Protein Assay Lab

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Introduction:

Proteins are present in a cell and they are responsible for almost every task of the cell life. They also are responsible for determining the cell shapes and its internal organizations, for receiving signals from outside the cell, and for transducing those signals into intracellular responses.

Cow milk, which has a content of approximately 8 grams of protein per serving, has been a part of American's everyday-meal and breakfast for decades, however, its consumption has declined by 25% in the last 20 years since people started to consume plant milks, which has a better taste and nutrition values, and has cheaper costs of production compared to the usual cow milk.

The purpose of this lab is to use a simple and accurate colorimetric assay, which is called the Bradford method, in order to determine the amount of protein that is present in different types of milk; cow and soy.

Soy milk is made from soy beans and it is known as an alternative milk for vegans and for people who are lactose intolerant. Since it is made from plants, it does not have cholesterol and lactose, and low in saturated fat, but it is a good source of protein. Therefore, it is hypothesized that the amount of protein in soy milk will be different from that in cow milk but will be close.

Materials and Methods:

Prepare duplicate samples containing various concentrations of BSA in the range from 0 to 10 μg/ml. Using micropipettes, place volumes of water and BSA solution into cuvettes, shown below, in order to achieve the desired final concentrations to be used as standards.

- 1a – 800 µl water with 0 µl BSA solution to make concentration of 0 µg/ml
- 2a and 2b – 720 µl water with 80 µl BSA solution to make concentration of 2 µg/ml
- 3a and 3b – 640 µl water with 160 µl BSA solution to make concentration of 4 µg/ml
- 4a and 4b – 560 µl water with 240 µl BSA solution to make concentration of 6 µg/ml
- 5a – 480 µl water with 320 µl BSA solution to make concentration of 8 µg/ml
- 5b – 400 µl water with 320 µl BSA solution to make concentration of 8 µg/ml
- 6a and 6b – 400 µl water with 400 µl BSA solution to make concentration of 10 µg/ml

Then, use the equation; $C_i \times V_i = C_f \times V_f$, where $C_i = 25$ µg / ml and $V_f = 1000$ µl, to calculate the BSA concentration in each tube.

After preparing all the tubes, add 200 µl of Bradfor Reagent to each and use parafilm to cover the cuvette and invert it to mix the sample. Measure Abs at 595 nm of each sample. Once you got all the numbers, make a graph with absorbance on the Y axis and the BSA concentration on the X axis. Insert the trendline and get the equation of the line; the slope indicates the Abs.

Take 200 µl of regular cow milk sample, and 3 eppy tubes and label them Tube A, Tube B, Tube C. With micropipette, put 400 µl of H_2O and 100 µl of the regular milk sample to Tube A, and mix it. Then, transfer 100 µl of the sample from Tube A to Tube B and add 900 µl of H_2O, and mix it. Finally, transfer 10 µl of the sample from Tube B to Tube C and add 990 µl of H_2O, and mix it. Repeat the steps for the soy milk. Then, use the dilution in Tube C for both regular milk and soy milk in order to determine the protein concentration. Measure the absorbance in 595 nm twice for each type of milk and get the average absorbance in 595 nm.

By using the Abs from the graph and the numbers you got for absorbance, find the protein concentration in μg/ml with using the equation; Y = slope * X, where Y = Absorbance of the sample, slope = Abs in μg/ml, and X = protein concentration in μg/ml.

After getting the protein concentration in μg/ml for both regular milk and soy milk, calculate the experimental value of protein concentration in g of protein / serving.

- Start with the Concentration in the Cuvette (μg/ml)
- Calculate the Concentration per ml of sample (μg/ml) by dividing the previous number by 0.8
- Calculate the Concentration of Undiluted Milk (μg/ml) by multiplying the previous number by 5000
- Calculate the Concentration of Undiluted Milk (g/ml) by diving the previous number by 1,000,000
- Calculate the Experimental Value of Protein Concentration (g protein/serving) by multiplying the previous number by 240

Compare the numbers you got for the experimental values for both regular milk and soy milk to the reported value, and discuss what those value mean and the possible errors.

Data:

Table 1 – Standard Curve Table

			Concentration	**Absorbance**	**Average Absorbance**
Cuvette	**Water (µl)**	**BSA Solution (Vi) (µl)**	**BSA Concentration (Cf) (µg/ml)**	**A (595nm)**	**A (595nm)**
1a	800	0	0	0.000	BLANK
2a	720	80	2	0.182	0.188
2b	720	80		0.193	
3a	640	160	4	0.342	0.345
3b	640	160		0.348	
4a	560	240	6	0.464	0.454
4b	560	240		0.443	
5a	480	320	8	0.566	0.580
5b	400	320		0.593	
6a	400	400	10	0.653	0.659
6b	400	400		0.664	

Table 2 – Determining the Protein Concentrations

			Absorbance	**Average Absorbance**	**Concentration in the Cuvette (µg/ml)**
Cuvette	Water (µl)	Milk Sample (µl)	A (595nm)	A (595nm)	

1	800	0	0.322	0.322	BLANK
2a Regular Milk	_	800 (dilution 1C)	0.703	0.697	9.78
2b Regular Milk	_	800 (dilution 1C)	0.691		
3a Soy Milk	_	800 (dilution 1C)	0.984	0.933	13.08
3b Soy Milk	_	800 (dilution 1C)	0.882		

Table 3 – Finding the Protein Concentration in µg/ml

Y = 0.0713X Y = Absorbance of sample, X = Protein concentration in µg/ml, Abs = slope = 0.0713 Protein Concentration in the Sample in µg/ml = X = Y/0.0713			
	Y = Absorbance of Sample	**Abs = 0.0713**	**X = Protein Concentration in µg/ml = Y/0.0713**
Regular Cow Milk	0.697	0.0713	9.78
Soy Milk	0.933	0.0713	13.08

Table 4 – Calculating Protein Concentration

Milk	Concentration in the Cuvette (µg/ml)	Concentration per ml of sample (µg/ml)	Concentration Undiluted milk (µg/ml)	Concentration Undiluted milk (g/ml)	Experimental value (g protein / serving)	Reported Value (g protein / serving)
Cow Milk	9.78	12.225	61125	0.061125	14.67	8
Soy Milk	13.08	16.35	81750	0.08175	19.62	7

Sample Calculations (Soy Milk):

Concentration per ml of sample (µg/ml) = 13.08 / 0.8 = 16.35

Concentration Undiluted milk (µg/ml) = 16.35 * 5000 = 81750

Concentration Undiluted milk (g/ml) = 81750 / 1000000 = 0.08175

Experimental value (g protein / serving) = 0.08175 * 240 = 19.62

Table 5 – Calculating the % Errors

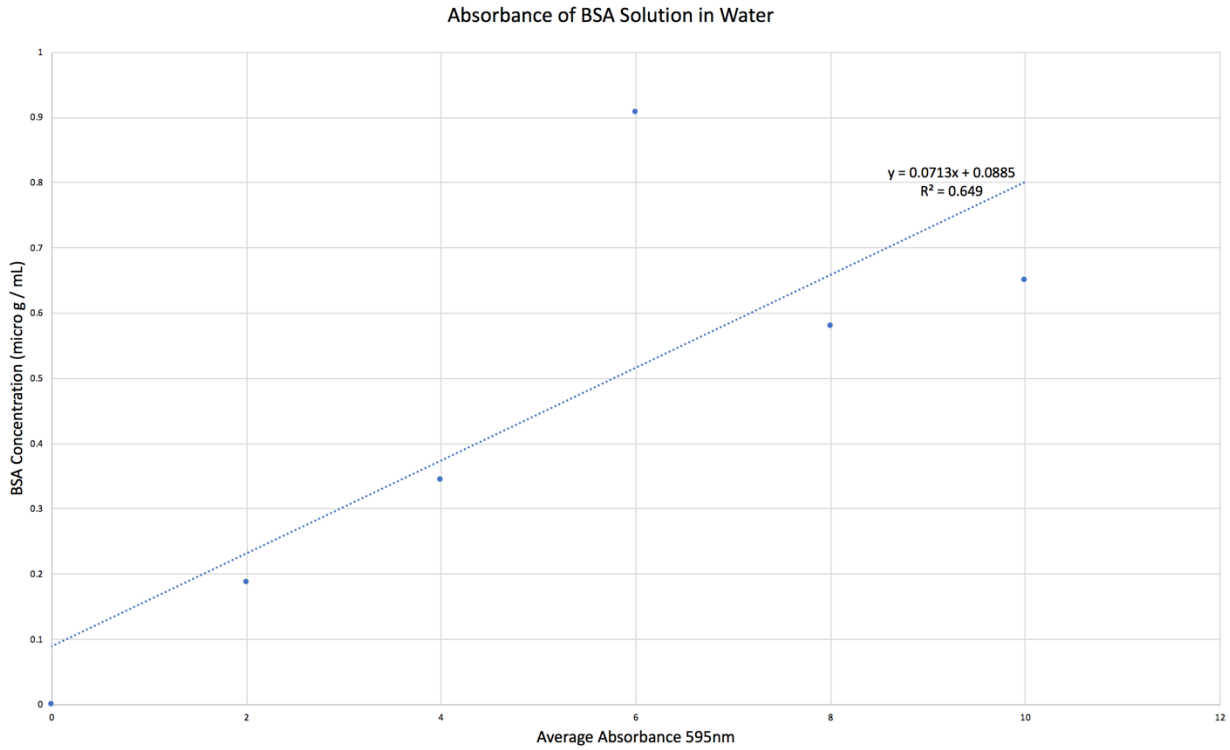
	Experimental value (g protein / serving)	Reported Value (g protein / serving)	% Error | (Experimental - Theoretical) / Theoretical | * 100 (%)
Regular Milk	14.67	8	83.375
Soy Milk	19.62	7	180.286

Sample Calculations (Regular Milk):

| (14.67 – 8) / 8 | * 100 = | 6.67 / 8 | * 100 = 0.83375 * 100 = 83.375%

Data Analysis:

Graph 1 – Absorbance of BSA Solution in Water



Graph 1 shows the Absorbance of the BSA Solution in Water, with the BSA Concentration in µg/ml as the dependent variable and the Average Absorbance in 595 nm as the independent variable. The slope of the trendline shows the Abs, which eventually will be used in the calculation to get the Protein Concentration in µg/ml.

Discussion and Conclusion:

From the Protein Assay Experiment, there was a huge error for both regular milk and soy milk; regular milk had an error of 83.375 % and soy milk had an error of 180.286 %. This means that we could not get close numbers to the reported values for protein concentration for both

regular and soy milk. Some possible sources of errors can be the human errors. Also, the R^2 value from the Graph 1 was 0.649 and it was below 0.9, so it indicates that we did not have a strong correlation between the BSA Concentration (μg/ml) and Absorbance (595nm). This might have caused by the impurities in the milk or the lab equipment, or the leftover in the micropipette tips.

In addition, our spectrophotometer gave us 0.322 Absorbance for 1a cuvette, which had 800 μl of water and 0 μl of BSA solution, where it is supposed to show 0.000 of absorbance; this also could be a source of error. When I subtracted this value; 0.322 from the Average Absorbance; 0.697 for regular milk and 0.933 for soy milk, and re-calculated for protein concentration for each solution, I got 7.889 for regular milk and 12.854 for soy milk, which are relatively close to the reported values. Since the base number of protein concentration of water was 0.322, instead of 0.000, subtraction was needed in order to find the difference between the protein concentration in water and the protein concentration in each sample of milk. Percent errors for these new re-calculated values were; 1.38 % for regular milk and 83.63 % for soy milk, which were better numbers compared to the numbers I got in Table 5.

Therefore, we could get relatively close number for the protein concentration of regular milk, however, we had far number from the reported value for protein concentration in soy milk.

References:

Introduction to Cellular and Molecular Biology Lab Manual: Emmanuel College 2018 Pages 13-25