Quantitation of Proteins by the Bradford, Lowry, and BCA Protein Assays Using the SmartSpec™ 3000 Spectrophotometer

Introduction

UV/visible spectrophotometry is among the most mature analytical techniques used in the laboratory. Since the commercialization of the spectrophotometer in the 1940s, methods have been developed for its use in many areas of scientific research. Today, spectrophotometers may be found in laboratories focusing on all major areas of research, such as analytical chemistry, functional genomics, and proteomics. Up until 1999, most laboratory spectophotometers were large instruments that relied on light emission and signal collection technology that has not changed dramatically in 50 years.

New advances in optics technologies have allowed the development of more affordable, compact spectrophotometers for routine laboratory use. Bio-Rad's SmartSpec 3000 spectrophotometer is one such benchtop instrument.

In this paper, results of colorimetric protein assays were compared for 2 different benchtop spectrophotometers and a more traditional high-end spectrophotometer. Protein concentration standards were assayed using the following protocols: Bradford,[1] Lowry,[2] and bicinchoninic acid (BCA).[3] Standard curves were generated using each spectrophotometer, and performance of the spectrophotometers was compared.

1. Bradford M, Anal Biochem 72, 248–254 (1976)
2. Lowry O et al., J Biol Chem 193, 265–275 (1951)
3. Smith PK et al., Anal Biochem 150, 76–85 (1985)

The SmartSpec 3000 spectrophotometer.

Method

Instruments

Two different spectrophotometers were compared to the SmartSpec 3000. One instrument, referred to as Spec A, is a benchtop spectrophotometer. Spec A has a user interface, operational features, and optics technologies that are similar to those of the SmartSpec 3000.

The other instrument compared to the SmartSpec 3000 is a high-end analytical spectrophotometer. This instrument, Spec B, has a more complex optics system and a traditional design. Spec B has a tungsten/deuterium lamp, a photomultiplier detection system, and dual-beam optics. Instruments like Spec B typically have a greater degree of accuracy and precision over a wider wavelength and absorbance range. In general, such instruments are used for a wider range of applications because they have more complex operating software and accessories packages.

Assay Types

Three popular colorimetric protein assay methods were used to compare spectrophotometer performance. They are:

- Bio-Rad protein assay kit I (Bradford)
- Bio-Rad *DC* protein assay kit I (modified Lowry)
- Pierce Chemical Company's BCA protein assay

The Bio-Rad protein assay kit is based on the Bradford dye-binding procedure.[1] The *DC* (detergent compatible) protein assay kit is an assay for protein solubilized in detergent. The reaction is similar to the well-documented Lowry assay,[2] modified to save time. The BCA protein assay is a third colorimetric assay method.[3]

Assay Protocols

Standards of bovine gamma globulin (provided in the Bio-Rad protein assay kits) were prepared at 7 concentrations, ranging from 0.2 mg/ml to 2.0 mg/ml. For the Bio-Rad assay kits, all 7 solutions were assayed using each instrument. For the BCA assay, 4 of the protein standard dilutions were assayed using each instrument.

Standards were prepared in triplicate for each assay method. The solutions were made and the assays performed according to the manufacturer's recommended protocol for preparation of a standard curve.

BIO-RAD

After color development, the samples were transferred to 1.5 ml disposable polycarbonate cuvettes. All 3 replicates of each standard were read using the 3 spectrophotometers included in the study. Each of the 3 protein assay methods used in this performance evaluation was strictly timed to ensure that changes in absorbance would not affect measurements.

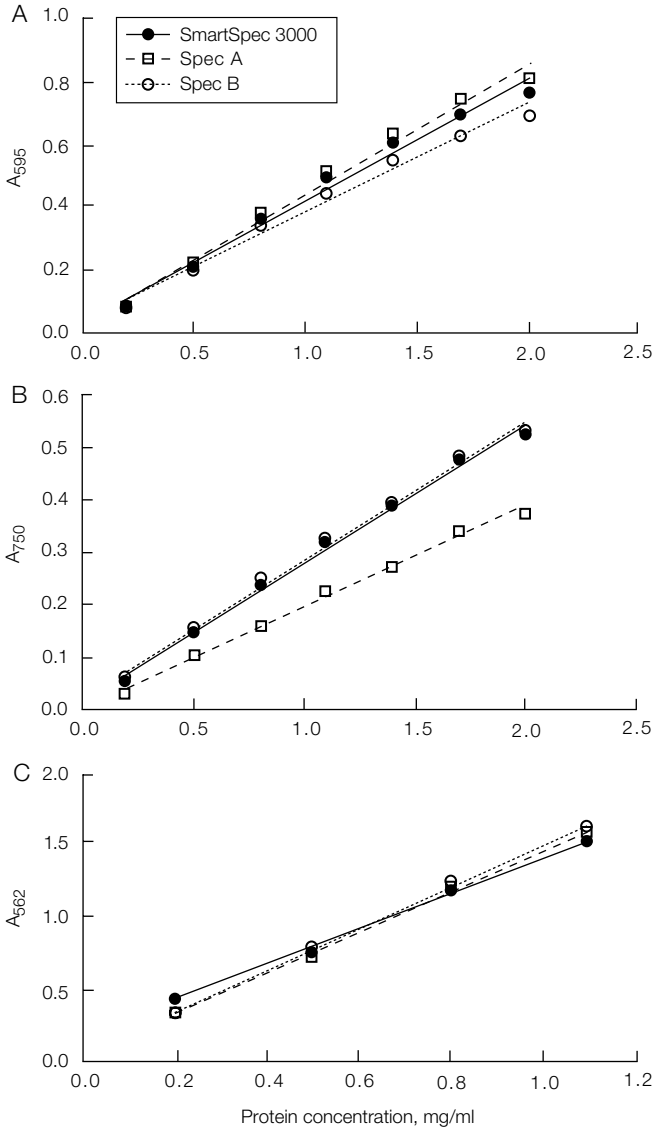

Fig. 1. A, Bio-Rad protein assay. B, Bio-Rad *DC* protein assay. C, BCA assay. Standard curves are from triplicate standards at each concentration (error bars showing standard deviation are smaller than the size of the symbols).

Results

Bio-Rad Protein Assay Kit I

The standard curves generated using each instrument have very similar slopes (Figure 1A). The standard curve generated by samples read in the SmartSpec 3000 falls between the curves generated by the other spectrophotometers. The correlation coefficient of the standard curve obtained using each instrument is >0.98. The standard deviation for each data point was <0.01 absorbance unit (AU).

Bio-Rad *DC* Protein Assay Kit I

For Bio-Rad's *DC* protein assay, the results using the SmartSpec 3000 and the high-end spectrophotometer, Spec B, are very similar (Figure 1B). The correlation coefficient calculated for each standard curve is >0.99. As with the Bio-Rad protein assay, the standard deviation for all data points from each instrument was <0.01 AU.

BCA Assay (Pierce Chemicals)

All 3 instruments showed values that were very close for the BCA assay. Figure 1C shows that the differences in the readings from each instrument were minor. The correlation coefficient calculated from the standard curve >0.99 for each instrument. The standard deviation calculated from data obtained in the BCA assay was <0.02 AU for all 3 instruments.

Discussion

For assays with a desired absorbance range between 0.1 and 1.5 AU, the benchtop spectrophotometers compare favorably to more traditional, high-end instruments. Although greater precision and accuracy can be achieved using high-end instruments for other applications, protein quantitation is limited more by the sensitivity of the assays than by the sensitivity of the spectrophotometer.

These results indicate that each of the spectrophotometers is acceptable for accurate protein quantitation by any of the 3 popular methods. The SmartSpec 3000 spectrophotometer performed similarly to the high-end instrument for all 3 assays.

Ordering Information

Catalog #	Description
Spectrophotometer and Accessories	
170-2501	SmartSpec 3000 Spectrophotometer
223-9950	Disposable Polystyrene Cuvettes, 3.5 ml, 100
223-9955	Disposable Polystyrene Cuvettes, 1.5 ml, 100
Bio-Rad Protein Assays	
500-0001	Bio-Rad Protein Assay Kit I, 450 standard 5 ml assays, bovine gamma globulin standard, based on the method of Bradford
500-0111	Bio-Rad *DC* Protein Assay Kit I, 500 standard 4 ml assays, bovine gamma globulin standard
500-0121	*RC DC* Protein Assay Kit I, reducing agent/detergent compatible protein assay, includes *DC* protein assay kit I components and *RC* reagents

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Web site *www.bio-rad.com* ***USA*** *(800) 4BIORAD* ***Australia*** *02 9914 2800* ***Austria*** *(01)-877 89 01* ***Belgium*** *09-385 55 11* ***Brazil*** *55 21 507 6191* ***Canada*** *(905) 712-2771* ***China*** *86-10-8201-1366/68* ***Denmark*** *45 44 52-1000* ***Finland*** *358 (0)9 804 2200* ***France*** *01 47 95 69 65* ***Germany*** *089 318 84-177* ***Hong Kong*** *852-2789-3300* ***India*** *(91-124) 6398112/113/114* ***Israel*** *03 951 4124* ***Italy*** *34 91 590 5200* ***Japan*** *03-5811-6270* ***Korea*** *82-2-3473-4460* ***Latin America*** *305-894-5950* ***Mexico*** *52 5 534 2552 to 54* ***The Netherlands*** *0318-540666* ***New Zealand*** *64-9-4152280* ***Norway*** *47-23-38-41-30* ***Russia*** *7 095 979 98 00* ***Singapore*** *65-2729877* ***Spain*** *34-91-590-5200* ***Sweden*** *46 (0)8-55 51 27 00* ***Switzerland*** *061-717-9555* ***United Kingdom*** *0800-181134*