Learning Scientific Topics by Experimental Application in Education

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Abstract

Today, where industrial waste is constantly increasing and causing many destructive changes in the ecosystem, it is substantial to minimize the detrimental effects of these wastes before they cause irreversible changes. Although some waste products cannot be recycled, many of them can be reprocessed and used in other industries for different purposes. Animal remnants abundant in favorable molecules, such as turkey feathers are a relevant example for this case. Turkey feathers take a long time to degrade in the environment, and possess a biohazard quality as they need to be burned or buried for disposal. However, the high amount of protein stored in these feathers is extremely valuable for industries such as medicine and cosmetics. In order to search for methods, observe, and test out the process and practicality of turkey feathers for their use in other areas, we, as 11th grade AP Chemistry students, conducted an investigation aimed to extract keratin protein from turkey feathers. The investigation was carried out in four steps: dissolving, protein precipitation, protein purification, and testing. Beer Lambert's Law was used to interpret our data. A biuret test was utilized to measure the concentration of proteins in different agents. Later the concentration was evaluated in terms of molars. All these processes helped us better understand AP Chemistry topics like absorption and molarity, and shown how they apply in lab conditions. Understanding the role of turkey feathers is critical in evaluating their contribution, and minimizing their pernicious effects on the environment. Though some wastes small in role seem harmless, it is crucial to note that all wastes, no matter what significance they hold, are the essential components that comprise the massive amount of global waste that threatens our environment. Performing this experiment on such a waste product using the knowledge we acquired from AP Chemistry, provided us with the opportunity to test out our lab skills and the proper way to implement our knowledge in experiments, in the meantime observing and determining how we can use this knowledge to turn wastes into useful instruments.

Keywords: *protein extraction, turkey feathers, feather waste, laboratory experiment, AP Chemistry;*

1. Introduction

The present research aimed to extract an unknown concentration of protein from turkey feathers. Turkey feathers pose a huge threat to the environment, mainly because of the techniques utilized in their disposal. Poultry processing plants and slaughterhouses produce massive amounts of feathers each year, considering the fact that 7% of a poultry's body weight is made up of feathers [1]. According to a research done in 2002 by the National Chicken Council of US [2] , 8.5 billion poultry animals are processed in the US annually, which would yield around 2.3 billion pounds of feathers. This is an incredible amount of feathers each year, all going to waste and harming the environment. However, these feathers are not actually total waste products considering their high keratin content. With the correct technology and equipment, these proteins can be extracted from the feathers, and used in many industries including cosmetics and medicine. Thus, using them as a protein source is both beneficial to us, and the environment. This experiment aimed to convert the theoretical knowledge on the extraction of proteins from turkey feathers into practical knowledge, affirming the presence of proteins, and observing the process with which they can be obtained. Protein in the feathers were extracted in several steps, tested for their presence via Biuret Test and evaluated through UV spectrophotometer. The measurement of the absorbance of turkey feather solution by UV Vis required further interpretation with Beer Lambert Law, which asserted the direct correlation between the quantity of light absorbed by a substance, and its concentration.

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2. Procedure

2.1 Pre-Treatment of Feathers

- 60 grams of turkey feathers were collected from a turkey farm in İstanbul.
- The feathers were separated into two groups of 30 grams. Each group was soaked in ether and left for 24 hours.
- The feathers were then dried under sunlight.
- 30 grams of dried feathers were blended into small pieces and weighed again.

2.2 Dissolving

- 2L of 0.5M sodium sulfide solution was prepared in a 2L volumetric flask. [3]
- The sodium sulfide solution was carefully added to the weighed turkey feathers, and the solution was heated to 30 degrees.
- A buffer consisting of 8M NH_3 and 1M NH_4Cl was prepared and added to the solution using a dropper.
- The solution was continuously stirred for 6 hours, and the pH was maintained around 11.
- After 6 hours, the solution was filtered and the liquid part was centrifuged at 10,000 rpm for 5 minutes. The supernatant liquid was filtered, put in a beaker, and stirred.

2.3 Precipitation of Proteins

- 35g of ammonium sulfate was dissolved in 500mL deionized water, and the resulting solution was filtered.
- The ammonium sulfate solution was carefully added to the filtered feather solution from before, using a dropper. The ratio of the two solutions were 1:1.
- The resulting solution was centrifuged at 10,000 rpm for 5 minutes, and then filtered. The supernatant liquid was discarded, and the solid particles were collected.
- The collected solid particles were washed by being added to 100mL deionized water and stirred until they dissolved. The resulting solution was once again centrifuged at 10,000 rpm for 5 minutes. The solid particles were again gathered.

2.4 Protein Purification

- The collected solid particles were dissolved in 100mL of 2M sodium hydroxide solution, and centrifuged at 10,000 rpm for 5 minutes.
- After centrifuge, the solution was filtered and the supernatant liquid was collected, where the solids were discarded this time.

2.5 Biuret Test

- 1% copper sulphate and 1% potassium hydroxide were prepared separately.
- 20mL of the supernatant liquid collected earlier was mixed with 20 mL of potassium hydroxide (1:1 ratio).
- Four drops of the copper sulphate solution was added.
- The solution was covered and left for observation. The changes were recorded.

2.6 UV Spectroscopy

- Absorbance values were measured between 0 and 1000 nm wavelength.
- 0.1M lithium nitrate solution was prepared in a 100mL volumetric flask.
- UV Spectroscopy machine was used to measure its absorbance. The results were recorded.
- The absorbance values of the final Biuret solution obtained by the turkey feathers was also recorded by the UV-vis. The results were recorded.
- Absorbance values of the two solutions were compared.
- The whole process was repeated until the Biuret test for the other group of 30g turkey feathers, using thioglycolate solution instead of sodium sulfide as a reducing agent.

3. Data

Type of Solution	Absorbance Value (A)
0.1M LiNO3	1,254
0.01M LiNO3	1,214
0.001M LiNO3	1,063
0.0001M LiNO3	0,596
Turkey Feathers	1,150

Table 1

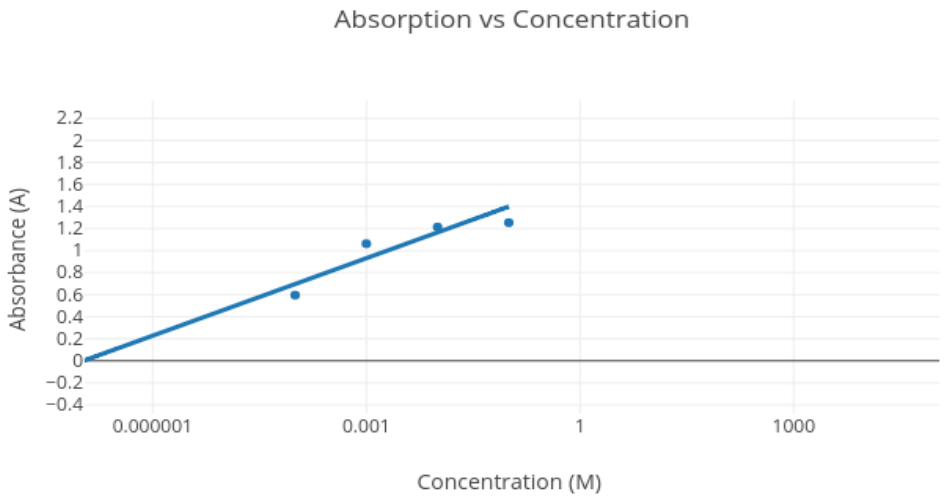

Figure 1.a

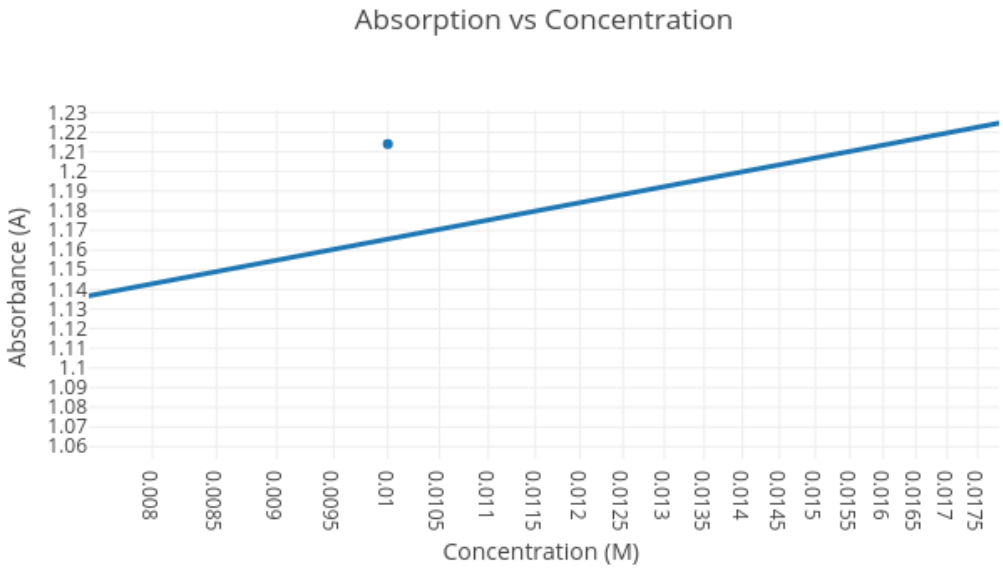

Figure 1.b

4. Results

In light of determining the presence of proteins in the final solutions that were obtained, two different methods were utilized. The first method, the Biuret Test, solely gave an insight to the overall concentration of the protein present in the solution, but did not provide any numerical data for the concentration of proteins. The contrast of the color specified the concentration; a darker color indicated a higher concentration of protein. The use of the first reducing reagent, Sodium Sulfide, produced a violet solution with medium contrast when subjected to the biuret reagent, asserting the existence of protein molecules in the solution in moderate concentration. The second reagent, thioglycolate, resulted in a lighter contrast of violet, which once again proved the presence of protein, though in a smaller concentration compared to the first sample.

The second method, the Ultra-violet visible spectrophotometry, provided a numerical data for a thorough examination of the concentration of proteins in the solution. The UV spectrophotometer calibrated the concentration of proteins by measuring the amount of light the solution absorbed as beams with varying wavelengths passed through the solution in the cuvette. The highest point in the absorption curve indicated the wavelength absorbed utmost by the solution, which referred to its concentration level. Despite the usage of thioglycolate during biuret testing, Sodium Sulfide was the only agent that had been subjected to UV spectrophotometer test for further measurement. A control group, Lithium Nitrate, in different concentrations was prepared to serve as referential data. Lithium Nitrate solutions of 0.1M, 0.01M, 0.001M, 0.0001M have been tested with UV spectrophotometer. The solutions indicated absorbance values of 1,254 A, 1,214 A, 1,063 A, and 0,596A respectively, as shown in Table 1. Next, the absorption value of the feather solution was measured, which was recorded as 1,150 A.

5. Discussion

The contrast of both solutions after the Biuret Test was observed, and a comparison was made, suggesting that a larger amount of protein keratin dissolved in the first agent, Sodium Sulfide, and a relatively low amount of protein dissolved in thioglycolate. Since Cu (III) ions give a violet color in the presence of peptide bonds, the intensity of the color corresponds to the amount of dissolved protein present in the solution. The biuret test validated the presence of proteins in both solutions, affirming our speculations. The UV spectrophotometer was utilized to pinpoint an approximate concentration of proteins in the solution. By comparing the absorbance of the protein solution extracted from turkey feathers with a similar solution containing a common compound, the approximate molarity of the protein solution was estimated. Lithium Nitrate was chosen as a reference due to its rich nature in nitrogen, the main component of the protein backbone. The similarity between the absorbance values of the sodium sulfide solution, 0.01M and 0.001M Lithium Nitrate solutions allowed us to estimate the concentration of the reduced feather solution to be between 0.01M and 0.001M. A graph had been constructed from the results of UV vis and a best line had been drawn to evaluate the concentration of the reduced feather solution. Inferred from the graph, the speculated concentration of the sodium sulfide solution was found to be 0.085M, seen in Figure 2.b. The UV Vis results referred to the principles of Beer Lambert's Law, exhibiting an increased rate of absorbance as the concentration of the solution increased. Both the biuret test and the UV spectrophotometer produced results that met our expectations and supported our hypothesis by demonstrating the existence of extracted protein in the reduced solution obtained from turkey feathers.

6. Conclusions

The experiment was done with the aim of obtaining keratin from an alternative source of protein: turkey feathers. Considering that turkey feathers consist of a high amount of protein, and that their slow decomposition as waste products cause a big problem to the environment, they are an ideal source of keratin. In this experiment, two different reducing agents were used to dissolve the feathers: sodium sulfide, and thioglycolate. A Biuret Test was done to measure the dissolving ability of these reducing agents. The proteins in the feathers were precipitated out of the solution by an ammonium sulfate solution. Two methods of testing were done to analyze the data and prove the presence of proteins in turkey feathers. The Biuret Test confirmed the presence of proteins when the Biuret solution turned purple because of the presence of peptide bonds in the added feather solution. The UV spectroscopy also confirmed this by showing correlating absorbance values with the prepared nitrogen-containing compound, Lithium Nitrate, and the feather solution, allowing us to estimate the concentration of protein in the sample. Thus, the results of this experiment showed that turkey

feathers contain protein, which is best dissolved by sodium sulfide, and may be used for many purposes.

References

[1] Arvind Soni, Sagar Chand, S. Talukder. “Feather Meal and Its Nutritional Impact.” PoultryWorld, 17 Feb. 2017
[2] McGover, Victoria. “Recycling Poultry Feathers: More Bang for the Cluck.” GreenBiz, GreenBiz Group Inc., 31 Mar. 2002
[3] Perumal, Ramanan. Bin Mohd Yunus, Rosli. Kamarudin, Nuruldiyanah. “Extraction of Keratin Protein from Chicken Feather.” Universiti Malaysia Pahang.