Biology and Biochemistry Department
BIOC311
Biochemistry Lab

Experiment #3
Title:(A)Quantitative Estimation of Amino Acids

(B)Biuret Assay

(C)The Folin-Lowry Protein Assay.

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Objective:

(A)

Using the ninhydrin reagent, determine the number of amino acids in a solution, and acquiring measurements of diluted and undiluted amino acid absorption.

(B)

Using an average absorbance vs. albumin concentration graph, determine the protein content in the sample by reacting albumin with a biuret reagent.

(C)

In alkaline conditions, copper tartrate combination and Folin reagent are used to react proteins. The objective is to measure the absorbance of a protein dilution using a spectrophotometer.

Introduction:

The building components of all proteins are known as amino acids. Proteins include a total of 20 distinct amino acids. A carboxyl and an amino group are linked to the same carbon atom in amino acids. Because of differences in their side chains, they differ in size, structure, electric charge, and solubility in water. In the study of proteins, the detection, measurement, and identification of amino acids in any sample are crucial stages. (1)

For proteins and peptides, the biuret method is a colorimetric approach. With compounds that have two or more peptide bonds, copper salts in alkaline solution create a purple complex. The amount of absorbance generated is proportional to the number of peptide bonds reacting and hence the number of protein molecules in the reaction system. As a result, spectrophotometry at 540–560 nm may be used to determine total protein using the biuret reaction with proteins. (2)

The biuret reaction is used in the Lowry protein assay, although it contains extra steps and chemicals to increase detection sensitivity. In the biuret reaction, copper binds to four nitrogen atoms in peptides to form a cuprous complex. Folin-Ciocalteu reagent, which is phosphomolybdic/phosphotungstic acid, is used by Lowry. This reagent produces a blue-green color at 650 and 750 nm when it interacts with cuprous ions as well as the side chains of tyrosine, tryptophan, and cysteine. (3)

Materials:

(A)

• Amino acids

• Ethanol 2L

• Ninhydrin reagent.

(B)

• Protein Standard

• Unknown protein sample

• Milk, Liver extract, plasma, and urine samples.

• Biuret reagent

• Water bath at 37

(C)

• Alkaline sodium carbonate solution

• Copper sulfate-sodium tartrate solution\

• Folin-Ciocalteu solution

• Protein dilutions from part B

Methods:

(A)

1) In 3 test tubes 2.5 ml of the amino acid solution were pipetted, then 0.5ml of ninhydrin reagent was added to the tubes.

2)the tubes were put in a hot water bath for 4 minutes while making covering them with a piece of parafilm.

3)the tubes were left to cool at room temperature, after that the absorbance was read at 570 or 4440nm.

4)3 serial amino acid dilutions were prepared by diluting each amino acid in half three times, and the previous steps were repeated again on the dilutions.

5)the absorbance reading of each dilution was obtained and plotted in an absorbance vs concentration graph.

(B)

1) 5 dilutions of albumin were prepared, and 2 dilutions of protein samples (1:5, 1:10) were prepared.

2) in a set of duplicate tubes, 3ml of biuret reagent was added to 1ml of the protein solution for each dilution.

3) the solution was mixed and warmed at 37 degrees for 10 minutes.

4) the dilutions were cooled and the absorbance was measured at 540nm.

5) a graph was prepared using the average absorbance vs albumin concentration and using the standard curve the concentration of the protein was determined.

(C)

1) 5 ml of the alkaline solution was added to 1 ml of the test solution.

2) The solutions were mixed thoroughly and allowed to rest at room temperature for 10 minutes or longer.

3) 0.5 ml of diluted Folin reagent was added rapidly followed by immediate mixing.

4) after 30 minutes the absorption was measured at 750nm while using an appropriate blank.

5) a standard curve of the protein concentration was prepared, and the protein concentration in the solution was estimated.

Data and results:

sample	*wavelength(nm)*	*Absorption (A)*
Proline	440	0.694
Aspartic acid	570	0.350
Arginine	570	1.316

Table 1 (A) sample amino acids (proline, aspartic acid, arginine) and their absorbtions at different wavelengths.

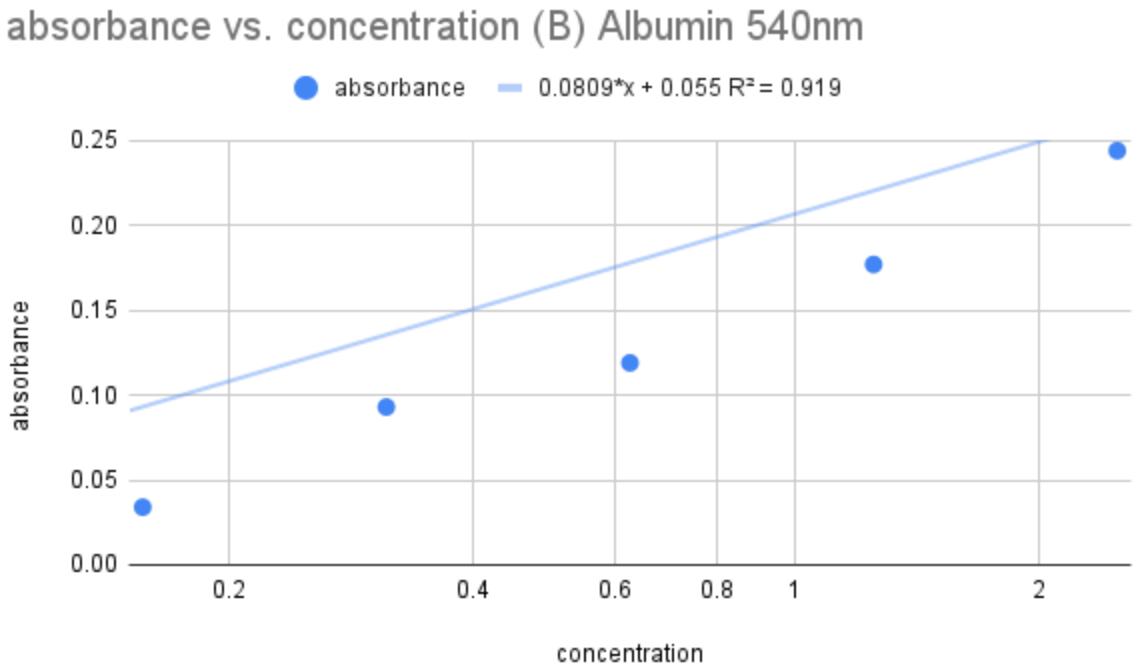

Chart 1 (B) absorption vs concentration curve of albumin with biuret reagent at 540nm.

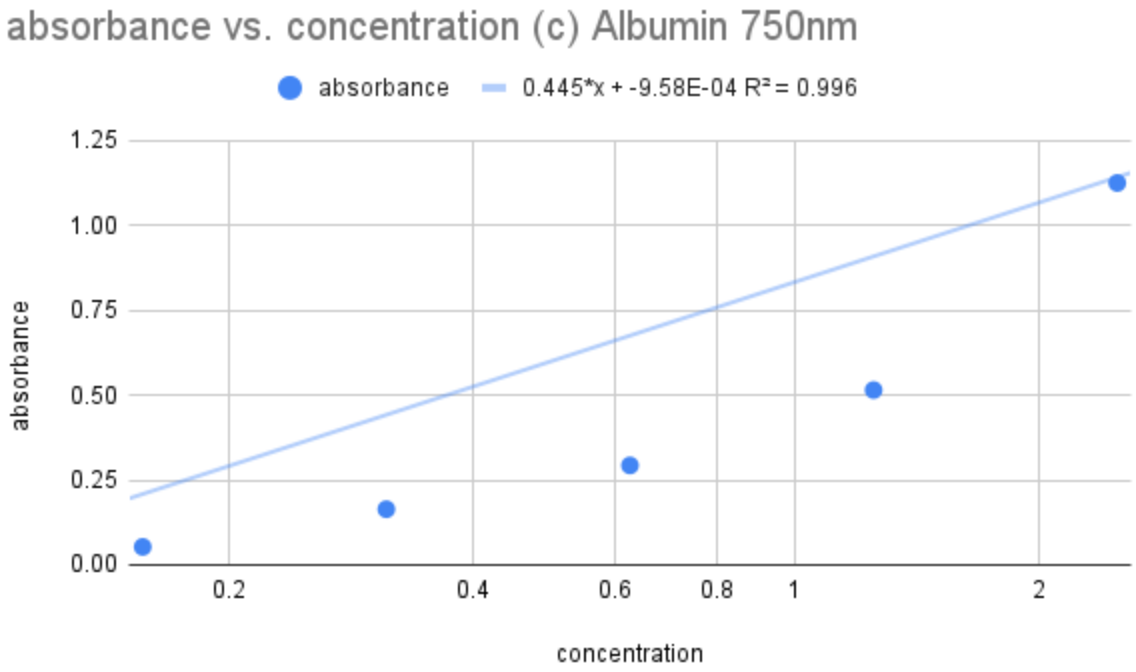

Chart 2 (C) absorption vs concentration curve of albumin with folin-Ciocalteu at 750nm.

Discussion:

For part A, ninhydrin was used to predict the amount of proteins present in argininem proline, and aspartic acid. As shown in table 1, it is shown that arginine had the highest absorption at 570nm. The positively charged guanidino group on arginine, an essential amino acid, is a good example. The amino acid arginine is fit to binding the phosphate anion, and it is commonly found in the active regions of proteins that bind phosphorylated substrates.(4)

As for part B, the biuret assay, a biuret reagent was added to different concentrations of 5mg/ml of albumin (1:2,1:4,1:8,1:16,1:32). And, a dilution of 1:5 and 1:10 of plasma. All the dilutions were read at an absorbance of 540nm. The albumin solutions were put in a standard curve since

they had known concentrations. The curve had an accuracy of 91.9%. Using the line equation y=mx+b that was produced by the curve, it was shown that the concentration of 1:5 of plasma is 14.26, and 1:10 is 9.80. These concentrations are quite high im comparison to those of albumin. This could be due to the fact that ammonium sulfate can frequently impede color development or produce colorful chemicals inside the sample, and it has low sensitivity. (5)

Now for part c; in this part of the experiment we used an alkaline solution Copper sulfate-sodium tartrate solution, and Folin-Ciocalteu solution and added them to our original solutions from part B then read their absorbances at 750nm. The standard curve show an accuracy of 99%. Using the equation y=mx+b, it was shown that the concentration of the 1:5 plasma sample is 5.08, and 4.04 for the 1:10 sample. This method is more sensitive than the biuret method, but this isnt always a good thing. The folin lowry method is usually more accurate for protein concentrations between 5- 100 microliters. Here, we have 5mg, which is clearly way higher than the experimental limit. Also, the reagent used could quickly disintegrate the proteins. So, the amount of protein detected wasnt as much as it couldve been.

Conclusion:

In conclusion, the best method to identify the concentration of proteins is the biuret method. That is because it doesnt disintegrate the peptide bonds in the proteins. The folin lowry method could be accurate if we had a lower concentration of protein (besides albumin). Also, it is concluded that peptide bonds are mostly responsible for protein wavelength absorbance. Otherwise, its the dissociation of the R-group.

References:

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Appendix:

$Y = mx + b$