Modifications of the Bradford Protein Assay

Hunter Davis, Dr. Jeffrey Boles
Tennessee Tech University

Introduction

The ability to measure protein concentration in solution is widely used in research and the ability to measure the concentration accurately is of great importance. The Bradford protein assay measures the change in absorbance that occurs when Coomassie dye complexes with protein under acidic conditions. The measured absorbance of the Bradford assay is slightly linear up to 2000 µg/mL of protein, and linearity decreases extensively at lower concentrations. An increase in linearity would allow for more accurate estimations of the amount of protein in an unknown solution. The original procedure involves using ethanol and phosphoric acid, and the goal of this study is to either reinforce the original, or to determine if there are better alternatives.

Materials and Methods

Modifications made to the original protocol:

- Citric acid
- Ascorbic acid
- Methanol
- Isopropanol
- 2-methyl-2-butanol

Procedure:

- 10mg of Coomassie Brilliant Blue G250 was dissolved in 5mL of the chosen alcohol.
- 40mL to 80mL of water was added, depending on the volume of the acid to be used.
- 10mL of 85% w/v of the chosen acid was added, then the total volume was brought to 100mL.
- 200µL of this reagent was added to each of the samples with a BSA concentration ranging from 0 to 20µg/mL.
- The samples were incubated at room temperature for 10 minutes before measuring absorbance at 595nm. The sample containing no BSA was used as a blank.
- Each reagent was tested in triplicates.
- This data then plotted and compared to two commercially available reagents, Thermo Scientific and Bio-Rad.

Results

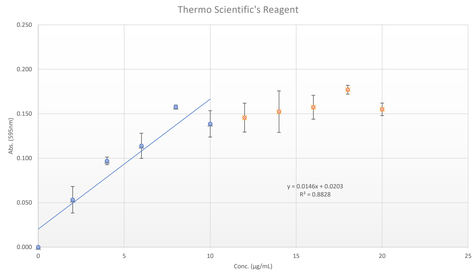

Figure 1. Absorbance at 595nm of Thermo Scientific's pre-made reagent.

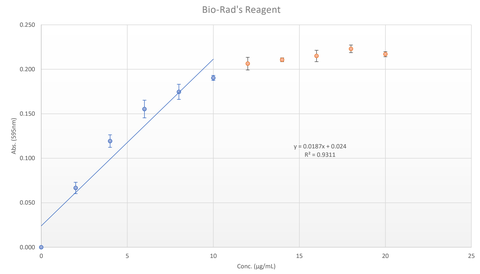

Figure 2. Absorbance at 595nm of Bio-Rad's pre-made reagent.

BSA (µg/mL)	Diluent (µL)	Reagent (µL)
0	800	200
2	780	200
4	760	200
6	740	200
8	720	200
10	700	200
12	680	200
14	660	200
16	640	200
18	620	200
20	600	200

Figure 3. Chart detailing the composition of each sample.

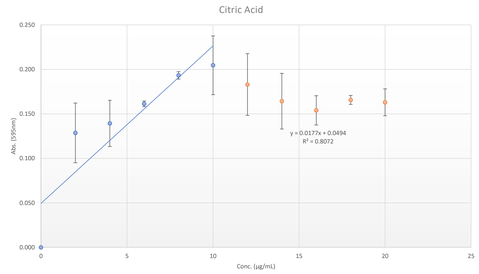

Figure 4. Absorbance at 595nm using the reagent made with citric acid.

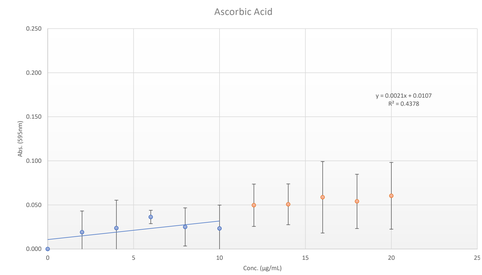

Figure 6. Absorbance at 595nm using the reagent made with ascorbic acid.

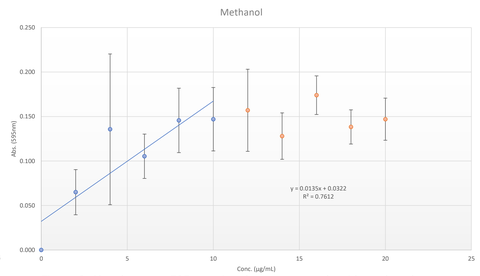

Figure 6. Absorbance at 595nm using the reagent made with methanol.

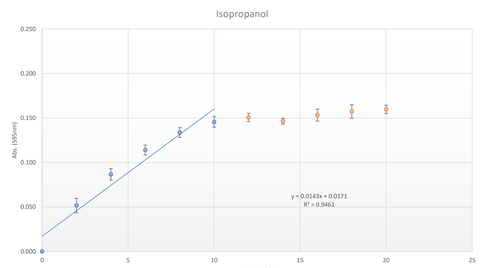

Figure 7. Absorbance at 595nm using the reagent made with isopropanol.

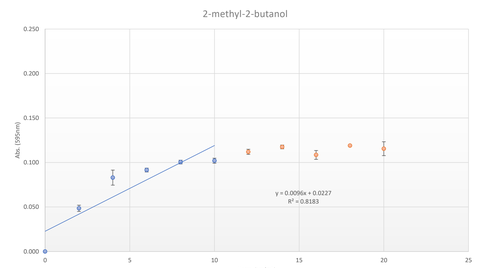

Figure 8. Absorbance at 595nm using the reagent made with 2-methyl-2-butanol.

Conclusions

While complete linearity was not achieved, the relative consistency is shown to vary between the different reagents. According to the data collected, the most consistent modified reagents appear to be isopropanol and 2-methyl-2-butanol.

By comparing the two commercially available reagents, it can be seen that Bio-Rad's appears to produce more consistent and linear results. However, this is only evaluated at lower concentrations.

The plotted absorbance of the reagent made with isopropanol fits the line of regression slightly better than Bio-Rad's reagent, but linearity beyond 10µg/mL is decreased.

Further testing could be done with 2-methyl-2-butanol, as it appears to become linear shortly after 5µg/mL. Of the tested reagents, this was the most successful at maintaining linearity at lower concentrations.

Further testing could also be done with alternative proteins. BSA is used for its stability, but it may not react in the same way as other proteins.

References

Bradford, M. "A Rapid And Sensitive Method For The Quantitation Of Microgram Quantities Of Protein Utilizing The Principle Of Protein-Dye Binding." *Analytical Biochemistry*, vol 72, no. 1-2, 1976, pp. 248-254.

Acknowledgements

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