Protein Concentration Measurement

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Introduction to Biology

BIO100

7/21/2022

INTRODUCTION

The objective of the experiment is to illustrate Bradford assays on various protein samples. The experiment also aims at drawing the standard curve of the standard protein with absorbance against concentration. The purpose of the experiment is also to demonstrate beer lambert's law. Another objective is to determine the concentration of the unknown.

HYPOTHESIS

If a known sample of protein concentration is used in the Bradford assay, then the concentration of an unknown protein can be extrapolated.

If the concentration is increased then the absorbance will also increase.

MATERIAL AND METHODS

B: Demonstration of Beer-Lambert Law. The methods and procedures used are:

1. 7 tubes were obtained and each labeled 1 to 7. In tube 1, 1 ml water was added. In tube 2 0.9 ml of water and 0.1 ml of albumin were pipetted. In test tube, 3 0.8 ml of water and 0.2 ml of albumin were pipetted. In tube 4 0.7 ml of water was pipetted with 0.3 ml of albumin. In tube 5, 0.6ml of water and 0.4ml of albumin was added. In tube 6, 0.5ml of water was pipetted with 0.5 ml of albumin and in test tube 7 0.4ml of water was pipetted with 0.6ml of albumin.
2. A second set of 7 tubes were obtained and each labeled 1 to 7. 0.1ml of the above solution was transferred to their respectively labeled tubes and 4 ml of CBB dye was added to each.

3. A parafilm was used to cover the second set and the solutions were mixed by inverting.
4. A wavelength of 595 was set and while tube 1 was used to zero the machine, the absorbance of each of the remaining 6 tubes (2-7) of the second set was read.

C: Determination of unknown protein concentration. The methods and procedures used are:

1. In a tube 0.2ml of unknown solution was obtained and 0.8ml of water was added. The solution was mixed.
2. 0.1ml was transferred to a clean tube and 4ml of CBB dye added.
3. The solution was enclosed with a parafilm and mixed by inversion.
4. Test-tube 1 of experiment B was used as a blank to zero the machine at a wavelength of 595 and the absorbance was obtained.

D: Linear Regression Analysis.

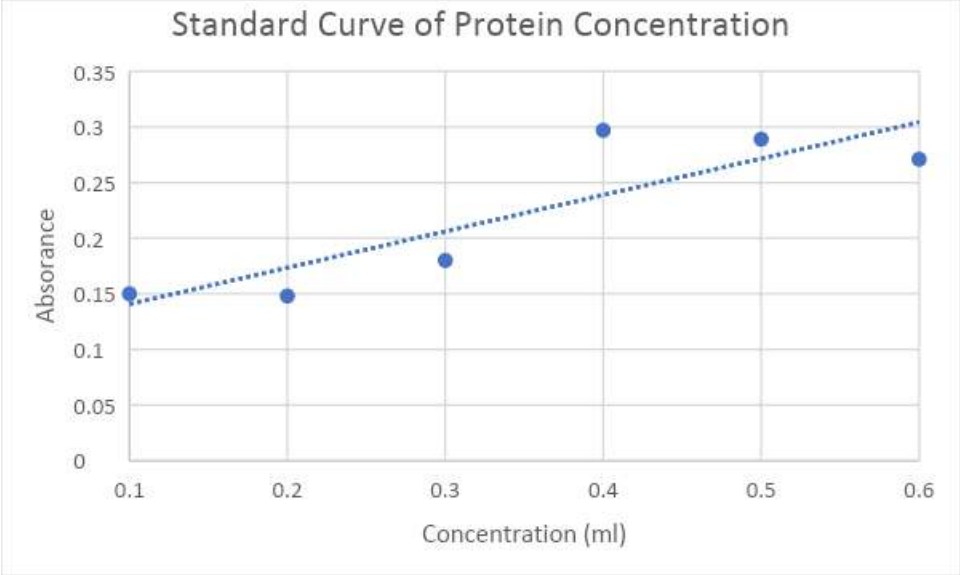
1. Excel was used to calculate the slope and Y intercept of the standard curve.
2. Concentration of unknown was also determined.

RESULTS

B: Demonstration of Beer-Lambert Law.

Tube	Concentration	Absorbance
2	0.1	0.15
3	0.2	0.148
4	0.3	0.18
5	0.4	0.297
6	0.5	0.289

7	0.6	0.271



C: Determination of unknown protein concentration.

Unknown (ml)	**Absorbance**
0.2	0.135

D: Linear Regression Analysis.

y= mx + c. From this equation x=(y-c)/m.

Yet from experiment B, y=0.3271x+0.108.

The Y value of unknown is 0.135. Thus, x= (0.135-0.108)/0.3271

Concentration of unknown is 0.08254.

DISCUSSION

The higher the concentration of the standard the darker the mixture and the higher the absorbance. This is evidenced by the standard curve of absorbance against concentration having a slope of +0.3271. This then proves our second hypothesis that if the concentration is increased then the absorbance will also increase. The R-value is 0.866603 which is close to 1 showing a strong linear relationship. The correlation does however not seem to be linear or proportional which may be suggestive of errors during experimentation. Errors may include cuvettes of varying lengths, interference by impurities in samples, and errors in pipetting.

Using the Bradford assay and Beer-Lambert's Law to quantify protein mixtures, the concentration of the unknown was found to be 0.08254. This then proves our second hypothesis that: if a known sample of protein concentration is used in the Bradford assay, then the concentration of an unknown protein can be extrapolated.