

Functional properties of Proteins

Reagents and Materials

1. Bovine Serum Albumin (BSA) in solution, 1.0 mg/ml
2. Bio-Rad dye reagent
3. Parafilm
4. Defatted soy flour
5. NaOH solution, 1.0 N
6. HCl solution, 1.0 N
7. CaCl₂ solution, 5.0 M

Equipment

1. Test tubes
2. Cuvettes (*remember to throw them away after you are done with the measurements!!!*)
3. Spectrophotometer
4. Pipettes
5. Stir plate and magnetic stir bars
6. Beakers, 50 ml and 200 ml
7. Centrifuge tubes with caps
8. Centrifuge
9. pH meter and pH standards
10. glass stirring rods

Procedure

Part I: standard curve construction

A standard curve for the dye-binding Bradford assay using bovine serum albumin (BSA) as standard protein will be constructed using the following procedure.

1. Label 4 test tubes and prepare approximately 1 ml of standard solutions of BSA in water having the final concentrations listed in the table below (**Note:** the initial concentration of the BSA solution provided for the lab is 1.0 mg/ml).

Final concentration (mg/ml)	Water addition* (ml)
0.25	
0.5	
0.75	
1.0	

*volume of water addition to the initial solution necessary to obtain the desired final concentration

2. Transfer 0.1 ml of each standard solution to labeled test tubes.
3. Add to each tube 5 ml of dye reagent.
4. Close each tube with parafilm and invert the tubes several times to mix the content.

- Place the tubes on a rack and allow about 30 minutes of incubation at room temperature.
- Zero the spectrophotometer by reading a blank at 595 nm (**Note:** the blank solution should be identical to the sample solutions except that it should not contain protein; for the purpose of the lab it should not make a big difference if you use the “pure” dye solution instead of adding 0.1 ml of water to 5 ml of dye reagent).
- Read and record the absorbance values at 595 nm of all the solutions at different concentrations.

BSA concentration (mg/ml)	Absorbance @ 595nm
0.25	
0.5	
0.75	
1.0	

Part II: protein extraction for quantification and tofu preparation

Soy protein will be extracted from soy flour samples (dissolved in water). Each group will perform the extraction at different pH levels and the extraction yields (at different pH values) will be compared. Also protein extraction for tofu production will be performed in parallel.

- Prepare a 1:15 mixture of soy flour into distilled water (e.g. dissolve 20 g of soy flour in 300 ml of distilled water; use the magnetic bar stirrer to obtain a uniform suspension of the flour).

This is the extraction for protein quantification (at the end proceed to “part III”)

- Transfer 15 ml of the soy flour suspension to a 50 ml beaker (**Note:** pipette the suspension with the bar stirrer on to prevent settling).
- Adjust the pH of your solution to the assigned value: add drop by drop either 1.0 N HCl or 1.0 N NaOH until you reach a value close to the assigned one.
- Place the magnetic bar into the beaker and allow 20 minutes of stirring at room temperature on the magnetic stirring plate.
- Transfer 10 ml aliquot of the solution to a centrifuge tube.
- Centrifuge at full speed for about 10 minutes (**Note:** be sure to have the tubes balanced on opposite sides of the centrifuge).
- Carefully transfer supernatant to a clean test tube.

Part III: protein extracts quantification

- Dilute 1:25 the supernatant obtained from “part II – 7.” (e.g.: add 2.4 ml of distilled water to 0.1 ml of supernatant).
- Follow the procedure in “part I – 2. to 7.” to determine protein content in the soy flour extract (e.g.: mix 0.1 ml of diluted supernatant with 5 ml of dye reagent).

Graphs & Calculations.

- Plot the standard curve obtained for BSA (absorbance vs. concentration).
- Plot the absorbance of the protein extracts vs. the extraction pH.
- Calculate the amount of soluble protein in the soy flour extract at the particular pH you used (consider all the dilutions made during the extraction and neglect the volume of acid or base added during the pH adjustment).

General comments.

Include all your observations on changes in consistence, texture, etc. during the steps of extraction and tofu preparation.