Associate of Science Programme
CCST4017
Molecular Biology and Biochemistry

LABORATORY REPORT

PRACTICAL 1

DETERMINATION
OF
PROTEIN CONCENTRATION

DUE DATE: **1 WEEK AFTER PRACTICAL**

ACADEMIC YEAR 20__24__ SEMESTER __1__

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Introduction

The Bradford protein assay is used to measure the concentration of total protein in a sample (He, 2011). In acidic conditions, the dye turns red. When the dye binds to a protein, the dye turns blue. Therefore, the Bradford protein assay is widely used for determining the quantity of proteins in a solution.

Objectives

To determine the concentration of the unknown protein sample by using the BSA standard curve.

Materials and procedures

Apparatus:

P1000 micropipette

P100 micropipette

P10 micropipette

100-1000µl pipette tips

10-100µl pipette tips

1-10µl pipette tips

1 beaker

2 Cuvettes

10 1.5 ml Eppendorf tube

A box of kimwipes

Spectrophotometer with 595nm wavelength

Procedures:

First, the apparatus needed are prepared and added BSA into 6 Eppendorf tubes with different concentrations, which are 0, 0.05, 0.075, 0.1, 0.25 and 0.5mg/ml respectively. The tubes are labelled from tube 1 to tube 6 afterwards.

Next, the samples are all diluted, and the final volume are at least 40 µl.

Then, 40 µl of an unknown sample is transferred into an Eppendorf tube and the tube is labelled as tube 8.

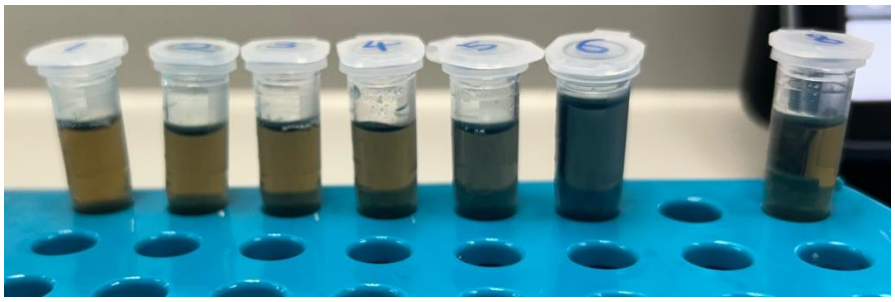
After that, 1 ml BioRad Protein Assay reagent (Bradford) is added to all samples respectively. After adding the reagent, the cap of the Eppendorf tubes are closed and are inverted several times to be mixed throughly for 7 to 10 seconds.

Subsequently, we waited for 10 minutes for the samples to be fully reacted.

Afterwards, one of the samples is poured from Eppendorf tube to the cuvette and is inserted into the spectrophotometer for measuring the A595 readings. Kimwipes are used to clean the cuvette after measurement for the next sample. Tube 8 requires a separate cuvette.

Finally, repeated the previous procedure for all samples until all 7 readings are recorded.

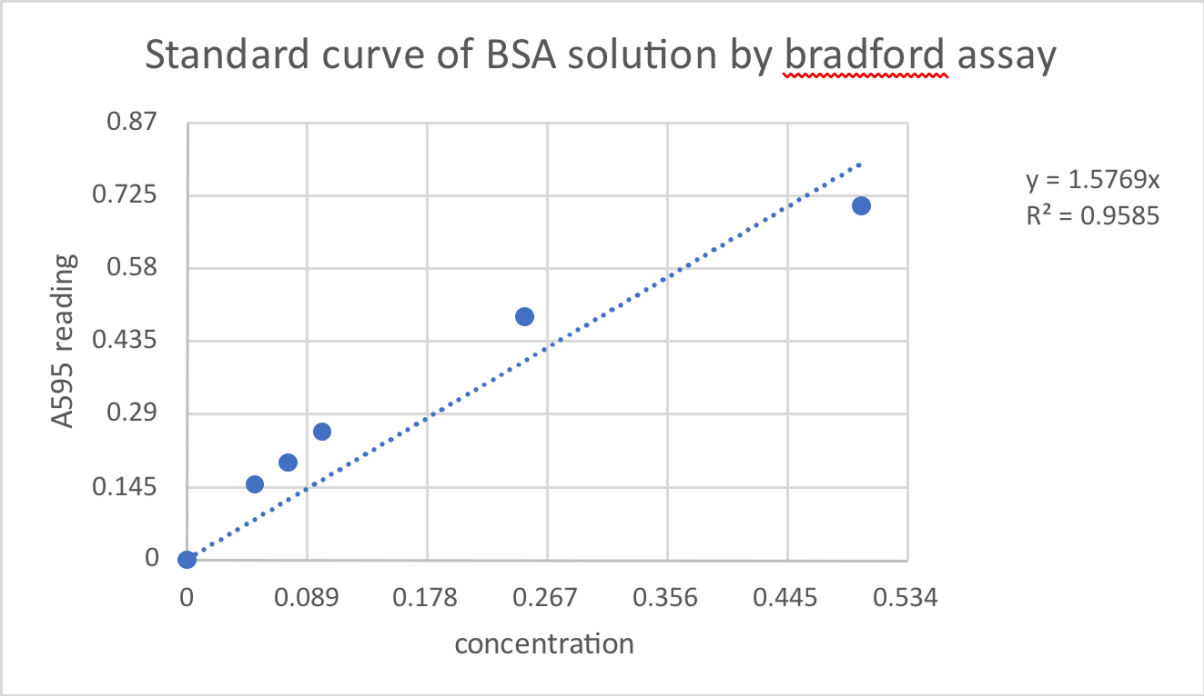
Results



The picture of the samples after 10 minutes

Table of data collected

Sample	1	2	3	4	5	6	8
Conc. (mg/ml)	0	0.05	0.075	0.1	0.25	0.5	Unknown
A595 readings	0	0.150	0.195	0.255	0.485	0.705	0.145



Therefore the concentration of the unknown sample:

$$\frac{0.145}{1.5769} = 0.091952565 \approx 0.0920$$

Discussion

According to the result, it shows that under the influence of the BioRad Protein Assay reagent, samples with higher concentration of protein has a higher A595 reading. The concentration curve shows that the curve rises steadily. The Bradford assay is based on the direct binding of Coomassie brilliant blue G-250 dye (CBBG) to proteins, producing an absorbance maximum at 595 nm (Olson & Markwell, 2007). There's a phenomenon that the graph shows that the rate is not completely linear and more likely to form a curve instead of a line. There might be errors in the colour changes or the assay did not fully react. Multiple experiments can be done to improve the accuracy.

According to Olson & Markwell (2007), the Bradford assay is simple, fast and sensitive protein assay compared to other protein assays currently available. Resulting it to become one of the most popular choices. However, inside the assay, there are two forms of dyes, one that is bound to the protein and one that is free in the solution. When both forms overlap in their color response, it causes the color change of the solution to be less predictable and causing inaccuracy.

It is assumed that higher A595 readings results in higher concentration of protein but affected by the curve. Which means in order to find out the concentration of protein in the unknown sample, the data collected should be inserted into the standard curve for comparison. During the experiment, even though it is noted that the Bradford assay is considered non-toxic for

most laboratory applications, the assay would still cause irritation to skin and eyes. Therefore, safety goggles and gloves should be worn during the experiment. In addition, micropipette with distinct size should be indicated and used correctly to prevent harm to the apparatus and inaccuracy to the experiment. Besides, cuvette should be wiped throughly with kimwipes to prevent any droplets of other samples are involved while recording the colour of the current sample. The wavelength of UV light with maximal absorbance by purified protein is 280nm and tryptophan has the highest absorbance at the wavelength of 280nm while other basic amino acids have minimal to no absorbance at this specific wavelength during protein quantification (Anthis & Clore, 2013). According to Westermeier & Marouga (2005), there are a lot more protein detection methods, which includes copper staining and immuno blotting.

Significance and application

This experiment can showcase the standard curve of Bradford protein assay and then determine the protein concentration of the unknown sample. This can provide an easier method to determine the protein concentration of samples and thus saves more time for future experiments.

Conclusion

The result shows that the A595 readings can showcase the protein concentration of the samples according to the standard curve. However, the graph shows that the standard curve of BSA solution by Bradford assay is not completely linear. Still, we can find out the protein concentration of the unknown solution is 0.092 mg/ml. Overall the experiment is still successful.

References

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