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## Determination of the Sensitivity Range of Biuret Test for Undergraduate Biochemistry Experiments

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### Abstract

Quantitation of the total protein content in a sample is a critical step in protein analysis. Molecular UV-Vis absorption spectroscopy is very efficient in quantitative analysis such as protein quantitation and has extensive applications in chemical and clinical laboratories worldwide. Among the various techniques for protein assay, biuret test is of particular interest in this study. This study sought to verify the sensitivity of biuret assay to protein samples with concentrations ranging from 0.0100 to 5.00 mg/mL. Albumin chicken egg was used as the protein sample. The assay was assessed to determine the range of concentration in which it will show linearity. The biuret test exhibited linearity in a wide range of concentrations ( $0.1000 \pm 0.0004$  to  $5.00 \pm 0.02$  mg/mL). The starting protein concentration range established for the calibration curve of this assay is lower than the found literature value (1 mg/sample). The study provides a more workable range of biuret test.

**Keywords:** *biuret test, UV-Vis absorption spectroscopy, protein quantitation*

### INTRODUCTION

The quantitation of protein content is important and has many applications in clinical laboratory practices and in research especially in the field of biochemistry. The accurate quantitation of protein content is a critical step in protein analysis. Over the past two decades, different protein assay techniques have been developed for the assessment of the protein concentration in a sample (Okutucu, Dınçer, Habib, & Zihnioğlu, 2007). Spectroscopic methods are the most common way to quantitate protein concentrations. UV-Vis Spectroscopy is primarily used for quantitative analysis in chemistry and one of its many applications is in protein assays. Classical methods such as the biuret test, Bradford test, spectrophotometric assay at 280 nm, Smith test, and Lowry test are some of the most commonly used techniques in protein quantitation.

Given the importance of protein assay, it is significant to choose the appropriate technique from the available methods. In doing this, several factors such as the nature of the protein, the nature of other components present in the sample, and the preferred speed, accuracy, and sensitivity of assay are considered. It is also of great importance to know which particular range of protein concentration an assay is sensitive to. In an ideal assay, the most preferred calibration curve generates a linear response to the standard solutions that covers the range of the concentration of the unknown. As the linearity

range for the calibration curve is known, it will make the assay more accurate, time efficient and cost effective. Biuret test is of particular interest in this study.

One of the earliest colorimetric protein assay methods developed for the determination of protein content is the biuret reaction (Gornall, Bardawill, & David, 1949). Compounds containing two or more peptide bonds react with the biuret reagent forming a purple colored complex (Boyer, 2000). The colored product is the result of the coordination complex of copper atom and two nitrogen atoms from each peptide chain as shown in figure1 (Switzer & Garrity, 1999). The color reaches its maximum intensity after about 15 minutes and the complex formed is stable for several hours. Several studies agree that the biuret reaction is reliable. For people with experience, the error was found not to exceed 5 percent. Furthermore, it was determined to be in good agreement with the Kjeldahl method in the determination of total protein for a wide range of concentration (Kingsley 1939). It is also more reliable for protein quantitation compared to techniques which are dependent on the content of some particular amino acid (Gornall, Bardawill, & David, 1949). Because of its simplicity, rapidity, and reliability, the method became a choice in clinical laboratories since the end of 19th century.

However, similar with other protein assays, the linear range for the biuret test found in different literature varies. The most common lower limit of the calibration curve for the biuret test is 1 mg/sample (Boyer, 2000; Switzer, 1999; Wilson & Walker, 1994). In addition, only a few recent studies were done to verify the lowest concentration of the linear range for biuret test. With these, the study presented herein aims to:

- determine the sensitivity of the protein quantitation technique
- verify the range of protein concentration at which the method for protein quantification is accurate
- provide the protein concentration range in which it will generate the best standard calibration curve

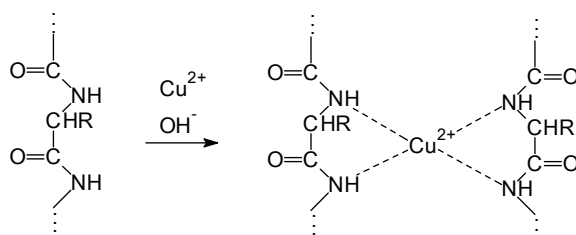
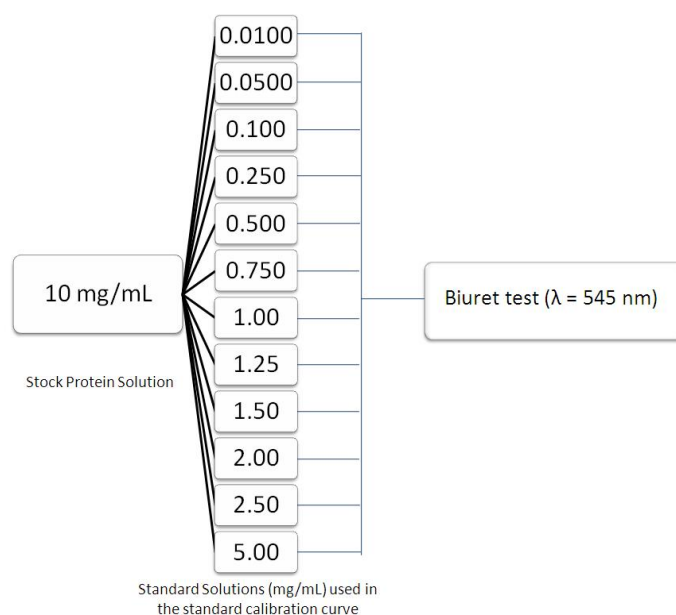


Figure1. Biuret test

## METHOD

### *Preparation of Protein Sample*

From the stock protein solution (10 mg/mL) as shown in the schematic diagram1, twelve 50-mL protein standard solutions with the following concentrations (mg/mL) were prepared: 0.0100, 0.0500, 0.100, 0.250, 0.500, 0.750, 1.00, 1.25, 1.50, 2.00, 2.50, and 5.00.



Schematic Diagram1. Methodology

#### *Preparation of Biuret Reagent*

To prepare the reagent, 0.375 g of cupric sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ) and 1.51 g sodium potassium tartrate ( $\text{NaKC}_4\text{H}_4\text{O}_6$ ) were dissolved in 100 mL distilled water. With constant swirling, 100 mL of 7.5% (w/v) NaOH (freshly prepared in  $\text{CO}_2$ - free, boiled water) was added to the solution. The resulting solution was then transferred to a 250-mL volumetric flask and then diluted to the mark using distilled water and mixed. The reagent was then transferred to an amber bottle and was stored at 2-8°C. The reagent must be discarded if precipitation will form.

#### *Biuret test*

The twelve protein standard solutions were subjected to biuret test. For the control, 1-mL of distilled water was added to 4.5-mL of biuret reagent in a test tube. For the test sample, 1-mL of the protein sample was added to 4.5-mL of biuret reagent. The solutions were shaken using a vortex. The color of the solution was observed to be light blue and the absorbances of the protein samples at 545 nm were determined using the UV-Vis spectrophotometer.

## RESULTS AND DISCUSSIONS

Spectrophotometric methods which utilize visible radiation are called colorimetric analyses (Harris, 2003). The absorbance of the analyte versus its concentration is plotted in a calibration curve, which shows the response of an analytical method to known quantities of analyte (Danzon & Currie, 1998). In an ideal assay, the most preferred calibration curve generates a linear response to the standard solutions that covers the range of the concentration of the unknown. A linear range is defined as the range in which the response of the analyte concentration is proportional to the concentration. It is best to work in a linear range calibration curve in determining the concentration of an unknown sample.

Aside from having a linear response in the calibration curve, an ideal protein assay should be reliable and accurate in the working sample concentration. The assay should also give a good result for different kinds of proteins.

Std	Protein concentration (mg/mL)	Trial (absorbance)			Average
		1	2	3	
1	0.0100	-0.001	0.001	-0.001	0.000
2	0.0500	-0.001	0.001	0	0.000
3	0.1000	0.001	0.003	0.002	0.002
4	0.250	0.009	0.01	0.009	0.009
5	0.501	0.017	0.02	0.019	0.019
6	0.749	0.031	0.03	0.029	0.030
7	1.000	0.038	0.041	0.042	0.040
8	1.250	0.05	0.052	0.053	0.052
9	1.501	0.06	0.063	0.063	0.062
10	1.999	0.08	0.083	0.086	0.083
11	2.504	0.099	0.106	0.106	0.104
12	5.00	0.201	0.206	0.212	0.206

Table1. Absorbance data for the Biuret test

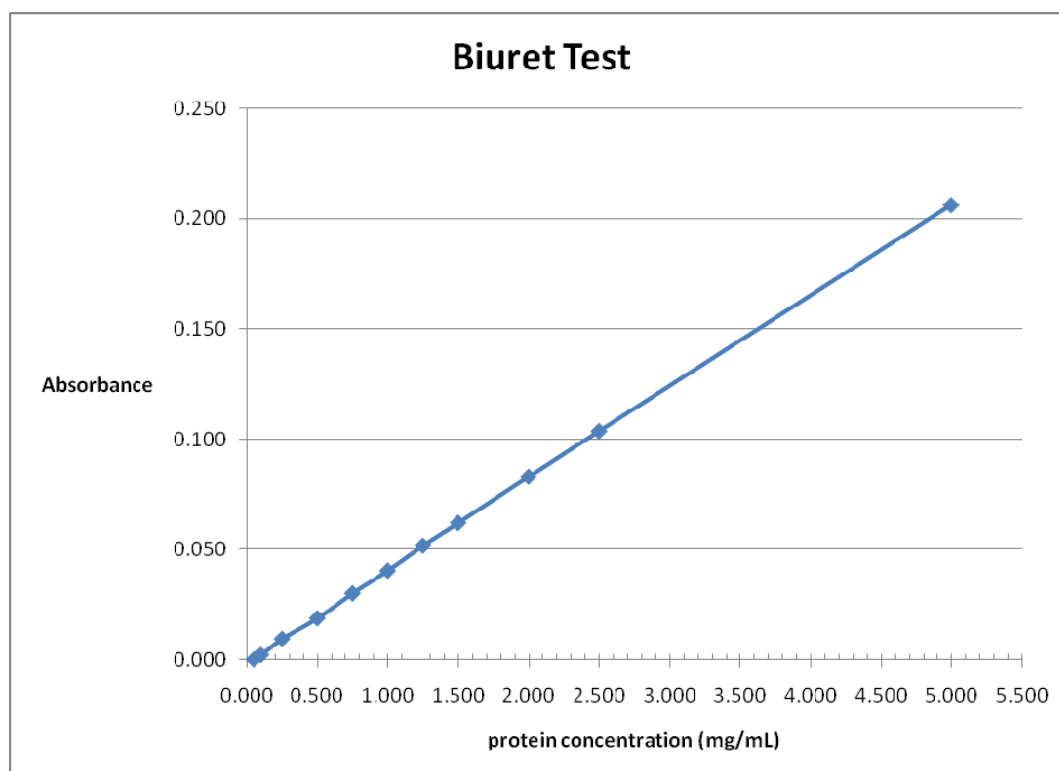


Figure1. Calibration Curve of the Biuret Test

From many techniques for protein quantitation, the determination of the sensitivity range is done only for biuret test. In the study, a calibration curve is constructed from the average absorbance. The experimental graph for the biuret test exhibited a linear calibration curve (Figure1). However in the standard solutions 1 and 2, with protein concentration of 0.0100 and 0.0500 mg/mL respectively, there is no measurable absorbance detected. This shows that the test is not recommended to use for proteins with very low concentration. The linear range (Figure2) for this test is from  $0.1000 \pm 0.0004$  –  $5.00 \pm 0.02$  mg/mL. The experimental  $R^2$  value (Figure3) obtained for this test is 0.9999 and the equation of the line is  $y(\pm 0.001) = [0.0406(\pm 0.0003)]x - [0.0017(\pm 0.0006)]$ . The  $R^2$  value obtained in this study suggests that the calibration curve generated using the said range is very linear. The result shows that even at protein concentrations of lower than that found in literature value (1 mg/sample) the calibration curve is still linear.

The study widens the range of the linear calibration curve, thus making it more useful to various applications. This will also help students and other researchers in the same field by providing an easy, rapid, and reliable method. Also in the range determined, the absorbance values for the three trials were consistent with one another (Table1) which will give the same calibration curve. This further suggests that the range obtained from the study can be best used in determining the protein quantity in an unknown sample.

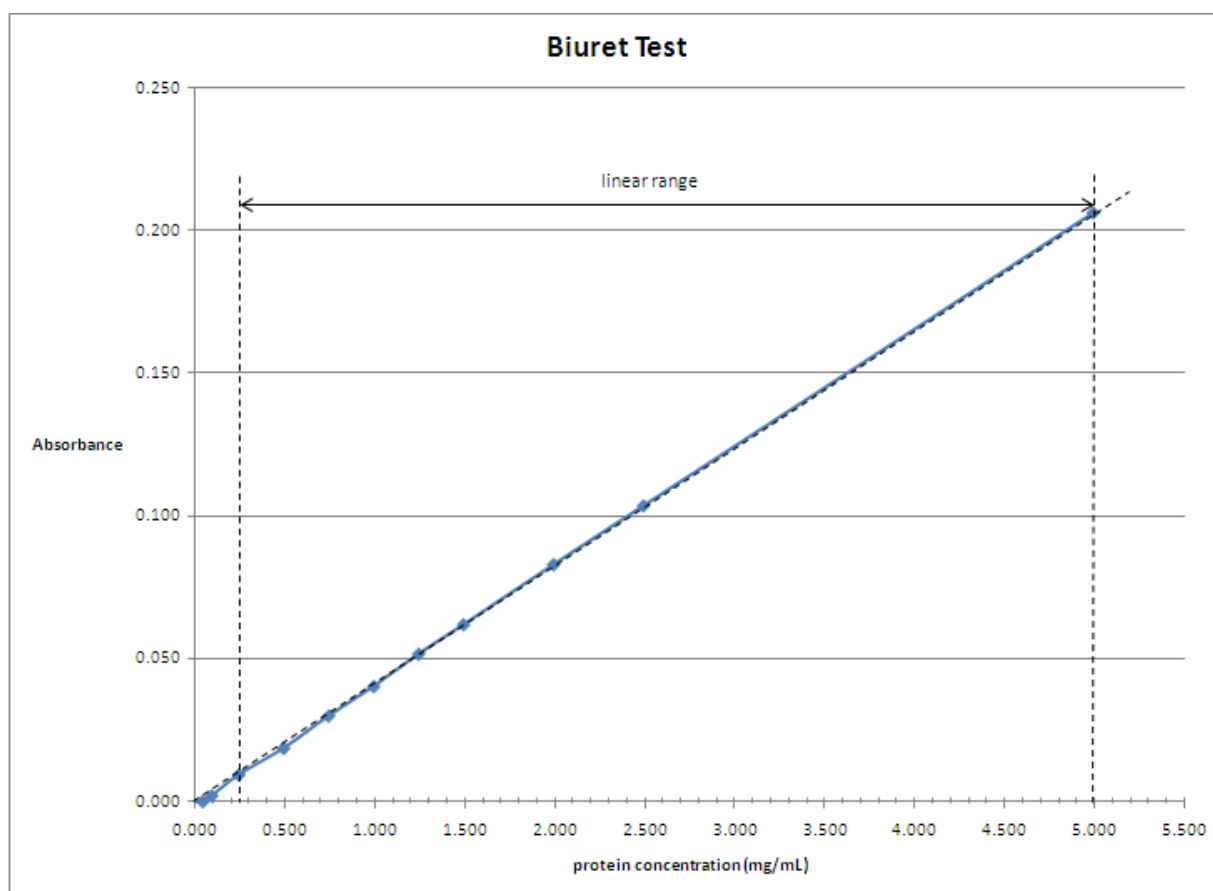


Figure2. Derivation of the Linear Range on the Calibration Curve for the Biuret Test

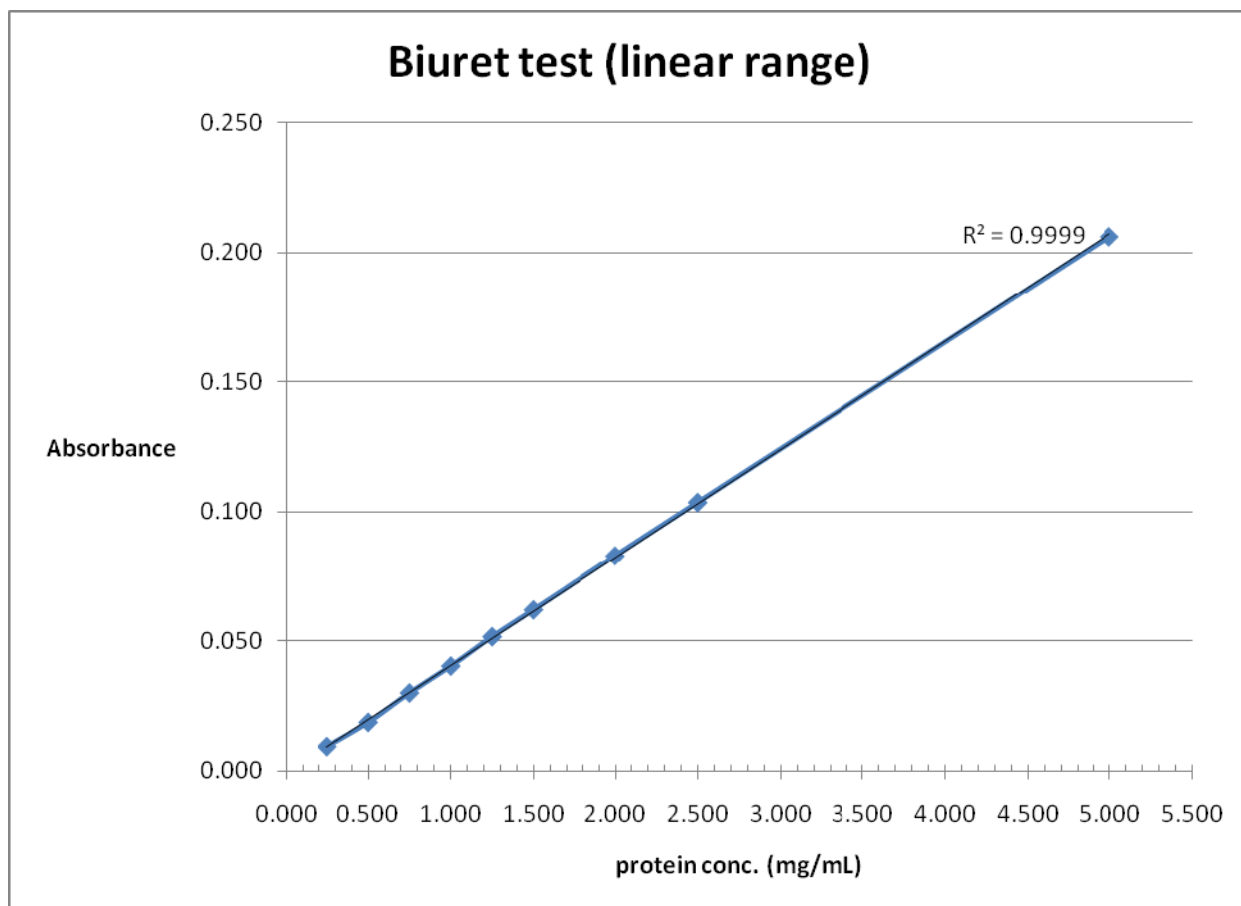


Figure3. Calibration Curve of the Linear Range for the Biuret Test

## CONCLUSION

The study was able to increase the known sensitivity for biuret test, making the starting protein concentration range used for the calibration curve lower than the found literature value (1 mg/sample). The study makes the biuret test more useful to various applications like clinical laboratory practices and biochemical researches. Also, since the calibration curve for biuret test obtained in the study exhibit an  $R^2$  value of 0.9999, the study provides the best linear protein concentration range for biuret test. It gives the future analyst, the best workable concentration for biuret test.

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