BRADFORD ASSAY - COMPARISON AMONG PHOTOPETTE®, SPECTROPHOTOMETER AND MICROPLATE READER

Pei She Loh and Volker Patzel, Department of Microbiology & Immunology, *National University of Singapore, Singapore.*

- *Photopette® enables measurement of protein concentration directly at the bench.*
- *The quality of measurements with Photopette was at least equal or better than with benchtop reference instruments.*

OBJECTIVE

In this application note we compare different readout instruments for the Bradford assay – The Photopette® device, a Shimadzu benchtop spectrophotometer and a BioTek microplate reader.

INTRODUCTION

The Bradford protein assay represents the gold standard method for protein quantification in solution. It finds applications in molecular biology, the life sciences and in the food and beverages industry.

It is calorimetric protein assay based on an absorbance shift of the Coomassie Brilliant Blue G-250 dye. The reaction is dependent on the amino acid composition of the measured protein. In acidic conditions, protein binds with the dye and alter its absorption maxima from 465 nm to 595 nm [1]. Figure 1 below illustrates the formation of the complex.

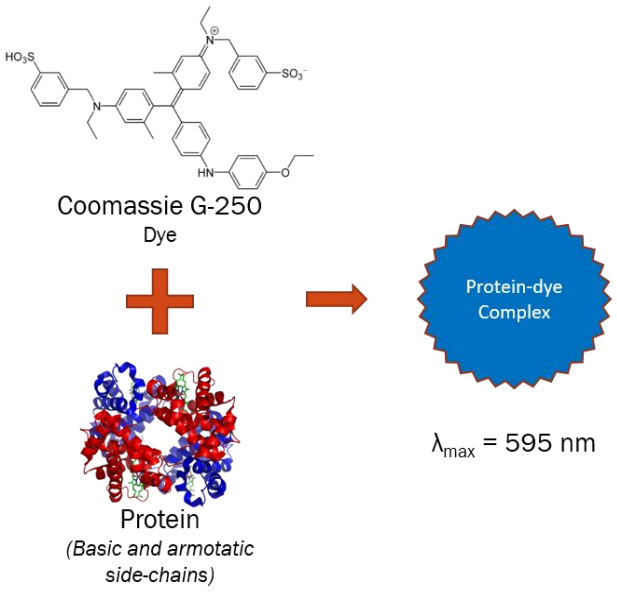

Fig 1: Working principle of Bradford assay for protein analysis.

A change in absorbance at 595 nm can be used to quantify protein concentration. Tip Biosystem's Photopette® devices OD600 and Cell support absorbance measurement at a peak-wavelength of 600 nm. Their design as portable handheld devices enable the users to bring the device to the sample and not the other way around.

MATERIALS AND APPARATUS

Instruments:

- Photopette® Cell or Photopette® OD600 with 600 nm wavelength.
- Spectrophotometer UV-1800 (Shimadzu).
- Microplate reader Synergy H1 (BioTek).

Reagents:

- Protein Standard (New England BioLabs, #B9000S).
- Bradford Reagent (Bio-Rad, #5000006).

METHOD

EXPERIMENTAL PROCEDURE

For the Bradford assay, absorbance at 600 nm (A600) was measured using a spectrophotometer (Shimadzu, UV-1800), a microplate reader (BioTek, Synergy H1) and Photopette® Cell and OD600 (Tip Biosystems) devices. Two independent measurements in duplicates were made with each of the three devices.

Sample preparations: The experimental protocol highlighted in Bio-Rad's Technical Bulletin (#5000006) for use of the Bradford Reagent in a spectrophotometer was modified for 1.5 mL volume [2]. This was necessary for measurements in cuvettes for the spectrophotometer. Photopette and the microplate reader only require 250 µL volume.

The Bradford reagent was prepared by diluting the Bradford stock reagent 1:40 with water. BSA solutions with

concentrations ranging from 0.05 to 1 mg/mL were prepared by serial dilutions from a protein standard (New England BioLabs, #B9000S).

The blank sample was prepared by mixing 30 µL water and 1.5 mL Bradford reagent, followed by an incubation for 5 minutes. The BSA protein samples were prepared by mixing 30 µL of the BSA serial dilutions with 1.5 mL Bradford reagent, followed by a 5 min incubation at room temperature.

Spectrophotometer: The double beam Shimadzu benchtop spectrophotometer was connected to the PC based software. Measurements were started 10 min after the device was switched on to allow the UV lamp to warm up and reach stable light output. The sample volume of 1.5 ml was placed in a plastic cuvette (10 mm pathlength) and A600 was measured. The blank sample was placed in the reference beam as negative control. The results of the measurements are displayed in Figure 2.

Photopette: The Photopette® device was connect to the Photopette® Android app and 'Bradford Assay' was selected as the measurement type. 'Dataset' was selected (additional settings might apply) and the measurement was started (*'Start Measurement'*). A pre-warming phase is not required for this LED-based device. A user manual for operating and safety precautions referring to the Photopette® is available online in video-tutorials at **www.tipbiosystems.com** [3]. A CuveTip™ was placed firmly on the device probe and was dipped into the blank sample to perform an auto-zero measurement. Subsequently the samples with different protein concentrations were measured using the same CuveTip. Between measurements, the CuveTip was washed by dipping into deionized water and dried by gentle contact with a wipe. All measurements were performed directly in the 2 mL cuvettes still holding the samples from the spectrophotometer measurements. Since longer incubation does not drastically increase absorbance for Bradford assay, the possibility of triggering systemic error can be excluded. The results of the measurements are displayed in Figure 3.

Microplate Rader: Sample volumes of 250 µL from the spectrophotometer readings were loaded into the wells of a 96 well microplate for the A600 reading with the microplate reader. 'Relative Absorbance' was calculated by deducting the baseline absorbance (blank) from the sample readings. The results of the measurements are displayed in Figure 4.

RESULTS

The measurement results obtained from the spectrophotometer, Photopette® and microplate reader are summarized in Table 1.

Concentration (mg/ml)	Spectro-photometer	Photopette®				Microplate reader
		#57	#58	#59	#303	
0.050	0.019	0.073	0.083	0.065	0.142	0.026
0.100	0.069	0.132	0.125	0.135	0.126	0.055
0.200	0.146	0.271	0.329	0.263	0.323	0.119
0.400	0.234	0.493	0.563	0.493	0.471	0.224
0.600	0.289	0.577	0.631	0.594	0.610	0.271
0.800	0.289	0.734	0.854	0.756	0.803	0.359
1.000	0.293	0.841	0.944	0.830	0.824	0.413

Table 1: Summary of average readings measured with a benchtop spectrophotometer, four Photopette® devices (manufacturing no. #0057, #0058, #0059 and #0303) and a microplate reader.

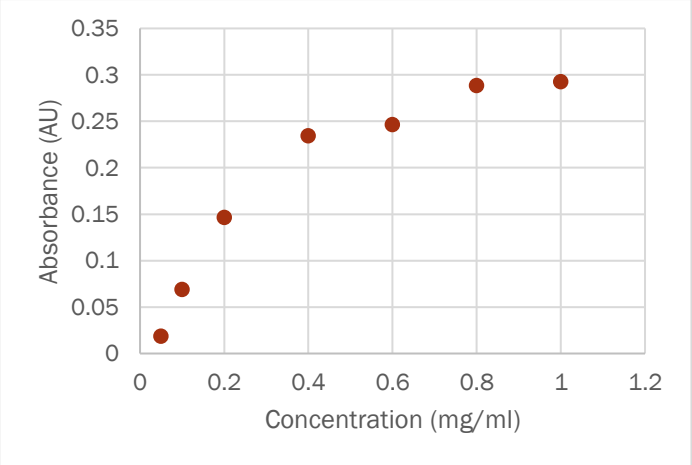

Fig 2: Absorbance (A600) of protein standards for Bradford Assay using the spectrophotometer.

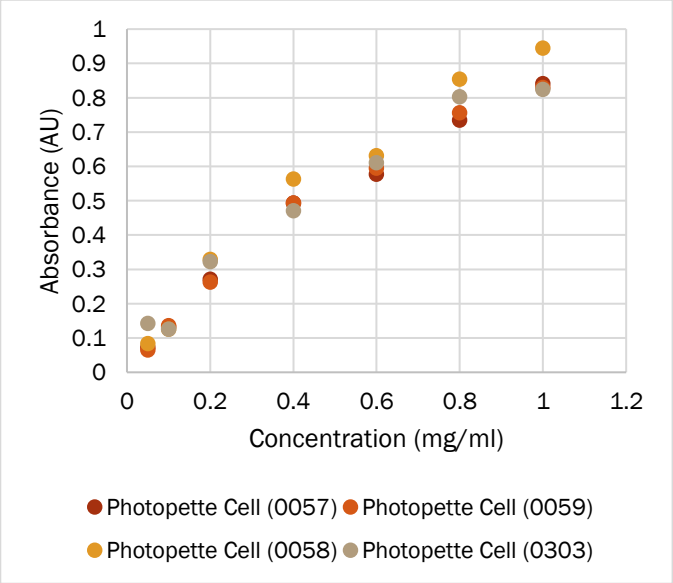

Fig 3: Absorbance (A600) of protein standards for Bradford Assay using four Photopette® devices.

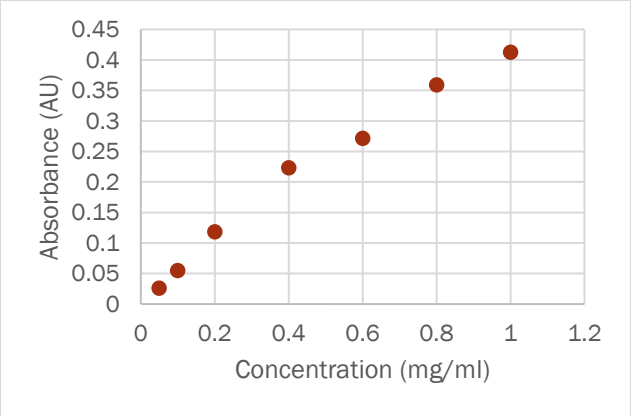

Fig 4: Absorbance (A600) of protein standards for Bradford Assay using the microplate reader.

RESULTS

EXPERIMENTAL PARAMETERS

Upper measurement-limit and linear range

According to Bio-Rad's recommendation for the use of the Bradford reagent, protein concentrations between 0.2–0.9 mg/ml were used to generate the standard curve. This is a limitation of the assay but not of any of the instruments.

STANDARD CURVE

Standard curves were measured with all devices using protein concentrations from 0.2 mg/ml to 0.8 mg/ml (Figures 5 to 7). A linear regression was performed on the data using Microsoft's Excel® software and the equations of the standard curves along with the R-squared values were obtained. The slopes of the standard curves obtained were 0.2195 (spectrophotometer), 0.8274 (Photopette) and 0.3846 AU/mg (microplate reader) respectively. The coefficient of determination (R^2) was highest for the microplate reader (R^2>0.982) followed by Photopette (R^2 = 0.973 to 0.962 for different devices) and lowest for the spectrophotometer (R^2>0.899).

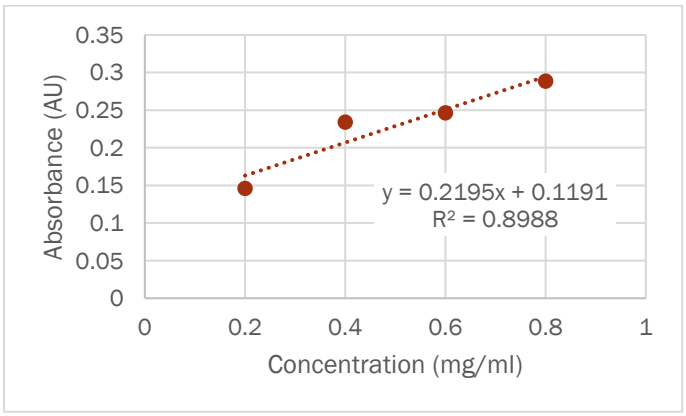

Fig 6: Standard curve for the Bradford assay measured with the spectrophotometer.

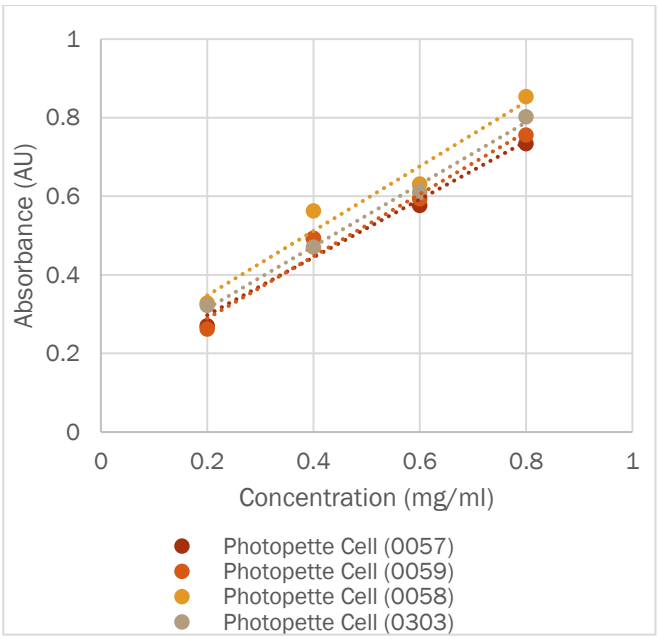

Fig 5: Standard curve for the Bradford assay measured with the Photopette® devices.

Unit #	Equation	R^2
0057	y = 0.8032x + 0.0842	0.9727
0058	y = 0.9126x + 0.0935	0.9633
0059	y = 0.8133x + 0.0818	0.9684
0303	y = 0.7803x + 0.1198	0.9623

Table 2: Summary of standard curve equations and R^2 values obtained from Photopette®.

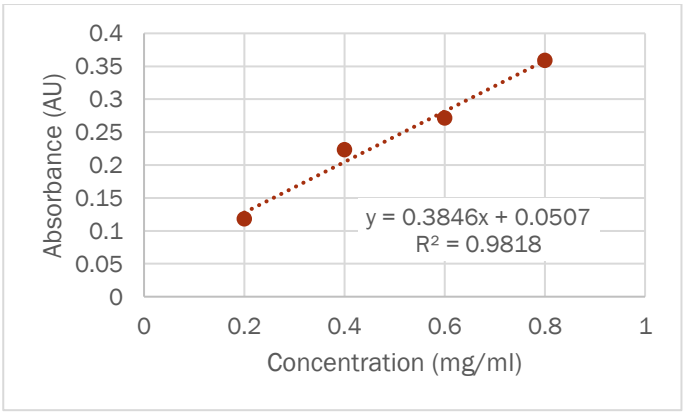

Fig 7: Standard curve for the Bradford assay measured with the microplate reader.

DETERMINING THE CONCENTRATION OF SAMPLES USING THE STANDARD CURVES

Using the standard curves, we quantified the protein concentrations of two samples. The concentration of Sample 1 was 0.33 mg/ml in the lower third of the concentration range used to measure the standard curve, the concentration of Sample 2 was 0.67 mg/ml in the higher third of the linear range. For the quantification, the measured absorbance (A600) was entered into the equation of the respective standard curve (y) *and the protein concentration (x) was calculated (Table 3).*

Instrument	Sample 1 concentration of 0.33 mg/ml)	Sample 2 Concentration of (0.67 mg/mL)
	Measured (error%)	Measured (error%)
Spectrophotometer	0.213 (-35%)	0.619 (-8%)
Microplate Reader	0.276 (-16%)	0.572 (-15%)
Photopette #57	0.324 (-2%)	0.547 (-18%)
Photopette #58	0.296 (-10%)	0.735 (+10%)
Photopette #59	0.296 (-10%)	0.578 (-14%)
Photopette #303	0.339 (+3%)	0.527 (-21%)

Table 3: Comparison of Photopette®, spectrophotometer and microplate reader.

SUMMARY & DISCUSSION

Bradford assays were performed using three different devices: a benchtop spectrophotometer, a benchtop microplate reader, and handheld Photopette devices. All instruments performed well but some differences are noted:

- Standard curves generated with the Photopette® devices exhibited a higher slope, indicating higher sensitivity of detection.
- Measurements performed with the Photopette and the microplate reader exhibited a larger dynamic range of linearity between protein concentration and absorbance compared to the spectrophotometer.
- Photopette® devices were most accurately quantifying the sample with the lower concentration, the spectrophotometer was most accurately quantifying the sample with the higher concentration.

Notably, the cost of the 3 instruments differ significantly: The Photopette® is by far the cheapest device. The microplate reader is about 4x and the spectrophotometer about 5x more expensive than the Photopette® device.

Measurements performed with the Photopette® devices were found to be at least as accurate as those performed with the other two much more expensive instruments. The measurement errors of the 3 different instruments are tabulated in Table 3. Taking the average of the errors for both samples; the spectrophotometer resulted in 21.5% error, the microplate reader in 15.5% error and the Photopette in 10 to 12% error (depending on the device) to the reference Samples 1 and 2. The high quality of Photopette® measurements is attributed to the way how the device works and analyses the data. Each Photopette® measurement is generated from a series of short measurements (LED flashes) and results are calculated by deduction of environmental light. Therefore, a result displayed at the Photopette® app is in fact an average of several measurements, in this way the random error is reduced. The LED technique of the Photopette does not call for a warmup period and the device were immediately ready to measure after switching it on.

The Photopette measures at environmental light conditions and there is no need to transfer the sample from the assay incubation tube into a cuvette or microplate; this saves considerable time.

ADAPTATION FROM MANUFACTURER'S PROTOCOL

Manufacturer's protocol for Bradford assay is designed for detection with spectrophotometer or microplate reader. To adapt the protocol for the use of Photopette®, the total volume of each assay can be reduced to as low as 250 µl.

REFERENCES

[1] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Anal. Biochem.*, vol. 72, no. 1–2, pp. 248–254, May 1976.

[2] Bio-Rad, "Bio-Rad Protein Assay."

[3] Tip Biosystems Pte Ltd, F. Omar, "Photopette User Manual V1.0.0," Singapore, 2017.

Conflict of Interest Statement

Coauthor Patzel is shareholder of Tip Biosystems. This shareholding did not affect the results, interpretations and conclusions provided in this application note.

Disclaimer

The views and opinions expressed herein are those of the authors and do not represent the views and opinions of the National University of Singapore or any of its subsidiaries or affiliates.