Quantification by SpectroArt200 Bio-Spectrophotometer

INTRODUCTION

SpectroArt 200/200S* Spectrophotometer equipped with high intensity xenon flash lamp provides fast and stable spectral analysis. Functions include protein and nucleic acid quantification, bacterial growth, kinetic assay, specific wavelength and full spectrum scanning, FOI function, and Single Drop, applicable with all kinds of bioscience experiments. Based on different detection principles, common used protein assays with wide concentration detection range methods like Bradford, Lowry, and BCA assays were determined through sub micro cuvette. Applicable with different concentration DNA samples were also applied within various sample cuvettes for confirming the performance from sub micro cuvette, ultra-micro quartz cuvette, to fibre-optics ultra-micro quartz cuvette. SpectroArt was proved to have very stable and reliable performance among various sample detections.

*SpectroArt 200S supplied with built-in Single Drop analysis mode and Traycell, which applicable with 0.7 µl sample volume measurement.

MATERIALS

- SpectroArt 200/200S (Wealtec)
- Micro cuvette, 700 µL, 10 mm light path, quartz (Wealtec)
- Micro cuvette for 30 – 50 µL , 10mm light path, z-8.5 mm, black wall (Wealtec)
- Single Drop Fiber-Optic Ultra-Micro Cell 0.7 – 5 µL, light path 1.0 mm/0.2 mm (Wealtec)
- Calf Thymus DNA (Invitrogen)
- Bradford agent (Sigma-Aldrich)
- BCA protein assay kit (Merck)

PROCEDURES

Protein assay

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 micro cuvette 700 µL, 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 200.
4. Detection of the absorbance at 280 nm, the diluted protein samples were 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.

Nucleic acid detection

1. Dilute the Calf Thymus DNA solution from 10 to 100 µg/mL by sterilized distilled water.
2. Sample loading:
   a. Micro cuvette 700 µL: load 700 µL DNA sample into quartz cuvette.
   b. Micro cuvette for 30 – 50 µL: add 30 µL and 50 µL DNA sample into cell.
   b. Single Drop Fiber-Optic Ultra-Micro Cell: load 3 µL of DNA sample on top of the cuvette and then put on the 1.0 mm cap.
3. Insert the cell into the cuvette holder.
4. Measure DNA concentration with SpectroArt 200/200S.
5. After gathering the measurement data, the standard curve and R square value was analyzed in Microsoft Office Excel software.

RESULT

Protein assay

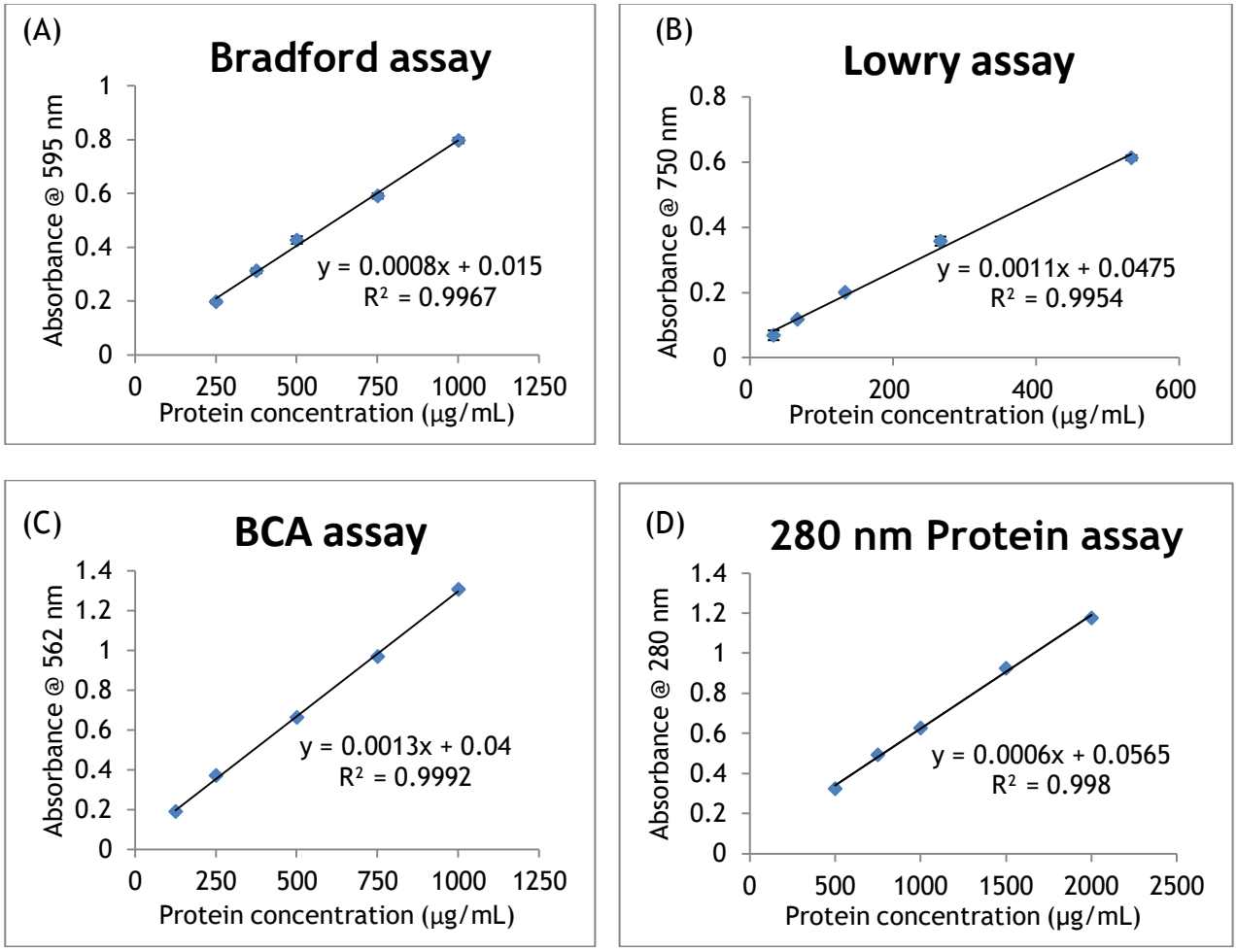

Figure 1. *Protein quantification by using SpectroArt 200. (A) Bradford, (B) Lowry, (C) BCA and (D) UV 280 nm assay were used.*

Nucleic acid detection

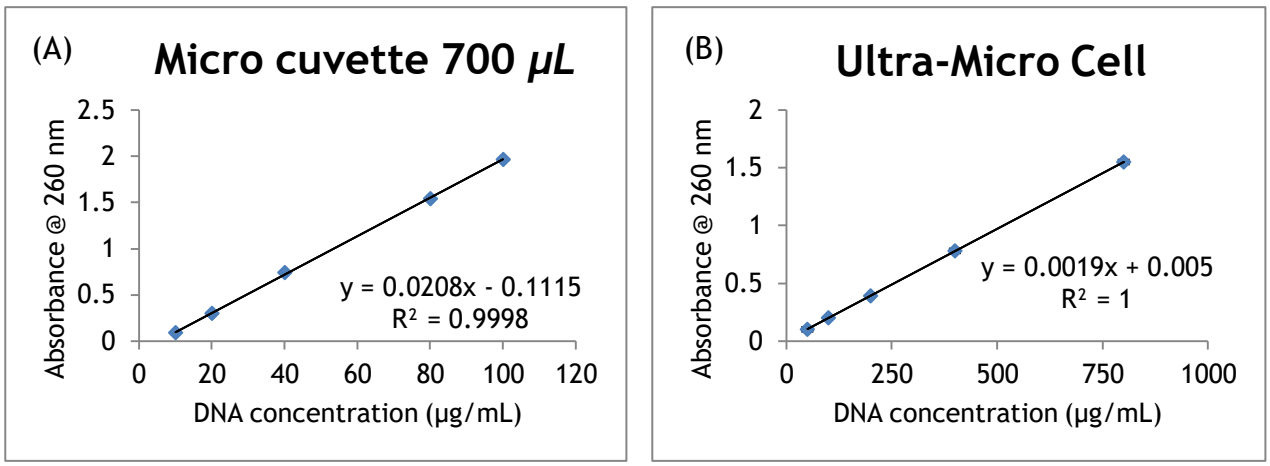

Figure 2. *The effective range of ds-DNA measurement by using SpectroArt 200S. (A) Micro cuvette 700 µL and (B) Single Drop Fiber-Optic Ultra-Micro Cell were used.*

Table 1. *ds-DNA measurements with micro cuvette for 30 - 50 µL.*

DNA sample (µg/mL)	Sample volume			
	50 µL		30 µL	
	Measurement	RSD%	Measurement	RSD%
10	10.46	3.90	10.82	3.24
20	20.72	2.10	19.57	2.74
40	39.66	0.79	41.07	1.96
80	81.34	0.53	82.31	0.65
100	100.05	0.78	101.90	0.62

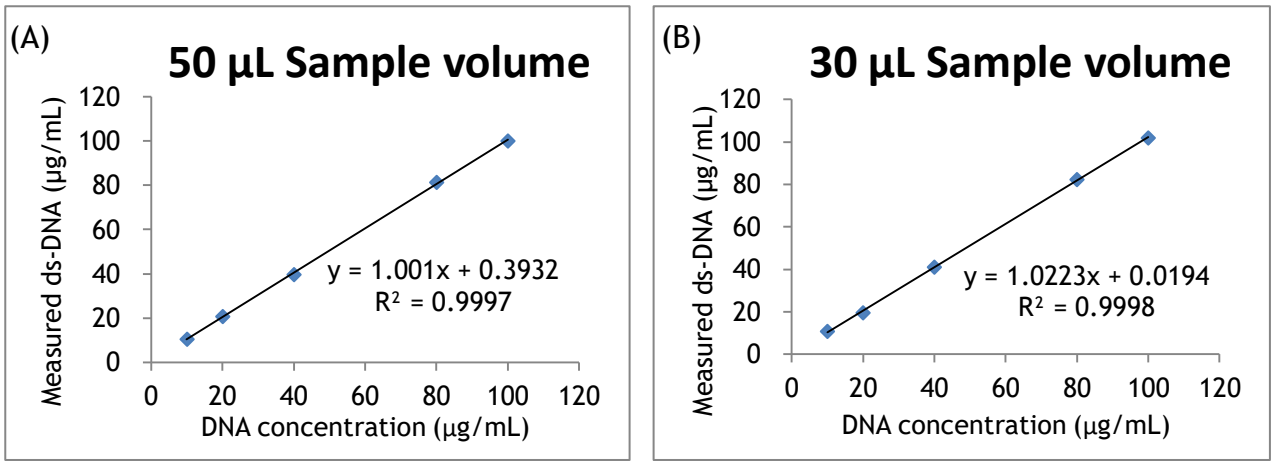

Figure 3. *Comparison data for ds-DNA concentrations by SpectroArt 200 with (A) 50 µL and (B) 30 µL sample volume.*

DISCUSSION

Bradford, Lowry and BCA assay are the most common used methods for protein quantification. In Bradford assay, the spectral shifting from 465 nm to 610 nm after reacted between Coomassie blue and aromatic amino acids. SpectroArt 200 was proved to have working range from 200 to 1000 μg/mL with the R square value around 0.99 at 595 nm (*fig*. 1A). For the other two methods which based on protein-copper chelation, SpectroArt 200 was also proved to have a very good linear regression working range from 33 to 533 μg/mL in Lowry assay, and from 125 to 1000 μg/mL in BCA assay, respectively (*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. Through UV transparent quartz cuvette, protein concentration was also determined in SpectroArt 200 by measuring the absorbance at 280 nm (*fig*. 1D).

According to Beer-Lambert's law, $A = \varepsilon bc$, the most reliable linear range between sample concentration and absorbance unit (AU) of spectrophotometer ranges from 0.2 to 1.2. With micro cuvette 700 μL, SpectroArt 200 can measure DNA concentration from 10 to 80 μg/mL with a perfect linear correlation, R square value 0.999, between AU and concentration in fig. 2A. Once the AU is over the working range, the concentration will be underestimated. For instance, as measure with 100 μg/mL sample, measurement result got lower, but the R-square value was still over than 0.999.

As applied with Ultra-Micro Cell in Single Drop function, SpectroArt 200S can measure samples with extremely small volume down to 0.7 ~ 5 μL. The measurement result was presented as in fig. 2(B) with excellent R-square value 1.0. Due to more delicate design in light path, it should be notice that slight movement of the cell would largely affect the measurement.

In order to lower down the applying sample volume, the Micro cuvette for 30 – 50 μL was designed to applicable for 50 μL sample volume. As in this experiment, the sample volume of Micro cuvette for 30 – 50 μL was proofed can be applied lower to 30 μL level. Referring to fig. 3C and 3D, all RSD was less than 3.9% (Table 1).

SpectroArt 200 was proved to have excellent performance on both kinds of common samples in this article. Equipped with high intensity flash Xenon lamp and super high sensitivity linear CCD detecting module, SpectroArt 200 provides the most accurate and reliable measurement result with the easiest but completed operation interface.

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