APPLICATION NOTE

UV/Visible Spectroscopy

Determination of Total Protein Using the LAMBDA UV/Vis Spectrophotometer: Lowry Method

Introduction

The Lowry and Biuret methods are standard methods for protein quantification. Though the latter is more sensitive and is used for investigative work, it is limited by (1) poor stability of the combined reagent, (2) non-reproducibility of color, especially at low protein concentrations, and (3) a non-linear chromogenic response with protein concentration. Ohnishi and Barr[1] modified and simplified the Biuret combined reagent for the Lowry procedure and at the same time improved its stability. This application note describes the modified Lowry procedure for protein analysis.

Principle

The blue color which appears during the Lowry method results from (1) the reaction between Cu^{2+} and a peptide bond and (2) reduction of the chelating reagent (Phenol reagent, Folin reagent, Lowry reagent or Folin-Ciocalteu reagent) which includes phosphomolybdic acid from the tyrosine and tryptophane cystein residues in the protein.

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Reagents and Apparatus

1. Protein standard (BSA) 1 mg/ml
2. Biuret reagent: cupric sulfate 75 mmol/L, sodium chloride 94 mmol/L plus sodium tartrate, iodide and carbonate
3. Folin-Ciocalteu's phenol reagent: 2.0 N
4. Unknown protein
5. Sodium chloride solution (0.85%)
6. LAMBDA™ 465 UV/Vis Spectrophotometer
7. UV Lab™ Software
8. Cuvettes (10 mm pathlength)

Procedure

1. Prepare the protein solutions, mixing protein and saline solution in each of the six test tubes as in Table1.
2. Add 2.2 ml of Biuret reagent into the standards and unknown sample, mix and leave for 10 minutes.
3. Add to each tube 0.1 ml of Folin-Ciocalteu's phenol reagent. Mix each tube thoroughly immediately after addition.
4. Select the Lowry method among method files in Quantification mode.
5. In Quantification standard mode, measure the absorbance of test tube standards 2 to 5 with reference to tube 1 at a wavelength of 725 nm.
6. In Quantification Sample mode, measure the unknown sample (tube 6).
7. Plot the absorbance of standards vs. their concentration.
8. Compute the concentration of the unknown sample.

Table 1. Concentration of protein standards.

Reagent	Test Tube No.					
	1	2	3	4	5	6
1 mg/ml Standard Protein (ml)	-	0.05	0.1	0.15	0.2	-
Unknown Protein (ml)	-	-	-	-	-	0.2
Saline Solution (ml)	0.2	0.15	0.1	0.5	-	-
Concentration (mg/dl)	0	25	50	75	100	-

Instrument Parameters

The instrument parameters of the LAMBDA 465 are as follows:

Experiment Setup

Data type: Absorbance
Sampling: Single cell
Mode: Fast (Spectra no.: 1 / Scan no.: 50/ Integration no.: 1 / Gain no.: 1)

Experiment Method

Quantification method: Lowry method
Wavelength used: 725 nm
Curve dimension: 2

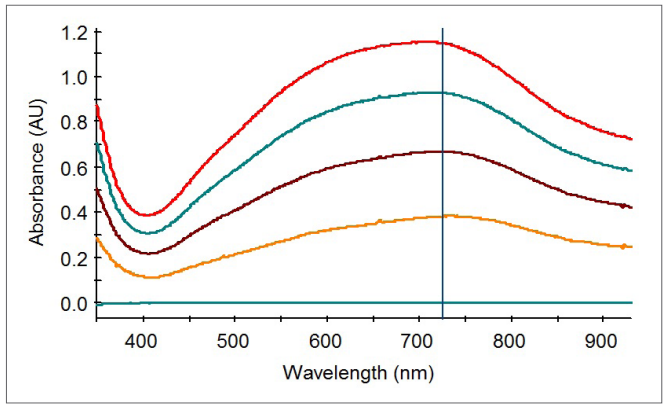

Figure 1. Spectra from the Lowry method.

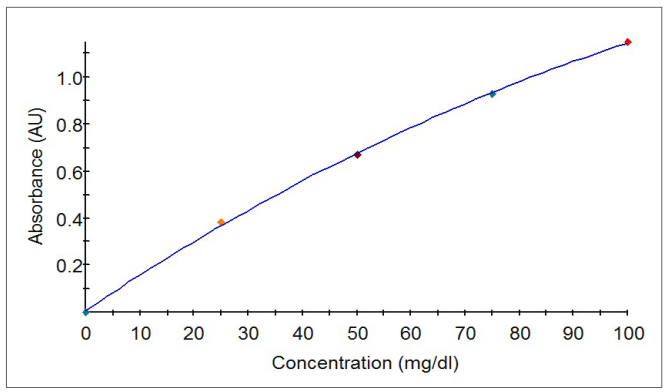

Figure 2. Calibration curve from Lowry method.

Result

1. Calibration curve

Figure 1 shows the spectra of the protein standard solutions. They represent the max absorbance at 700-750 nm and measured absorbance at 725 nm. Figure 2 shows the resulting second-order (quadratic) calibration curve. The correlation coefficient R^2 is 0.9995.

2. Unknown Protein sample

The concentration of the unknown sample was calculated to be 74.23 mg/dl as shown below.

Table 2. Calculated concentration for the unknown protein sample.

Name	Concentration (mg/dl)	Au (725.00 nm)
Unknown	74.23	0.9288

Conclusion

Quantitative analysis of protein was performed using the LAMBDA 465 and UV Lab software. Rapid acquirement of spectra and good sensitivity were obtained with the LAMBDA 465. The Quantification mode in the UV Lab software was used effectively for the quantitative analysis and to process the data efficiently.

References

1. Ohnishi ST, Barr JK : A simplified method of quantifying proteins using the Biuret and phenol reagent. Anal Biochem 86: 193, 1978

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