Available online at www.abhipublications.org
Review Article
International Journal of Pharmacy and Engineering (IJPE)
ISSN 2320-849X

A review on determination of the concentration of solubilised protein

Sudipta saha[1]*, Sumit biswas[1], Parikshit Banerjee[1], Goutam Mukhopadhyay[1]

*Corresponding author at: Sudipta Saha, BCDA College of Pharmacy & Technology, Kolkata-700127, India. Email: sahasudipta24@gmail.com (S.Saha), Contact: +918961224404,

Abstract

The Bradford protein assay is a simple and accurate procedure for determining the concentration of protein in solution. It provides ready-to-use convenience by supplying the dye reagent at 1x concentration and two protein assay standards at seven prediluted concentrations. The pre diluted standards are conveniently packaged in 2 ml screw cap vials, eliminating wasteful and sharp ampoules, and ensuring protein stability over the shelf life of the product. It involves the addition of an acidic dye to protein solution, and subsequent measurement at 595 nm with a spectrophotometer or micro plate reader. We are trying to find out the comparison to a standard curve provides a relative measurement of protein concentration through a literature review in this paper.

Key Words: pre diluted standards, spectrophotometer/micro plate reader

1. Introduction

The Bio-Rad Protein Assay is a dye-binding assay in which a differential colour change of a dye occurs in response to various concentrations of protein.[1]The absorbance maximum for an acidic solution of Coomassie Brilliant Blue G-250 dye shifts from 465 nm to 595 nm when binding to protein occurs.[2,3,4] The Coomassie blue dye binds to primarily basic and aromatic

amino acid residues, especially arginine[5].Spector [6] found that the extinction coefficient of a dye-albumin complex solution was constant over a 10-fold concentration range. Thus, Beer's law may be applied for accurate quantitation of protein by selecting an appropriate ratio of dye volume to sample concentration. Interferences may be caused by chemical-protein and/or chemical-dye interactions.(Note: Basic buffer conditions and deter- gents interfere with this assay.) Since every protein-chemical reagent combination has not been assayed, it is possible that some of the listed reagents produce interference in combination with certain proteins. However, with respect to proteins such as bovine serum albumin and gamma globulin, the listed reagents show little or no interference. The acceptable concentrations of reagents for the Standard Procedure are shown in Table 1. Interference may be caused by chemical-protein and/or chemical-dye interactions. Table 1 lists those chemical reagents not directly affecting the development of dye colour. Since every protein- chemical reagent combination has not been assayed, it is possible that some of the listed reagents produce interference in combination with certain proteins. However, with respect to proteins such as bovine albumin and globulin, the above listed reagents show little or no interference.

Table 1 Reagents Compatible with the Bio-Rad Protein Assay When Using the Standard Procedure.*

Acetate, 0.6 M Acetone Adenosine, 1 mM Amino Acids Ammonium sulfate, 1.0 M Ampholytes, 0.5% Acid pH ATP, 1 mM Barbital BES, 2.5 M Boric acid Cacodylate-Tris, 0.1 M CDTA, 0.05 M Citrate, 0.05 M Deoxycholate, 0.1% Dithiothreitol, 1 M DNA, 1 mg/ml EDTA, 0.1 M EGTA, 0.05 M Ethanol Eagle's MEM Earle's salt solution Formic acid, 1.0 M Fructose Glucose Glutathione Glycerol, 99% Glycine, 0.1 M Guanidine-HCI Hank's salt solution HEPES buffer, 0.1 M	KCI, 1.0 M Malic acid, 0.2 M MgCl2, 1.0 M Mercaptoethanol, 1.0 M MES, 0.7 M Methanol MOPS, 0.2 M NaCl, 5 M NAD, 1 mM NaSCN, 3 M Peptones Phenol, 5% Phosphate, 1.0 M PIPES, 0.5 M Polyadenylic acid, 1 mM Polypeptides (MW<3000) Pyrophosphate, 0.2 M rRNA, 0.25 mg/ml tRNA, 0.4 mg/ml total RNA, 0.30 mg/ml SDS, 0.1% Sodium phosphate Streptomycin sulfate, 20% Triton X-100, 0.1% Tricine Tyrosine, 1 mM Thymidine, 1 mM Tris, 2.0 M Urea, 6 M Vitamins

*Interference may be caused by chemical-protein and/or chemical-dye interactions. Table 1 lists those chemical reagents not directly affecting the development of dye color. Since every protein-chemical reagent combination has not been assayed, it is possible that some of the listed reagents produce interference in combination with certain proteins. However, with respect to proteins such as bovine albumin and globulin, the above listed reagents show little or no interference.

2. Product Description

Protein Assay Dye Reagent Concentrate contains 450 ml of solution containing dye, phosphoric acid, and methanol. One bottle of dye reagent concentrate is sufficient for 450 assays using the standard assay procedure, or 2,250 assays using the microassay procedure. The Dye Reagent Concentrate can be purchased in a kit with one of two standards: Bovine gamma globulin (Kit I, catalog number 500-0001) or bovine serum albumin (Kit II, catalog number 500-0002).The Bio-Rad Protein Assay is for research use only.

2.1. For standard assay

Spectrophotometer set to 595 nm Cuvettes with 1 cm path length matched to laboratory spectrophotometer. Bio-Rad's disposable polystyrene curettes (catalogue number 223-9950) are recommended 13 x 100 mm test tubes Test tube rack for 13 x 100 mm test tubes Vortex mixer Whitman #1 filter (or equivalent) and funnel for dye reagent preparation Graduated cylinders, pipettes, and containers for reagent preparation and storage Pipettes accurately delivering 100 µl and 5.0 ml

2.2. For micro plate assay

Microtiter plates Pipets accurately delivering 200 µl and 800 µl

3. Instructions

Reconstituting the Standard: 20 ml of deionized water and mix until dissolved to reconstitute the lyophilized bovine gamma globulin and bovine serum abumin standards, and used within 60 days, otherwise should be aliquoted and frozen at -20 °C.

3.1. Standard Procedure [1]

1) Prepare dye reagent by diluting 1 part Dye Reagent. Concentrate with 4 parts distilled, deionized (DDI) water and filtered (Whatman #1 filter or equivalent). If in room temperature, should be used within two weeks.

2) Prepare three to five dilutions of a protein standard, which is representative of the protein solution to be tested. The linear range of the assay for BSA is 0.2 to 0.9 mg/ml, whereas with IgG the linear range is 0.2 to 1.5 mg/ml.

3) Pipet 100 µl of each standard and sample solution into a clean, dry test tube. Protein

solutions are normally assayed in duplicate or triplicate.

4) Add 5.0 ml of diluted dye reagent to each tube and vortex.

5) Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.

6) Measure absorbance at 595 nm.

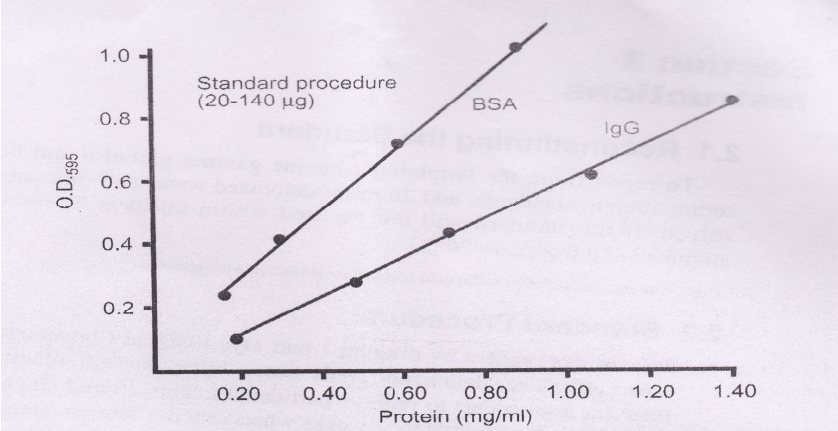

Fig. 1. Typical standard curve for the Bio-Rad Protein Assay, bovine gamma globulin (standard I), bovine serum albumin (standard II). $O.D._{595}$ corrected for blank - 200-1,400 µg/ml x 0.1 ml = 20-140 µg protein.

4. Microassay Procedure [1]

1) Prepare three to five dilutions of a protein standard which is representative of the protein solution to be tested. The linear range of the assay for BSA is 1.2 to 10.0 µg/ml, whereas with IgG the linear range is 1.2 to 25 µg/ml.
2) Pipet 800 µl of each standard and sample solution into a clean, dry test tube. Protein solutions are normally assayed in duplicate or triplicate.
3) Add 200 µl of dye reagent concentrate to each tube and vortex.
4) Incubate at room temperature for at least 5 minutes. Absorbance will increase over time; samples should incubate at room temperature for no more than 1 hour.
5) Measure absorbance at 595 nm.

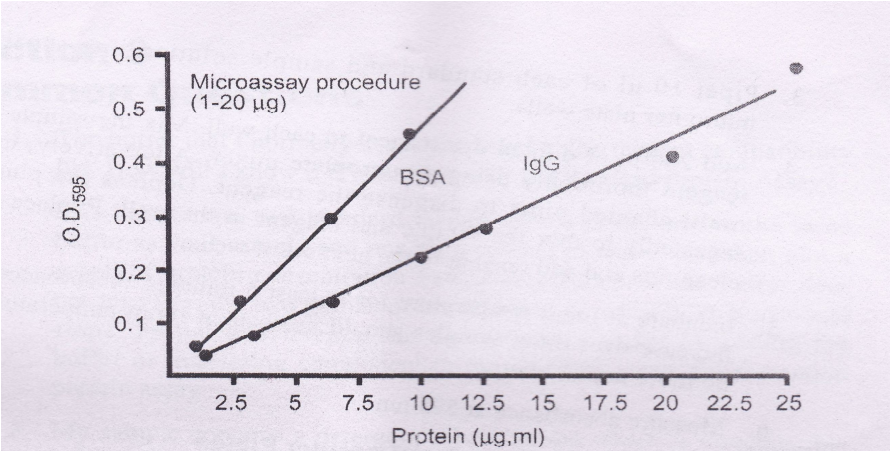

Fig. 2. Typical standard curve for the Bio-Rad Protein Microassay (1-20 µg/ml), bovine gamma globulin (standard I), bovine serum albumin (standard II).
$O.D._{595}$ corrected for blank. 1.25-25 µg/ml x 0.8 ml = 1-20 µg protein.

Example standard curves for the Thermo Scientific Pierce BCA Protein Assay Kit are shown in the above fig2**.** Eight concentrations of bovine serum albumin (BSA) and bovine gamma globulin (BGG) were assayed. The response values (absorbance) were plotted and a best-fit line drawn through the points. If unknown samples had been tested at the same time, their concentrations could be determined by reference to the one of these standard curves.

Sample assay response are directly comparable to each other if they are processed in exactly the same manner. Variation on amount of protein is the only possible cause for differences in final absorbance(colour intensity) if all three of the follow conditions are met:

- Samples are dissolved in the same buffer
- The same lot and stock of assay reagent is used for all samples
- All samples are mixed and incubated at the same time and temperature
- No pipetting errors are introduced

Of course, because of differences in the chemistry of protein assay methods, proteins will generate different absorbance values even at the same concentration. This is called “protein variation” or “protein uniformity”.

5. Microtiter Plate Protocols

The Bio-Rad Protein Assay can also be used with a micro plate reader, such as Bio-Rad's Model 450 and 3550 Micro plate Readers. The linear range of the Standard and Micro assay

procedures when used in the microtiter plate format is slightly changed, since the ratio of sample to dye is modified.

6. Conclusion

The Bio-Rad Protein Assay compares favorably with two other protein assay methods, the Biuret and the Lowry. The literature results showed that Bio-Rad method is most suitable and accurate compares to others assay methods. Bio-Rad Protein Standard I (bovine gamma globulin) was used for the Lowry and Bio-Rad Protein Assays, with bovine serum albumin was used for the Biuret method. All three assays considerable variation in response to different proteins, but the averages were comparable better in Bio-Rad method.

7. Acknowledgement

Authors are thankful to Principal of BCDA College of Pharmacy and Technology for technical support.

8. References

[1]. Bradford, M., *Anal. Biochem.*, **72**, 248 (1976).

[2]. Reisner, A. H., Nemes, P. and Bucholtz, C., *Anal. Biochem.*, **64**, 509 (1975).

[3]. Fazakes de St. Groth, S. et al., *Biochem. Biophys. Acta,* **71**, 377 (1963).

[4]. Sedmack, J. J. and Grossberg, S. E., *Anal. Biochem.*, **79**, 544 (1977).

[5]. Compton, S. J. and Jones, C. G., *Anal. Biochem.*, **151**, 369 (1985).

[6]. Spector, T., *Anal. Biochem.*, **86**, 142 (1978).

[7]. Duhamel, R. C., Meezan, E. and Brendal, K., *J. Biochem. Biophys. Methods,* **5**, 67 (1981).

[8]. Macart, M. and Gerbaut, L., *Clin. Chim. Acta*, **122**, 93 (1982).