B. Bradford Protein Assay

This assay is based on the color change (red to blue) of the dye Coomassie Brilliant Blue G upon binding to added protein. The method is relatively free from interference from most buffers but does bind to detergents. All cuvetts must be scrupulously clean since the reagent will stain dirty glass or plastic. The binding of dye to protein occurs because of hydrophobic and electrostatic interactions (look at structure below), and the nature of the complex is a property of the amino acid composition of the protein. Therefore, standards should be as similar to the proteins being assayed as possible. Free amino acids, peptides, EDTA, and reducing agents such as monosaccharides and mercaptoethanol do not interfere with the assay.



Procedure

The standard procedure is applicable up to 0.20 mg/mL protein. An aliquot of protein solution is combined with 4.00 mL of the reagent and shaken vigorously. The absorbance of the sample is read at 595 nm after 15 minutes and before one hour. Color is fully developed in 15 minutes, however, and the protein will start to precipitate after 45 minutes.

- 1) Determine a standard curve by preparing a blank solution (4.00 mL Bradford Reagent and 0.08 mL distilled water, and three **standard albumin** solutions (0.02, 0.04 and 0.08 mL of **standard** plus water to give 0.08 mL in each case) plus 4.00 mL Bradford Reagent.
- 2) Prepare three solutions of the **crude protein extract** by mixing 4.00 mL Bradford Reagent, 0.04 mL of extract and 0.04 mL of distilled water.
- 3) Prepare three solutions of the **redissolved precipitate** by mixing 4.00 mL Bradford Reagent and 0.08 mL of redissolved precipitate.

Report:

- 1) Include a brief summary of the methods of the experiment in your notebook.
- 2) Prepare a Table and Figure for the standard curve with linear regression.
Spreadsheet Table will be provided that calculates items in 3) and 4).
- 3) Use the standard curve slope and y-intercept, along with the sample absorbances to determine to protein concentration of the crude extract and redissolved precipitate (mg/mL). Calculate the percentage of protein precipitated by the 40% ammonium sulfate solution.
- 4) Calculate the mean deviation and percentage deviation for the Bradford Assay based on your two different protein samples.
- 5) Write an abstract to report the results of your study that contains:
 - a) a statement of the purpose of the experiment (hypothesis)
 - b) a statement of the experimental plan used (refer to authors' names and year of publication)
 - c) a statement of the principal results (standard curve slope with units, average concentrations found with uncertainties)
 - d) a statement of your conclusion (% protein precipitated in a 40% saturated (NH₄)₂SO₄ solution)
- 6) Hand in your abstract and the 2 tables and the figure.