Bradford Test for Protein:
How can the protein concentration of milk (mg/ml) be determined

Introduction

In your previous biology classes you should have determined the presence or absence of various organic molecules with specific testing reagents such as Benedicts (tests for reducing sugars) or Lugol's Iodine (tests for starch) or even Biuret (tests for proteins). These reagents were qualitative in nature. They gave a color change but that was only used to determine if they were present or absent. There are methods that can be used to determine their concentration. Qualitative measures determine if they are there or not, by color or some other quality. Quantitative measures determine the amount or quantity present.

Quantitative estimation of the total protein content of a sample is frequently necessary in cell physiological and biochemical studies. Several methods of determining the total protein content of a sample have been developed and widely used during this century. One of the simplest and most sensitive is the "Bradford" assay, which was introduced in the mid-1970s. This assay is based on the binding specificity of the dye Coomassie Brilliant Blue-G250 for protein molecules but not for other cellular constituents. This organic dye binds specifically to tyrosine side chains.

The binding of the dye to protein shifts the peak absorbance of the dye. Unbound Coomassie Blue absorbs light maximally at a wavelength of 465 nm, while the absorption maximum is at 595 nm when the dye is bound to protein. Applying Beer's law: The absorbance of light by the dye-protein complex at 595 nm is proportional to the amount of protein bound (over a limited range); i.e. there is a linear relationship between absorbance and the total protein concentration of the sample over a narrow range.(source: http://www.acad.carleton.edu/curricular/BIOL/resources/rlink/lab1p4.html)

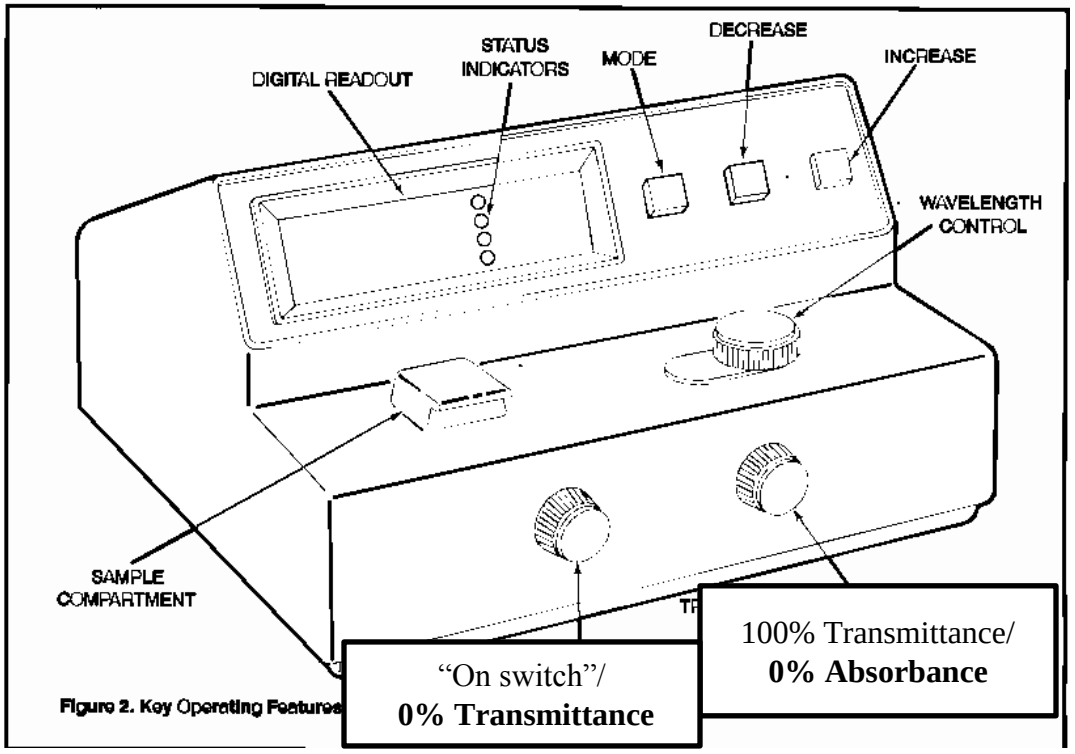

Figure 2. Key Operating Features

So the main idea is to have a series of proteins that you know the concentration of protein and mix them with Bradford reagent. Once you determine the absorbance with a series of known proteins concentrations, how could you use that data to determine the concentration of an unknown, diluted milk? How could you evaluate the labs precision? How could you evaluate the labs accuracy?

Procedure

Note: do all determinations in duplicate by working in collaboration with another group.

Construction of Standard Curve

1. You will be 4 of using Bio-Rad's Quick Start Standards. They have known concentrations ($\frac{mg\ protein}{ml}$) of Bovine Gamma Globulin (BGG) or Bovine Serum albumin (BSA) solutions. Did you note which ones you are using?
    a. *Option: you could make protein standards by starting with $\frac{2.0\ mg\ protein}{ml}$ stock concentrated solution. Using the formula C1V1=C2V2 make 100 ul of each of the following BSA dilutions. .2, .4, .6, .8 mg/ml(ug/ul) Use 1 X Phosphate Buffered Saline to do the dilutions.*
2. Add 50 µl of known concentration BGG or BSA protein solution to the cuvette. *What tool did you use? Can you determine the uncertainty of this device?*
3. Add 2.5 ml of Bradford Reagent to each cuvette. Mix well and let sit 5 minutes. *What tool did you use? Can you determine the uncertainty of this device?*
4. Set Spectrophotometer to 595nm and let warm up for a min of 15 minutes. Set Transmittance to 100 using a cuvette that has 50 ul of 1X PBS and 2.5ml of Bradford reagent. This is the negative control/ "Blank" and is used to make the machine ignore the glass and the unbound Bradford reagent.

"Old style" spectrophotometer	Spectrovis spectrophotometer & computer

1. Turn on (& check filter toggle set to 340-599nm)
2. Mode: select Transmittance
3. Make sure sample compartment does NOT have a cuvette in it, then SLOWLY move Knob **"0% Transmittance"** to 0.000
4. Turn "Wavelength Control" knob to set wavelength to 595nm
5. Let warm up 15 min (if just turned on)
6. Place the "Blank" (negative control) cuvette in the sample compartment
  a. Use the knob for "**100% Transmittance**", adjust dial to 100.0 (this tells the spectrophotometer to ignore the cuvette glass and the unreacted Bradford.
7. Remove "Blank" cuvette
8. Change Mode to Absorbance (should read zero if you put blank back in ☺, but empty it goes "funky")
9. Put in first known concentration of protein, determine absorbance
  a. determine how much readings fluctuate – per sample take a total of 3 readings
10. repeat steps 9 with other known samples
	1. Plug in USB connection
	2. Open Logger Pro
	3. Configure Spectrophotometer (icon that looks like a rainbow graph)
	  a. Click "absorbance v. concentration"
	  b. Check off wavelength closest to 595nm
	4. Very set by going to "Experiment tab" → Data Collection: it needs to be "Events with entry". Click done
	5. To Calibrate go back to "experiment tab" →calibrate→spec 1
	  a. Place the "Blank" (negative control) cuvette in the sample compartment…and follow computer prompts
	6. Remove "Blank"
	7. Put in first known concentration of protein, click on green "start collection" button. To determine absorbance click "Keep"
	  a. determine how much readings fluctuate – per sample take a total of 3 readings
	8. Repeat for other known protein concentration values and unknown too.

Warning: by the time you are ready to determine absorbance of unknown; it is a good idea to first use the "blank" cuvette and re "zero" to spectrophotometer.	

Testing Unknown Solutions:

1. Obtain a pre-dilluted milk sample or dilute the milk sample 1/100 by adding 1 µl of milk to 99 µl 1x PBS.
2. Take 50 µl of the diluted milk sample and mix with 2.5 ml of the Bradford reagent and let sit 5 min.
3. Use the blank again as in steps 5 and then place your milk tubes in as you did in steps six and seven above.

Can you Determination of Unknown Protein Concentration in Milk?