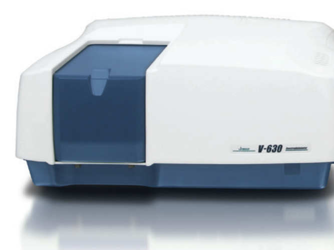
Application Note
UV-0003

Quantitative Determination of Proteins

Introduction

One of the easiest and most accurate spectroscopic tool for determining protein concentration is by UV-Visible spectrophotometers. The V-630 is designed for biochemical analysis and equipped with 6 quantitative calibration curves based on UV absorption spectrophotometry including Lowry, Biuret, BCA, Bradford, and WST methods.



V-630
UV-Visible Spectrophotometer

Table 1 shows the features of each of the six quantitation methods. The preferred method can be selected based on the desired concentration range and whether any contaminants or agents present in the sample will influence the quantitation results. Five of the quantitative analysis methods utilize a chromogenic reaction. While reagent manufacturers produce chromogenic kits for BCA, Bradford, and WST methods, as well as an instruction manual explaining the measurement procedures, the Lowry and Biuret methods require the reagent to be prepared by the user. Therefore, the measurement procedures for the Lowry and Biuret methods can differ and the quantitative results will depend on the types of proteins and differences in the concentration ranges used.

This application note will describe how to create calibration curves for the Lowry, Biuret, and UV-Visible absorption analysis methods to determine protein concentration.

Keywords

V-630, UV-Visible/NIR, Calibration curve, Quantitative, Biochemistry

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Table 1. Protein quantitation methods.

Methods	Principle	Concentration Range*	Advantages	Disadvantages
UV Abs	The absorption maximum at 280 nm corresponds to the response of the tyrosine and tryptophan residues used for the analysis method.	50 - 2000 µg/mL	Simple method, sample can be used after measurement.	Absorbance differs for each protein. Collagen and gelatin do not have absorption at 280 nm and cannot be measured. Nucleic acids with UV absorption can obscure measurement.
Biuret	An alkaline solution of Biuret reagent including copper sulfate and Rochelle salt is added to a protein solution, which turns purple after the polypeptide chain chelates with a copper ion. Uses the absorption maximum at 540 nm.	150 - 9000 µg/mL	Simple procedure, chromogenic rate is constant for most proteins.	Low sensitivity. Sample with low protein concentration cannot be measured. Chromogenic reaction is influenced by high concentrations of trisaminomethane, amino acids, and NH_4^+.
Lowry	An alkaline copper solution is added to the protein solution. Tyrosine, tryptophan, and cysteine reduce the molybdenum acid and phosphotungstic acid of the phenol reagent, turning the solution blue. Uses the absorption maximum at 750 nm.	5 - 20 µg/mL	High sensitivity, widely used.	Complicated procedure with long preparation time. Chromogenic reaction occurs via reduction reaction so contamination of reduction material interferes with quantitative determination. Chromogenic rate differs for each protein.
BCA	Combines the Biuret and Bicinchoninic acid (BCA) methods. When a protein reacts with 2 molecules of BCA, a copper ion is formed and turns the solution purple. Uses the absorption maximum at 560 nm.	20 -2000 µg/mL	Simple procedure, high sensitivity, wide concentration range.	Thio, phospholipid, and ammonium sulfate interfere with measurements.
Bradford	The absorption maximum shifts from 465 to 600 nm when the protein binds to Coomassie Brilliant Blue G250. Uses the absorption maximum at 600 nm.	10 -2000 µg/mL	Simple operation, little influence from blocking agents.	Chromogenic rate differs for each protein. Contamination by the surfactant can interfere with chromogenic reaction.
WTS	WST-8 reduces upon interaction with high pH proteins, turning the sample solution blue. Uses the absorption maximum at 650 nm.	50 -5000 µg/mL	Simple operation, little influence from surfactant.	Chromogenic rate differs for each protein.

**Bovine Serum Albumin*

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Results

UV-Vis Absorption Method

The absorbances of bovine serum albumin (BSA), hen egg lysozyme (HEL), and chymotrypsin from bovine pancreas were measured at 280 nm. Calibration curves were created using the follow concentrations. Bracketed concentrations used the 10 mm rectangular quartz cell while all other values were measured using the micro cell. The results of the calibration curves are listed in Table 2.

BSA	0.02, 0.025, 0.05, 0.1. 0.2, 0.25, 0.4, 0.5, 1.0, (1.5, 2.0) mg/mL
HEL	0.02, 0.025, 0.05, 0.1. 0.2, 0.25, (0.4, 0.5) mg/mL
α-chymotrypsin	0.0, 0.1. 0.2, 0.25, 0.4, 0.5 mg/mL

Table 2. Quantitative results for the UV-Vis absorption method.

	Cell	Concentration Range	Calibration Curve	Correlation Function	Standard Error	Detection Limit	Determination Limit
BSA	10 mm quartz	to 2 mg/mL	Y = AX + B A=0.6652 ±0.0079 B=-0.0130 ±0.0064	0.9994	0.0219	0.0097 mg/mL	0.0470 mg/mL
	Micro cell	to 1 mg/mL	Y = AX + B A=0.6713 ±0.0043 B=-0.0016 ±0.0008	0.9999	0.002	0.0012 mg/mL	0.0218 mg/mL
HEL	10 mm quartz	to 0.5 mg/mL	Y = AX + B A=0.6474 ±0.0459 B=-0.0150 ±0.0109	0.9991	0.0076	0.0041 mg/mL	0.0680 mg/mL
	Micro cell	to 0.25 mg/mL	Y = AX + B A=2.7499 ±0.0429 B=-0.0060 ±0.0055	0.9995	0.0031	0.0020 mg/mL	0.0096 mg/mL
α-Chymotrypsin	10 mm quartz	to 0.5 mg/mL	Y = AX + B A=1.904 ±0.0237 B=-0.0035 ±0.0070	0.9997	0.0042	0.0037 mg/mL	0.0615 mg/mL
	Micro cell	to 0.5 mg/mL	Y = AX + B A=2.1279 ±0.0655 B=-0.0202 ±0.0193	0.9983	0.0104	0.0091 mg/mL	0.1263 mg/mL

Biuret Method

To prepare the Biuret reagent, 60 mL of 10% NaOH was added to 100 mL of aqueous solution to dissolve 0.3 g $CuSO_4$ and 1.2 Rochelle salt. Then 200 mL of water was added to this mixture.

2.0 mg of the Biuret reagent is added to 500 µL of an aqueous protein solution. To determine the length of time necessary for the chromogenic reaction to stabilize, the absorbance of human serum albumin (HSA) was monitored at 540 nm and shown on the left in Figure 1. The data indicate that the Biuret reagent should react with protein samples for at least 60 minutes before absorbance measurements are obtained.

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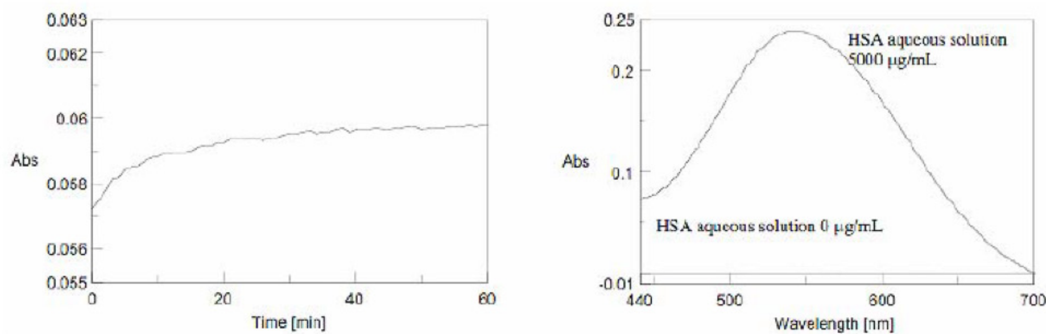

Figure 1. Time course measurement (left) of the chromogenic reaction and the absorption spectrum (right) of HSA reacted with the Biuret reagent. The absorption maximum is 540 nm.

Figure 2 illustrates the color change upon addition of the Biuret reagent to protein solutions.

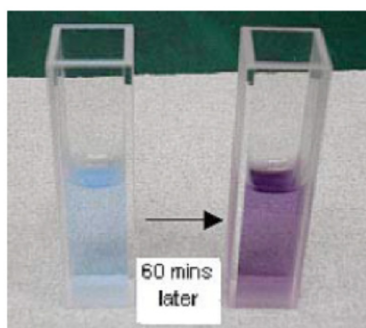

Figure 2. Color change in protein solutions after the addition of the Biuret reagent.

The absorbances of bovine serum albumin (BSA), hen egg lysozyme (HEL), and chymotrypsin from bovine pancreas were measured at 540 nm. Calibration curves were created using the follow concentrations and the results of the calibration curves are listed in Table 3.

BSA	10 mm rectangular quartz cell	0.0, 0.25, 0.5, 1.0, 5.0, 9.0 mg/mL
	Micro cell	0.0, 1.0, 3.0, 5.0, 9.0 mg/mL
HEL	10 mm rectangular quartz cell	0.0, 1.0, 3.0, 5.0. 9.0 mg/mL
	Micro cell	0.0, 1.0, 3.0, 5.0. 9.0 mg/mL
α-chymotrypsin	10 mm rectangular quartz cell	0.0, 1.0, 3.0, 5.0. 9.0 mg/mL
	Micro cell	0.0, 1.0, 3.0, 5.0. 9.0 mg/mL

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Table 3. Quantitative results for the Biuret method.

	Cell	Concentration Range	Calibration Curve	Correlation Function	Standard Error	Detection Limit	Determination Limit
BSA	10 mm quartz	to 9 mg/mL	Y = AX2 + BX + C A=-0.0005 ±4.3444•10-5 B=-0.0548 ±0.0004 C= 0.0505 ±0.0004	1	0.0109	0.008 mg/mL	0.1483 mg/mL
	Micro cell	to 9 mg/mL	Y = AX2 + BX + C A=-0.0016 ±0.0003 B=-0.0653 ±0.0027 C= 0.0509 ±0.0008	0.9998	0.0775	0.0696 mg/mL	1.0127 mg/mL
HEL	10 mm quartz	to 9 mg/mL	Y = AX2 + BX + C A=-0.0009 ±4.9343•10-5 B=-0.0591 ±0.0005 C= 0.0509 ±0.0008	1	0.0144	0.0133 mg/mL	0.2420 mg/mL
	Micro cell	to 0.9 mg/mL	Y = AX2 + BX + C A=-0.0053 ±0.0005 B=-0.0983 ±0.0048 C= 0.0476 ±0.0063	0.9999	0.0786	0.0640 mg/mL	-
α-Chymotrypsin	10 mm quartz	to 9 mg/mL	Y = AX2 + BX + C A=-0.0012 ±0.0004 B=-0.0064 ±0.0035 C= 0.0578 ±0.0060	0.9996	0.0958	0.0934 mg/mL	1.2987 mg/mL
	Micro cell	to 0.9 mg/mL	Y = AX2 + BX + C A=-0.0010 ±0.0003 B=-0.0614 ±0.0025 C= 0.0343 ±0.0043	0.9998	0.0697	0.0695 mg/mL	0.9987 mg/mL

Lowry Method

Dissolve 2 g of anhydrous sodium carbonate and 0.4 g caustic soda in 100 mL water to obtain a 2% Na_2CO_3 solution. Then dissolve 50 mg copper (II) sulfate pentahydrate and 0.1 g potassium sodium tartrate tetrahydrate in 10 mL of water to create a 0.5% $CuSO_4$ solution. To prepare the Lowry reagent, mix 50 mL of the 2% Na_2CO_3 solution with 1 mL of 0.5% $CuSO_4$ solution. Immediately add 2.5 mg the solution to 500 µL of an aqueous protein solution, then add a phenol reagent, rapidly mix, and allow the solution to react for 60 minutes before obtaining absorbance measurements.

The protein solutions reacted with the alkaline copper and phenol reagent will turn blue and absorb at 750 nm. The time course measurement of the chromogenic reaction was measured at 750 nm and the absorbance became stable after about 60 minutes (Figure 4, left). The spectrum of HSA aqueous solutions that were measured 60 minutes after the reaction with the Lowry reagent are shown on the right in Figure 4.

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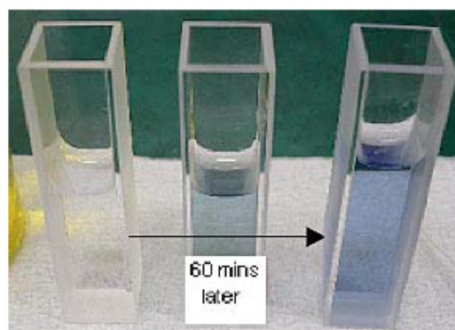

Figure 3. Color change in protein solutions after the addition of the Lowry reagent.

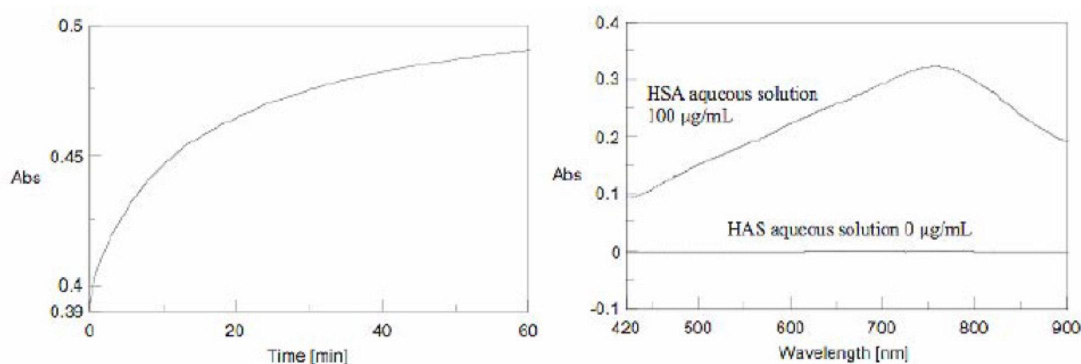

Figure 4. Time course measurement (left) of the chromogenic reaction and the absorption spectrum (right) of HSA reacted with the Lowry reagent. The absorption maximum is 750 nm.

The absorbances of bovine serum albumin (BSA), hen egg lysozyme (HEL), and chymotrypsin from bovine pancreas were measured at 750 nm in both the 10 mm micro cell and rectangular cell. Calibration curves were created using the follow concentrations and the results of the calibration curves are listed in Table 4.

BSA	0.0, 2.0, 290, 50, 100, 200 mg/mL
HEL	0.0, 1.0, 5.0, 10, 20, 50, 100, 200 mg/mL
α-chymotrypsin	0.0, 2.0, 20, 50, 100, 200 mg/mL

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Table 4. Quantitative results for the Lowry method.

	Cell	Concentration Range	Calibration Curve	Correlation Function	Standard Error	Detection Limit	Determination Limit
BSA	10 mm quartz	to 200 mg/mL	Y = AX2 + BX + C A=-4.4663•10-6 ±5.5049•10-7 B=0.0041 ±0.0001 C= 0.0250 ±0.0034	0.9999	1.2336	0.8385 mg/mL	3.9441 µg/mL
	Micro cell	to 200 mg/mL	Y = AX2 + BX + C A=-4.0578•10-6 ±1.3689•10-6 B=0.0041 ±0.0003 C= 0.015- ±0.0097	0.9994	2.5325	2.3903 mg/mL	10.1765 µg/mL
HEL	10 mm quartz	to 200 mg/mL	Y = AX2 + BX + C A=-5.6033•10-6 ±7.2903•10-7 B=0.0049 ±0.0001 C= 0.0293 ±0.0037	0.9998	1.3861	0.7598 mg/mL	3.5722 µg/mL
	Micro cell	to 200 mg/mL	Y = AX2 + BX + C A=-4.8873•10-6 ±8.2675•10-7 B=0.0047 ±0.0002 C= 0.00076 ±0.0042	0.9997	1.6514	0.8911 mg/mL	4.1471 µg/mL
α-Chymotrypsin	10 mm quartz	to 200 mg/mL	Y = AX2 + BX + C A=-1.0948•10-5 ±4.3250•10-7 B=0.0061 ±8.8164•10-5 C= 0.0112 ±0.0027	1	0.685	0.4371 mg/mL	7.6764 µg/mL
	Micro cell	to 200 mg/mL	Y = AX2 + BX + C A=-8.8298•10-6 ±8.8527•10-7 B=0.0054 ±0.0002 C= 0.0167 ±0.0055	0.9999	1.3341	1.0214 mg/mL	16.3006 µg/mL

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