Protein Quantification by Fast Scanning (Flash Xenon Lamp) Spectrophotometer

INTRODUCTION

SpectroArt 252 spectrophotometer is Wealtec's newly launched model which was upgraded from SpectroArt 200. In addition to the existing features such as flash Xenon lamp light source, narrow bandwidth (< 3 nm), reliable repeatability and compatibility with micro volume sample, SpectroArt 252 is upgraded with a faster and highly sensitive detection system, linear CCD detector. Adopted from previous version, SpectroArt 252 conserves six menu-driven analytic modes which provide fast analysis of protein/DNA quantification, bacterial growth, kinetics assay, specific wavelength detection and full spectrum scanning. In this article, the protein analysis function of SpectroArt 252 was examined by common-used protein quantification methods like Bradford, Lowry and BCA method. Furthermore, the protein concentration by detecting the absorbance at 280 nm was measured as well.

MATERIALS

- SpectroArt 252 (Wealtec)
- Bradford protein assay (Bio-Rad)
- BCA protein assay kit (Novagen)
- Lowry protein assay

PROCEDURES

1. Bovine serum albumin (BSA) samples were diluted to proper concentration.
2. Bradford, Lowry and BCA protein quantification assays were performed according to the instructions.
3. After loading into plastic cuvettes, the absorbance at relative wavelength (595 nm for Bradford assay, 750 nm for Lowry assay and 562 nm for BCA assay) was measured through the protein analysis category in SpectroArt 252.

4. Detection of the absorbance at 280 nm, the diluted protein samples were loaded into the 700 µl quartz cuvette (Starna®) directly and measured under "UV 280" option in protein analysis category.
5. After gathering the measurement data, the standard curve and R square value was analyzed in Microsoft Office Excel software.

RESULT

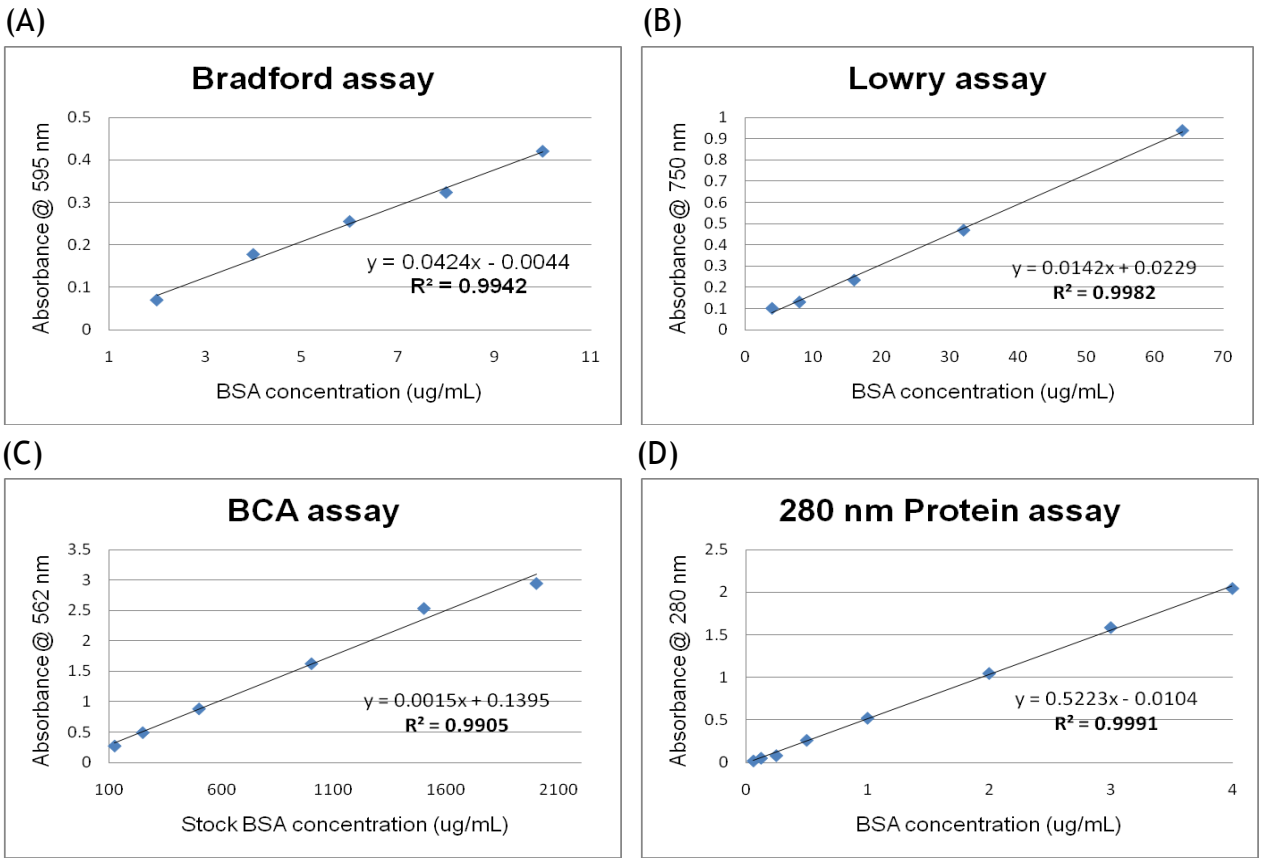

Figure 1. *Protein quantification by using SpectroArt 252. (A) Bradford, (B) Lowry, (C) BCA and (D) UV 280 nm assay were used.*

DISCUSSION

Bradford, Lowry and BCA assay are the most common used methods for protein quantification. For Bradford assay which has spectral shifting from 465 nm to 610 nm after reacting between Coomassie blue and aromatic amino acids, SpectroArt 252 was proved

to have effective range from 2 to 10 μg/ml and the R square value around 0.99 at 595 nm (*fig*. 1A). In addition, for the other two methods which based on protein-copper chelation, SpectroArt 252 was also proved to have effective range from 4 to 64 μg/ml in Lowry assay, and 125 to 2000 μg/ml (stock concentration) in BCA assay, respectively, with a very good linear regression (*fig*. 1B and 1C). Furthermore, protein itself has the maximal absorption at wavelength of 280 nm on those aromatic amino acids, such as tryptophan and tyrosine. By using of UV transparent quartz cuvette, protein concentration also can be determined in SpectroArt 252 by measuring the absorbance at 280 nm (*fig*. 1D).

To sum up, newly upgraded spectrophotometer, SpectroArt 252, was proved to have good performance on various protein quantification methods by using built-in protein functions of Bradford, Lowry, BCA assay and UV 280 nm detection. Moreover, with more stable UV module in SpectroArt 252, sample detection becomes much accurate and reliable.

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