

Extraction and characterization of proteins assignment

[Art & Culture](#)



**ASSIGN
BUSTER**

Abstract The experiment, entitled Extraction and Characterization of Proteins, aims to isolate casein from milk and albumin from egg; to explain the methods employed for protein extraction; to apply spectrophotometric methods in characterizing and quantifying extracted casein and albumin. The experiment was divided into 2 parts; the extraction of Albumin from egg and the determination of protein concentration via the Warburg-Christian method and Bradford Assay method.

In the first part, egg white underwent the addition of a weak acid, disruption of cell membrane, addition of ammonium sulfate and centrifugation. The product obtained was not weighed because of its relatively small amount and was considered a failure by the experimenters. In the second part of the experiment, the protein concentration of the product acquired from the first part was calculated to be 5.1 mg/mL using the Warburg-Christian method. In the Bradford assay method, however, a negative value for the concentration was derived (-0.16) which led to the use of another solution provided by the instructor. The concentration of the new solution was determined to be 0.024 mg/mL using the Bradford assay method. The use of different solutions in the two methods accounts for the difference in protein concentration calculated. From these results, it can be concluded that the experiment failed to extract the desired amount of proteins, although it was shown that there are different relatively accurate ways in the determination of protein concentration.

Discussion Data and Results Proteins are polymer organic molecules made up of monomers known as amino acids. First discovered in 1838, it was coined from the Greek word *proteios*, meaning primary??? a word which <https://assignbuster.com/extraction-and-characterization-of-proteins-assignment-essay-samples/>

describes its role in the composition of living organisms since it is the primary component of basic biological parts like the cell. Humans alone are composed of 15% proteins (Pauling et al, 1954) and have about 30, 000 different kinds of proteins, of which only 2% have been adequately described (Hendrix, 2007).

Proteins are composed of about 20 amino acids made up of carbon, hydrogen, nitrogen, oxygen and, sometimes, sulfur molecules. An amino acid is a short carbon skeleton with an amino group on one end and a carboxylic acid on the other. All amino acids have identical structures, all have amino groups, carboxylic acids and a hydrogen directly bonded to the alpha carbon, except for their side chains. These differences dictate the structure and the function of proteins. Proteins are abundant in nature. In a typical human diet alone, various proteins can be detected from different food items.

Such proteins include albumin, a simple protein which constitutes an important part of the diet and can be found in egg white, milk, muscle, blood plasma and plant seeds. These albumin-containing food items are great sources of protein. Aside from its importance in nutrition, it has also been used in different industries. During the Spanish colonial period in the Philippines, albumin containing materials like egg whites were used as cement in building churches and other edifices because of the ability of albumin to form mass of great hardness when mixed with slaked lime.

It is also widely used in sugar refining processes because of its ability to coagulate when heated to 70°C, where it can remove cloudy precipitates and clarify solutions. Albumin is also used as an antidote for toxins like

<https://assignbuster.com/extraction-and-characterization-of-proteins-assignment-essay-samples/>

bichloride of mercury, sulfate of copper, and nitrate of silver because of its ability to form insoluble compounds with some metallic salts. Another example of a commonly used protein is casein. Casein, in its pure form is actually a group of proteins (Redmond, 2007) called phosphoprotein, a group of proteins bonded to a substance containing phosphoric acid.

Its name was coined from the latin word caseus, which means cheese, because it accounts for nearly 80% of the proteins in milk and cheese. With the help of chymosin, an aspartic protease, specific peptide bonds of casein (specifically k-casein) are hydrolyzed; a reaction considered in the most effective procedure in the cheese-making industry (Rao et. al 1998). Aside from its use in cheese-making, casein is also used in making adhesives, binders, protective coatings, food additives, and is commonly used by bodybuilders because of its great source of slow digesting amino acids.

In order to study the structure, function and behavior of proteins, it is important to take a look at them at their purest form. Since proteins do not usually occur unadulterated, protein-containing materials must undergo protein purification. Protein purification is a series of processes which aim to isolate a protein from a complex mixture. Although there is still some trial-and-error experimentation that must be completed, the process of protein purification has become routine and standardized (Boyer, 1998). There are two types of protein purification, preparative and analytical.

Preparative protein purification aims to isolate a relatively large quantity of protein while analytical protein purification aims to isolate a relatively small quantity of protein for analytical, structural, functional, identification and

functional purposes. In the experiment, analytical protein purification method was used. The objectives of the experiment were to isolate casein from milk and albumin from egg; to explain the methods employed for protein extraction; to apply spectrophotometric methods in characterizing and quantifying extracted casein and albumin.

The experiment was divided into 2 parts, the first part of which was the extraction of albumin from egg and casein from milk, and the second part was the determination of protein concentration. In the first part of the experiment, the experimenters were assigned to extract albumin from egg. 20mL egg white was measured out and 1.0 M acetic acid was added to it dropwise. The addition and concentration of the weak acid was based on the fragility and strength of the proteins. After the addition of the acid, soluble proteins would be in the solvent and can be separated from cell membranes.

The solution was then filtered and squeezed through cheesecloth to break the cell membranes. The collected filtrate was then added with ammonium sulfate solution to alter the solubility of the proteins. Since the solubility of proteins depends on the ionic strength of the solution, it would depend greatly on salt concentration. In a solution of low salt concentration, an increase in the salt concentration or ionic strength would increase the solubility of the proteins; a process known as salting in.

Therefore, the addition of ammonium sulfate was to increase the ionic strength of the solution and to increase the solubility of the proteins in the mixture. The mixture was then allowed to stand for 30 minutes and was centrifuged. Centrifugation is a process which uses centrifugal force to

separate particles with varying mass. In the procedure, the solution is placed in a plastic tube and was rotated at a very high speed. During this process, the angular momentum exerts an outward force that drags bigger and more massive particles at the bottom side of the tube.

The lighter particles, however, would remain in the liquid solvent. Since it was noted that the solubility of the proteins increased because of the addition of ammonium sulfate prior to centrifugation, it could be concluded that the proteins were still in the supernatant. After centrifugation, the supernatant was immersed in an ice bath and was added with 50% saturated ammonium sulfate. It is important to note that prior to this period, the mixture already has high salt concentration because of the addition of ammonium sulfate before centrifugation.

The addition of more ammonium sulfate would extremely increase the salt concentration which would then lead to the decrease in concentration of proteins; a process known as salting out. This would lead to the precipitation of the proteins. The mixture was then centrifuged and the precipitate was collected since it was noted that the proteins were already precipitated prior to centrifugation. 5mL 0.9% NaCl was then added to the precipitate and was stored in a refrigerator.

It is important to take note that the temperature of the extracted albumin should be kept low since it is known that high temperature can cause the coagulation of the protein. The result of the first part of the experiment was not quite satisfactory. After collecting the precipitate in the last part of the procedure, it was shown that the precipitate was relatively of small amount.

<https://assignbuster.com/extraction-and-characterization-of-proteins-assignment-essay-samples/>

The amount of proteins extracted was also considered to be small since the precipitate collected was predicted to weigh about less than 1g.

Because of technical reasons, the experimenters were allowed not to weigh the collected crude precipitate anymore. In the second part of the experiment, the experimenters determined the protein concentration of the product acquired from the first part. This part of the experiment was divided in to two subparts, the first part uses the Warburg-Christian method while the second part uses the Bradford assay. The Warburg-Christian method was developed to correct nucleic acid contaminations so the derived absorbance would only be that of proteins. The method consists of two parts, the measure of absorbance at 280 nm and at 260 nm.

The measure of absorbance at 280 nm runs on the principle that amino acids tyrosine and tryptophan absorb light at wavelength 280 nm. In using this method, the concentration of pure protein can be calculated using the Beer-Lambert's Law, $A = ELc$ or $c = A/(EL)$ Where A is the absorbance at 280 nm, E is the molar absorptivity, L is the distance of the path traveled by light and c is the concentration of the solution. However, this method is not perfectly accurate since proteins are not the only ones that absorb light at 280 nm. However, other contaminants may interfere with the absorption.

Since nucleic acids are the primary opponents of proteins in absorption, they are the primary concern in aiming for accuracy. Nucleic acids strongly absorb light at 260 nm, while proteins do not. This is the reason while the Warburg-Christian method includes the measurement of absorption at 260 nm. In the experiment, the absorbance of the protein extracts was 4.0 at 280 nm and

1. 469 at 260 nm. From these values, we can calculate the protein concentration using the equation $\text{Protein concentration (mg/mL)} = \frac{1.55A_{280}}{0.76A_{260}}$ Where A_{280} is the absorbance at 280 nm and A_{260} is the absorbance at 260 nm.

This equation was derived from the correction factor calculated from the ratio of absorbance at 280 to that at 260. Substituting the values derived from the experiment, $\text{Protein concentration} = \frac{(1.55)(4)}{(0.76)(1.469)}$
 $\text{Protein concentration} = 5.08356$ or 5.1mg/mL Also using the values of the absorbance of the protein extracts, the % purity of protein isolate can be determined. This can be done by getting the A_{280}/A_{260} ratio and getting the difference between 100% and the corresponding % nucleic acid estimated from the % Protein and Nucleic acid Estimation by UV Spectrophotometry Table (Table 3, p. 1 of the Biochemistry Laboratory Manual, U. P. I. C. , 2007). In the experiment, the value of A_{280}/A_{260} is calculated to be 2.7. This value does not correspond to any given data in the table and it only means that the protein extract is of relatively small % purity since the table can only be used for protein extracts with percent purity of not less than 80%. This adds to the conclusion in the first part of the experiment that the protein extracted was of relatively small amount. In the Bradford assay part of the experiment, 9 test tubes were prepared from different solutions.

Tube 1 was used as blank and tubes 2-6 are for the construction of the calibration curve for protein quantification. On the other hand, tubes 7-9 are for the different concentrations of the isolates. The Bradford assay method, also known as the Coomassie blue method, was developed by Bradford in

1976. Coomassie blue G-250 binds to protein when it is in acidic solution. The peak absorbance then changes from 465 to 595 nm. Although it is not yet known to which amino acid Coomassie blue binds with, the protein concentration can easily be calculated by measuring the absorbance at 595 nm.

In the experiment, the 9 test tubes were prepared based on the table of Volume of Solutions (p. 14, Biochemistry Laboratory Manual, U. P. I. C. , 2007). The absorbance of each tube was then read at 595 nm. The results of the readings were summarized in the table:

Test Tube #	Concentration of Standard BSA (mg/mL)	Absorbance
1	20	0.0020
2	0.004	0.0040
3	0.008	0.0080
4	0.010	0.0080
5	0.0080	0.0110
6	0.010	0.0147
7	0.011	-0.0116
8	0.014	0.0149
9	0.025	0.025

(*NOTE: In the experiment, test tube 7 yielded a negative absorbance value, -0.116.

To compensate for the error, another solution given by the instructor was used in the Bradford assay method and in the calculations.) Based on the data given above (specifically on the data results of test tube 2-6), a calibration curve is made: From the graph, a linear equation can be derived. The equation $y = 1.15x + 0.0025$ can then be used to compute for the concentration of the mixtures in test tubes 7-9 substituting their absorption as y to compute for x which is the corresponding value for their concentration. From this assumption it can be known that the concentration of test tube 7 is 0.074 mg/mL, the concentration of test tube 8 is 0.01 mg/mL, and the concentration of test tube 9 is 0.02 mg/mL. It should be noted, however, that these are not the concentration of the proteins in the

solution since the solution was diluted with water and Bradford reagent. To get the theoretical concentration of the proteins, the dilution factor should be given consideration. The dilution factor can be calculated using the formula $\text{Dilution factor} = V_f/V_i$ Where V_f is the final volume and V_i is the initial volume before the dilution.

From that formula, the dilution factors of the calculations for the protein concentrations of the solutions of test tube 7-9. The dilution factors calculated are as follows: test tube 7 = 3.3; test tube 8 = 2; test tube 9 = 1.4. From these dilution factors, the protein concentrations can be calculated by multiplying the concentration of the solution in each test tube by their corresponding dilution factor. The calculated protein concentrations are as follows: test tube 7 = 0.024 mg/mL; test tube 8 = 0.02 mg/mL; test tube 9 = 0.028 mg/mL.

Although these calculated values for the concentration of protein in the 3 test tubes are almost identical with an average of 0.024 mg/mL, it can be noted that it differs a lot from the calculated value for protein concentration using the Warburg-Christian method which is 5.1 mg/mL, a value that differs greatly from that of the Bradford assay method. This difference can be attributed to the fact that the protein-containing solution used in the Warburg-Christian method was not the same as the solution used in the Bradford assay method. The protein used in the former yielded a negative value in the latter.

Because of this complication, another solution given by the instructor was used. The negative value of the original protein solution in the Bradford

assay method signals that there is an obvious error in the procedure. This may mean that there have been certain errors encountered (errors will be discussed later in this paper). Aside from the Warburg-Christian method and the Bradford Assay method, there are other quantification methods useful in determining protein concentrations. An example would be the A280 method. It rests upon the basic principle that amino acids tryptophan and tyrosine easily absorb light at 280 nm.

However, this method does not take into consideration the absorption of contaminants, a problem virtually solved by the Warburg-Christian method. Another example would be the Biuret method which makes use of the principle that proteins containing 2 or more peptide bonds form a purple complex with copper salts. The absorbance of the derived product could then be read at 550 nm. Although it was considered to have fewer deviations than other methods like Lowry method (Layne, 1957), it still has certain disadvantages like requiring numerous materials making the process expensive.

Although the process is also considered fast and sensitive, it still depends on large quantities of proteins which makes it difficult to be used especially if the protein extractions being used are extracted using the analytical protein purification method. Another protein quantification method is the Lowry procedure developed in 1951. It basically increases the sensitivity of the assay and is more dependent on the amino acids with reducing power like tyrosine, tryptophan and cysteine.

In the procedure, the protein extract is mixed with copper and acid solution used in Biuret method and react it with Folin-Ciocalteu reagent which divides the protein into its amino acid components. In doing the experiment, it was inevitable to experience difficulties that could lead to erroneous results. During the whole course of the experiment, contamination of equipments, glasswares and reagents have great impact on the results. The glasswares and equipments used might contain contaminants that could denature the protein under study and might be the reason for acquiring a low yield of product.

Also, contamination and wrong preparation of the reagents could affect the concentration of the extracted protein. Suppose the reagent ammonium sulfate was not prepared according to the procedure, the solubility of the proteins to be extracted would be at stake since their solubility greatly depends on the concentration of ammonium sulfate. Contamination also plays a vital role in the 2 protein quantification methods done. It should be given importance that the cuvettes used should be clean enough so no contaminants might interfere with the absorption of light.

Some contaminants might absorb or block the light leading to a greater absorption value. Also, if the cuvettes used have been contaminated with proteins prior to its use, an increase in the absorption value might happen. As for the difficulties encountered, it should be noted that the protein extracted in the experiment was of relatively small amount. This might be because the cell membranes were not fully destroyed during the filtration or the salt concentration of the solution leading to a deviation from what the

solubility of the proteins should have been, making it difficult to separate them from the mixture.

Because of this problem of low yield, the percent purity and the protein concentration of the sample of the experimenters became difficult to compute. It should also be noted that in the experiment, the value absorbance of test tube 7 was negative; a result that may be attributed to the low protein product yield. This also led to the substitution of another solution, the concentration of which was used in the calculations. Because of these results, the experimenters recommend to make several trials in order to ensure that there would be a chance to extract the desired amount of proteins.

This would also ensure that if there were errors done on one of the trials, the other trials could compensate for that error. This would also assure the accuracy of the experiment because the results would not depend on only one trial which can be erroneous. Also, the glasswares, equipments and reagents to be used should be monitored and cleaned well so as to avoid contamination. The preparation of the reagents should also be carefully done to avoid mistakes. References " Albumin. " Microsoft?? Student 2008 [DVD]. Redmond, WA: Microsoft Corporation, 2007. Biochemistry Laboratory Manual, UP Diliman. pp11-14.

Boyer, R. F. 1998. Concepts in Biochemistry. Brooks/ Cole Pub. Co. Caprete, D. R. Biuret Protein Assay. ; <http://www.ruf.rice.edu/~bioslabs/methods/protein/biuret.html>; Gill, SC & von Hippel, PH (1989), "(PubMed abstract) Calculation of protein extinction coefficients from amino <https://assignbuster.com/extraction-and-characterization-of-proteins-assignment-essay-samples/>

acid sequence data”, Analytical Biochemistry 182 (2): 319-26, ; <http://www.ncbi.nlm.nih.gov/sites/entrez> (PubMed abstract); Hendrix, M. L. “ Protein. ” Microsoft?? Student 2008 [DVD]. Redmond, WA: Microsoft Corporation, 2007. Layne, E. 1957. Spectrophotometric and Turbidimetric Methods for Measuring Proteins.

Methods in Enzymology. Linus P. , R. B. Corey and R. Hayward. 1954. The Structure of Protein Molecules. Scientific American, Inc. McMurry, J. 2004. Organic Chemistry. Brooks/Cole, Thomson Learning, Inc. Protein Analysis- Determination of Protein Concentration. ; <http://public.clunet.edu/~revie/biochemistry/Protein-analysis-lab.pdf>; Rao, M. B. , A. M. Tanksale, M. S. Ghatge, and V. V. Deshpande. 1998. Molecular and biotechnological aspects of microbial proteases. Microbiol. Mol. Biol. Rev. 62: 597??? 635 Santos, G. C. and Danac, A. C. 1999. Biology for High School. Rex Book Store, Inc.