5. Protein Assays and Error Analysis

5.1. Competent Chemist Rating: *"What's in a Cow's Heart Anyway?"*

Techniques Checklist:
- Pipetting with pipetman ❑
- Calibrating pipetman ❑
- Preparation of a standard curve ❑
- Serial dilution ❑
- UV-Vis Spectroscopy ❑

Pre-Lab:
- Discussion of Protein Assays

Equipment:
- Pipetman: 100 P, 1000 P
- Pipet tips - large and small
- 8 Test tubes
- Eppendorf tubes and holder
- Disposable UV-Vis cuvettes - 5 mL

Goal:
- You will be given a sample of a solution of bovine heart cytochrome c. You will use the Coomassie$^®$ Plus Protein Assay from Pierce to determine the concentration of protein in the sample.

Note:
- You will receive a tray of Eppendorf tubes: one containing stock solution, three containing 50 μL each of bovine heart cytochrome c, and several empty tubes for mixing solutions. You will also be provided with a bottle of 25mM MOPS buffer, pH 7.

Experiment Outline:

Pipetman Calibration

Prior to beginning any experiment with a pipetman, it is necessary to first calibrate it. This procedure will determine exactly how much liquid is delivered when a certain amount is "dialed-in" to the instrument. To calibrate your pipetman, simply draw up a certain amount of water, empty it into a tared container, and obtain a weight. Knowing that water has a density of 1.00 g/mL, you can perform a calculation to tell you the accuracy of your pipetman. Most instruments will need no correction, and ones that are incorrect will usually be off by no more than 1 μL.

The Coomassie®-Protein Reaction Scheme

This protein assay works by forming a complex between the protein and the Coomassie® dye. When bound to the protein, the absorbance of the dye shifts from 465 nm to 595 nm (A_{595}). You will first generate a standard curve using the protein Bovine Serum Albumin (BSA) by measuring the absorbance at 595 nm of a series of standards of known concentration. Next, you will measure the A_{595} of your sample and determine its concentration by comparison to the standard curve.

Protein + Coomassie®G-250 in acidic medium---> Protein-Dye complex (blue; measured at 595 nm)

1. Preparation of diluted BSA standards

• Prepare a fresh set of protein standards by diluting the 2.0 mg/mL BSA stock standard (Stock) as illustrated below. There will be sufficient volume for three replications of each diluted BSA standard, if necessary.

Vol of the BSA to Add	Vol of Diluent (buffer) to Add	Final BSA Conc.
300 μL of Stock	0 μL	**Stock** - 2000 μg/mL
375 μL of Stock	125 μL	**A** - 1500 μg/mL
325 μL of Stock	325 μL	**B** - 1000 μg/mL
175 μL of A	175 μL	**C** - 750 μg/mL
325 μL of B	325 μL	**D** - 500 μg/mL
325 μL of D	325 μL	**E** - 250 μg/mL
325 μL of E	325 μL	**F** - 125 μg/mL
100 μL of F	400 μL	**G** - 25 μg/mL

2. Mixing of the Coomassie® Plus Protein Assay Reagent:

      • Allow the Coomassie® Plus reagent to come to room temperature. Mix the Coomassie® Plus reagent solution just prior to use by gently inverting the bottle several times. Do not shake.

3. The Standard Protocol

      • Pipet 0.05 mL of each standard solution into appropriately labeled Eppendorf tubes. Prepare at least three samples of your unknown solution. Use 0.05 mL of the diluent (25 mM MOPS buffer, pH 7, provided by TA) to prepare two blank tubes.

      • Add 1.5 mL of the Coomassie® Plus reagent to each tube, mix well.

      • Measure the absorbance at 595 nm of each tube *versus* a water reference.

      • Subtract the average 595 nm reading for the blanks from the 595 nm reading for each standard or unknown sample.

      • Prepare a standard curve by plotting the average blank corrected 595 nm reading for each BSA standard *versus* its concentration in µg/mL. Using the standard curve, determine the protein concentration for each unknown sample.

Helpful Hints:

      • Keep all of your solutions until after you have plotted and analyzed your data. You may need to redo some of your UV absorptions.

Results:

      • To obtain your "CC Rating" in Protein Assays and Error Analysis, the line fit for your standard curve must have a 0.930 correlation coefficient (R value) or higher. Additionally, the results from your absorbance values of the unknown should have a standard deviation of less than 0.048. Finally, you must determine the concentration of your unknown protein.