MEASURING PROTEIN CONCENTRATION DIRECTLY USING PHOTOPETTE® BIO

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- *Photopette® enables fast protein measurements directly at the bench.*
- *Protein samples can be directly measured at 280 nm without sample preparation and sample transfer.*

OBJECTIVE

The objective of this application note is to demonstrate how the Photopette® Bio device can directly measure protein concentrations in an unknown sample. Furthermore, it acts as a handy guide to get you started with Photopette® Bio and outlines application-specific parameters for reference.

INTRODUCTION

Proteins are building blocks in all lifeforms. Plants, animals and microorganisms contain proteins in form of enzymes, tissues, hairs, etc. This application note introduces the quantitative measurement of protein concentrations by directly measuring the ultra-violet light (UV) absorbance at 280 nm using Photopette® Bio.

Determination of protein concentration in an aqueous solution is common procedure in life-science, biomedical and pharma research. A purified protein-sample can be analyzed directly and quantified by measuring its optical absorbance at 280 nm (i.e. the "Protein A280 method") [1]. This method does not require additional reagents or sample-prep or even generation of a standard curve for a known protein type. A typical UV absorbance spectrum for a protein is shown in Figure 1.

Aqueous solutions of proteins have absorbance maxima at 220 nm and 280 nm. Amino acids with aromatic rings (such as tyrosine and tryptophan) and/or Cys-Cys di-sulphide bonds within the proteins are the primary reason for the absorbance peak at 280 nm. Peptide bonds are primarily responsible for the peak at ~220 nm [3], [4].

In comparison, the DNA absorbance maximum is at 260 nm. As seen from Figure 1, the contamination of protein samples with DNA will increase the UV 280 absorbance and cause errors. For accurate measurements, the protein sample shall not contain other components such as nucleic acids or phenolic or aromatic components that absorb in the UV region. Different purified proteins such as BSA or immunoglobulins have slightly different absorbance as given in Table 2. Other factors such as pH, ionic strength and the 3D-structure of protein etc. can also affect the absorbance spectrum of proteins [5].

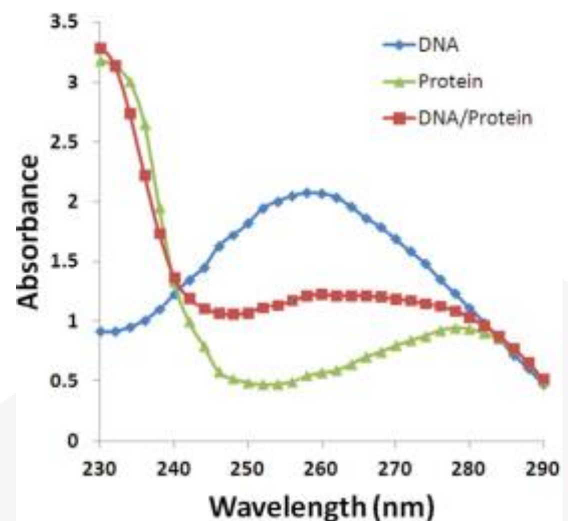

Figure 1: Typical UV absorption spectrum for a protein and a DNA sample and DNA/protein mixture [2]

MATERIALS AND APPARATUS

Instruments:

- Photopette® Bio with 280 nm wavelength.

Reagents:

- Protein Standard (Sigma Aldrich #P0834)

METHOD

Highlighted in this section are the steps to perform a direct-protein concentration measurement at 280 nm. It is advisable to perform an application specific risk-assessment analysis for the sample (e.g. clinical samples) before performing an experiment. Please refer to the "Photopette User Manual" for operating and safety precautions [6].

EXPERIMENTAL PROCEDURE

Turn-on the Photopette® device and connect to the Photopette® iOS/Android app. Select 'Direct-Protein' as the measurement type. Select dataset and set additional settings (if needed) before selecting *'Start Measurement'*. Please follow the video-tutorials on the Tip Biosystems webpage to get familiar with the measurement process.

Deionized water was used as measurement-blank. Alternative the respective buffer of you sample can be used. A CuveTip™ was placed firmly on the device probe and dipped into the blank sample to perform an auto-zero measurement. Please ensure that there is no air-bubble trapped in the CuveTip™ cavity as presence of air-bubbles can disrupt the optical-path and can result in erratic values.

Bovine serum albumin (BSA) solutions with concentrations ranging from 0.25 to 8 mg/mL were prepared by serial dilutions. Absorbance values for the samples were measured with the Photopette® Bio device and three repeats were taken for each measurement. The results of the measurements including the standard deviation (SD) and coefficient of variation (CV) are tabulated in Table 1.

Concentration (mg/ml)	AU 280	SD	CV
8	2.727	0.004	0.15%
7	2.602	0.012	0.44%
6	2.505	0.003	0.10%
5	2.420	0.003	0.10%
4	1.926	0.003	0.16%
3	1.662	0.002	0.10%
2	1.246	0.006	0.51%
1	0.624	0.002	0.33%
0.5	0.363	0.002	0.57%
0.25	0.120	0.001	0.48%

Table 1: Absorbance at 280 nm for BSA standard protein solutions

The same measurements as in Table 1 were performed (data not shown) with 4 other Photopette® Bio devices and all data from the 5 devices was plotted in Figure 2.

DETERMING CONCENTRATION OF AN UNKNOWN SAMPLE

Following the similar approach, absorbance of an unknown sample can be conveniently measured using the Photopette® device. The protein concentration can be calculated by substituting the absorbance value in the equation of the standard curve deduced in the next section.

DISCUSSIONS

The data from 5 different Photopette® Bio devices was plotted in Figure 2 as an overlay plot to demonstrate device to device performance.

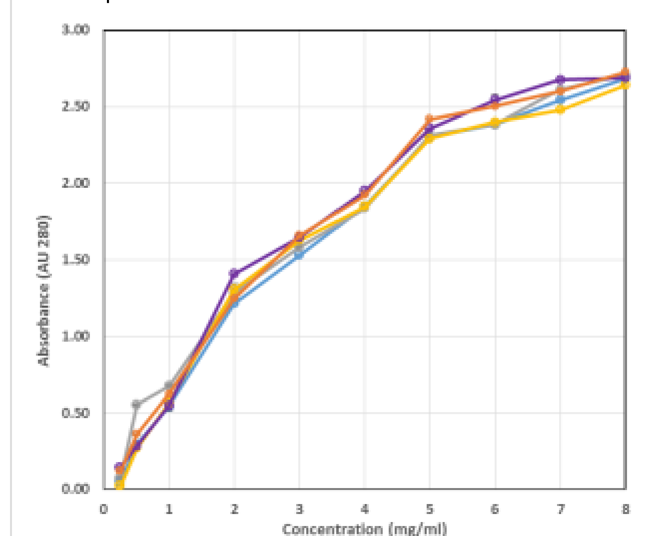

Figure 2: Absorbance as a function of protein concentration of 5 different Photopette devices.

Device to device variation was in an acceptable range. For any analytical instrument, it is advisable to perform a calibration and use the same instrument for measurements of an unknown sample.

EXPERIMENTAL PARAMETERS

STANDARD CURVE

A standard curve for BSA absorbance at 280 nm was plotted for Photopette (blue solid line) and a conventional spectrophotometer using a 10 mm cuvette (orange solid line) in Figure 3. In addition, the theoretical BSA absorbance was plotted as a dotted line in Figure 3.

Upper measurement-limit and Linear Range

An acceptable linear relationship was observed up to 2 mg/ml protein concentration for both instruments. By using the calibration curve for Photopette®, concentrations of up to ~8 mg/ml can be measured without dilution. In comparison, the measurement range of the conventional spectrophotometer was limited to about ~5 mg/ml at where the calibration curve starts to plateau. Regression analysis of the Photopette® calibration curve at its linear range using Microsoft's Excel® software resulted in a sloop of 0.666 AU ml/mg and an R-squared value of 0.992.

Photopette® users may download a pre-configured worksheet for this experiment from our online resource section. The worksheet is compatible with Microsoft Excel® and similar worksheet software, and will aid users in

performing the calculations and generating the standard curve.

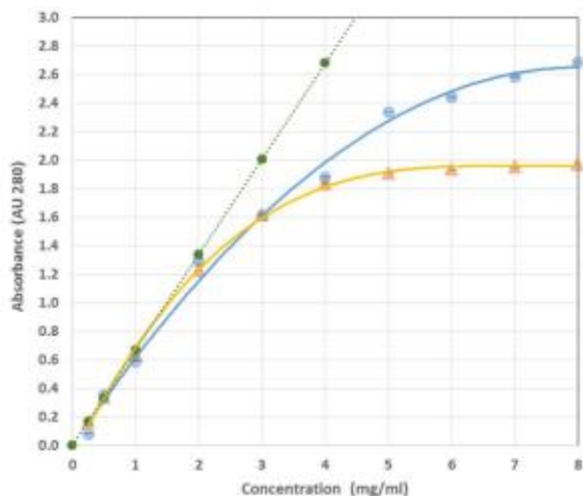

Figure 3: Standard Curve for direct protein measurement using Photopette®

Limit of Detection

The Limit of Detection (LOD) for this measurement using Photopette® is determined by factoring in the standard-deviation for blank measurements as well as experimental data using the equation given below [7]:

$$LOD = 3 \times SD_{blank} / Slope_{Standard\ Curve}.$$

The Standard Deviation (SD) for blank measurements (50 repeats) was found to be 0.005 AU. The limit of detection for direct protein measurement with Photopette® using the equation above was 0.023 mg/ml or 23 µg/ml.

$$LOD = 3 \times 0.005\ AU / 0.666\ ml\ AU/mg$$

$$= 0.023\ mg/ml$$

ABSORBANCE OF OTHER PROTEINS

Different proteins have slightly different absorbance values. Some examples are given in the following Table 2.

Protein solutions with absorbance A280 = 1	
Protein	Concentration (mg/ml)
BSA	1.50
IgG	0.71
Protein mixture	1.00

Protein solutions at 1 mg/ml concentration	
Protein	Absorbance A280
BSA	0.66
IgG	1.40
Protein mixture	1.00

Table 2: Absorbance values of selected proteins

Determining the concentration of an unknown protein sample

The concentration "C" of an unknown protein sample can be determined in two ways: A) By preparing a calibration curve of the same protein, or B) By using tabulated values of the UV absorbance of the unknown protein such as provided in Table 2. For method A, the purified unknown protein must be available and for method B the identity of the unknown protein and its absorbance values must be available. If this is not the case, the value for protein mixtures of Table 2 may be used.

For the example of the BSA measurement of this application note, both methods result in the same result. For example, the absorbance of an unknown BSA sample was measured as 0.85 AU. Using method "A", the absorbance value is divided by slope of the calibration curve 0.666 AU ml/mg:

$$C = AU / Slope_{Standard\ Curve}$$

$$C = 0.85\ AU / 0.666\ AU\ ml/mg$$

$$C = 1.276\ mg/ml$$

Using method "B", the absorbance value for BSA at a concentration of 1 mg/ml from Table 2 is used and the measured absorbance value is divided by the tabulated value:

$$C = AU / \text{Tabulated Absorbance}_{1\ mg/ml}$$

$$C = 0.85\ AU / 0.66\ AU\ ml/mg$$

$$C = 1.288\ mg/ml$$

LIMITATIONS

The A280 measurement approach is only applicable for samples free of any contaminants. It cannot be used for samples where impurities also have absorbance at 280 nm.

For direct measurements, without using a calibration curve, the nature and absorbance of the sample must be known. Otherwise, the values for a protein mix can be used, however this will result in a larger error.

SUMMARY

Photopette® Bio can be used for direct protein measurements of e.g. BSA up to protein concentrations of 2 mg/ml corresponding to an absorbance of about 1.4 AU with very minimal errors. For higher concentrations it is advisable to perform a calibration. Up to 8 mg/ml BSA corresponding to an absorbance of 2.6 AU can be measured in this way. In terms of its dynamic range, Photopette® Bio has outperformed the tested conventional spectrophotometer. Because Photopette® Bio does not require sample transfer or dilution, measurements can be

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performed in less than 10 seconds directly at the laboratory bench or in the field. The instrument was very simple and easy to use. The Photopette® App allowed data storage and instant data transfer to cloud storage and email. Photopette® data can be analyzed by existing software such as Microsoft Excel®. Allover, Photopette® is an attractive addition to the modern laboratory that improves productivity and enables non-trained personal to perform quality measurements.

REFERENCES

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[2] Peter Brescia, BioTek Instruments, Inc., Winooski, VT, USA

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[4] C. M. Stoscheck, “Quantitation of protein.,” *Methods Enzymol.*, vol. 182, pp. 50–68, 1990.

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[7] Using 3 x SD will result in a confidence of 99.86%.

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