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Protein Misfolding & Neurodegenerative Disease

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Abstract

The study of the impact of the pH of the protein egg albumin denaturing process on spectrophotometric absorbance is explored in the paper. One of the egg albumins was taken as a model protein, and it was exposed to the buffer solutions at pH levels of 2 to 10. 010 minutes, and the absorbance was calculated to obtain the degree of protein aggregation and turbidity. The stability of the proteins was observed to be greatest at approximately neutral pH (pH 6), in which the smallest absorbance was recorded, and so, denaturation was not significant. On the other hand, greater acidic (pH 2) and greater alkaline (pH 10) yielded greater absorbance, which is a sign of increased aggregation of proteins. The conclusions demonstrate that severe pH conditions result in severe disorganization of the protein structure and foster denaturation.

Key words: Protein Egg, Neurodegenerative disease, pH Level, Complex Arrangement

1 Introduction

Protein denaturation refers to the weakening of intermolecular forces, which maintain the structure of the protein under the influence of environmental conditions (pH, etc.). The reason that led to studying egg albumin as a model protein is the investigation of the impact of pH on the structure's stability, and provides insight into the fact of protein misfolding during biological and industrial applications.

1.1 Background and Context

Proteins are the compounds in chemistry that are complex, and the arrangement of proteins determines their functionality. Hydrogen bonding force, electrostatic force, hydrophobic force, and van der Waals force are the intermolecular forces that maintain their 3D structure. The forces can be affected by changes in environmental factors (temperature or pH), which will result in unfolding or denaturation of proteins. Particular attention is paid to protein misfolding, which has also been associated with several neurodegenerative diseases, including Alzheimer's and Parkinson's disease (Adam et al., 2023). Such diseases lead to a cell shutdown due to the formation of an abnormal conformation of proteins and, in the end, the aggregation of proteins. The egg white protein contains albumin, and in most instances, it is regarded as a model protein in biochemical studies because of the well-known protein structure and its behaviour of heating or denaturation.

Protein structure is usually analyzed using spectroscopies. With the assistance of molecular spectroscopy, variables like absorbance have been utilized to monitor the variation of proteins during the process of denaturation or aggregation (Wang et al., 2024). Changes in the protein structure can thus be given quantitatively by measurements of the absorbance at a particular wavelength. The previous studies established that the treatment of pH may modify the digestion behaviour and structure of the egg white proteins, which validates the vulnerability of albumin to the effects of acids and bases (Zhang et al., 2023). The significance of the examination of the effects of pH on protein denaturation cannot be related just to biochemistry but also to food science and medicine.

1.2 Problem Statement

Protein denaturation is one of the processes that is quite demanding and, therefore, affects both biological systems and industrial practices. However, a definite correlation between the environmental pH and the extent of the protein denaturation requires an intimate quantitative analysis. It is known that the extreme level of acidity or alkalinity of the conditions may actually lead to structural disruption; however, the explanations as to why such a tendency is observed in the range of a wide pH variation would be valuable to systematic experimental research. A model system is typically a protein of egg white, although structural changes cannot always be measured well. The traditional procedures traditionally rely on the visual impressions (coagulation), not providing the target quantitative data (Goryanin et al., 2022).

1.3 Objectives of the Study

1. To determine how different pH concentrations (pH 2-10) affect the denaturation of egg albumin.
2. To measure the extent of denaturation of the proteins in terms of absorbance change upon the conclusion of a 10-minute exposure at a wavelength of choice.
3. To establish the relationship between the changes in PH and the structural integrity of egg albumin.

1.4 Research Question

How does pH (pH 2–10) influence the extent of protein denaturation in egg albumin, determined by changes in absorbance at a specific wavelength after 10 minutes of exposure?

The proposed study is concerned with investigating the effect of the pH on the protein denaturation of egg albumin under laboratory conditions. The experiment will include testing of the protein samples, changing their pH between 2 and 10 in every case, and noting the shift in the absorbance in the 10 minutes' time interval. It entails the laws of acid-base chemistry, intermolecular forces, and spectroscopic analysis in the experiment.

However, several weaknesses are to be cited. To begin with, egg albumin is a denatured protein that cannot be considered representative of the complexity of the proteins that exist in a living organism. Secondly, the study is indirectly dictating denaturation on the absorbance basis rather than on the actual quantification of the complete thickness of the molecular setup of the protein. Third, the researchers may have influenced the research outcomes through the external factors such as the variations in temperature, the error in measuring the spectroscopic equipment, or minor changes in the sample preparation.

The study is useful as it contributes to information on how environmental factors impact the stability of proteins. Protein denaturation is one of the major factors that occur during different biological processes and diseases. The study of protein misfolding has shown that structural instability of protein is a component of neurodegenerative illness, which revealed that the factors that contribute to protein folding are significant and therefore should be researched (Adam et al., 2023). Proper behaviour of proteins under different conditions is relevant in food science in improving food processing procedures. Studies have also shown that any protein structural modification could increase the functional properties of egg white proteins, ensuring that they can be used in the food industry sustainably (Akbar et al., 2024). Further, the storage conditions and a specified environment are some of the factors that can affect the functional and nutritional properties of the protein that determine its structure (Li et al., 2023).

2 Literature Review

Protein folding is a major aspect in biology, where it helps in preventing the unfolding of proteins and the loss of their usual functions. Denaturation causes loss of the three-dimensional structure of proteins when they are exposed to environmental stresses such as changes in temperatures and result in subsequent misfolding or aggregation of the proteins. Unfolds have been typically linked with neurodegenerative diseases such as Alzheimer's, Parkinson's, and Huntington's, related to protein misfolding. The literature review that follows gives a summary of different key works relating to the topic of protein unfolding, aggregation, spectroscopic research, and correlation of protein misfolding and neurodegenerative illnesses.

Protein Unfolding under Environmental Stress: The article by Jia, Cocker, and Sampath (2025) assesses the impact of the environmental factors: pH, temperature, and mechanical stress on protein unfolding using molecular dynamics simulations. In their experiment, it is found that secondary and tertiary protein structures become destabilized by alterations in temperature, weakening of hydrogen bonds, and change of hydrophobic interactions. The simulations depicted that the protein backbone unfolds gradually with the rise in temperature, and this increases the flexibility of the protein. The paper has identified the fact that temperature-initiated unfolding typically occurs in several stages, in which there exist initial small-scale changes in the conformational changes, followed by extensive structural rearrangement. Such contracts are applicable in experimental research because they will prove that the high temperature of protein denaturing, such as albumin, rises more quickly. This theoretical knowledge plays a role in the study of the effect of temperature to denaturation of egg albumin in the lab.

Spectroscopic Techniques for Egg Protein Davari, Bahreini, and Sabzevari (2023) examined the use of micro-Raman spectroscopy, which is also used in conjunction with multivariate analysis in the assessment of the egg quality and structure of their protein. They have shown that through the experiment that the spectroscopic method can thus detect small alterations in the conformation of proteins by quantifying the alterations in the molecular vibration patterns. The authors have come to the conclusion that the spectroscopic method is highly promising in monitoring structural changes of proteins in the presence of denaturation. The findings support the absorbance-based values in the experimental studies. Although it has more advanced methods, such as Raman spectroscopy, more simplified methods to visualize structural changes, such as colorimetry, may be employed to observe a change in absorbance. That is why it is possible to demonstrate through the use of spectroscopic analysis the validity of the study of temperature-induced denaturation of egg albumin.

Albumin Unfolding and Refolding Mechanisms: Del Giudice and colleagues (2025) investigated the unfolding and refolding process of albumin when conditions were varied by altering PH. Their work indicated that albumin can make reversible changes in the conformational shape under the influence of alterations in the environmental conditions. When the pH suddenly changes, the albumin is quickly unfolded; however, it can re-fold in the event that the conditions come back to normal. These results would be significant; however, the changes indicated insight into the stability of albumin structures despite the study on the pH changes. The researchers found out that unfolding of proteins, in this case of albumin, is a consequence of the distortion of intramolecular interactions that contribute to the maintenance of the tertiary structure of the proteins. Exposure of proteins to high temperatures is brought about by the same processes. Therefore, their work contributes to the literature of the effects of environmental factors that cause stress to the environment, including the temperature, in causing structural destabilization and denaturation of albumin proteins.

Structural Dynamics of Albumin Proteins: Espinosa, Carlevaro, and Ferrara (2025) examined the dynamics of the structure of bovine serum albumin and the effect of chemical substances on protein structure, e.g., urea. They discovered that the dialogue of the albumin structure is highly sensitive to outside circumstances, which disrupts the relations among the molecules. It was revealed that destabilizing reagents induce slow unfolding of proteins, leading to increased exposure of hydrophobic groups. Acetate groups like these bare areas would have the capacity to aid aggregation of proteins as long as the denatured proteins have the capacity to interact. The authors stressed that similar structural processes occur in the event that proteins are exposed to heat stress. Consequently, their findings can be added to the explanation of the fact that the effect of the increase in temperature leads to an increase in the speed of rise of protein denaturation and aggregation.

Protein Misfolding and Neurodegenerative Diseases: Koszala and Solek (2024) came up with the linkage between neurodegenerative diseases and protein misfolding. Their study was caused by a quality control system of protein synthesis within cells that attempted to limit the accumulation of protein misfolding. During the misfolding of proteins, cellular chaperone apparatus, such as chaperone proteins, are endeavoring to abolish or restore the malfunctionally refolded proteins, and degradation machineries appear. However, when these systems become congested, proteins that have been misfolded accumulate and form aggregates, leading to the destruction of neurons. The article reveals the reality that the misfolding of proteins can be induced by factors that are stress-causing, which can be environmental, such as a change in temperature, destabilizing the proteins in their normal folds. Denaturation processes of proteins are, therefore, a useful source of the initial influence on disease pathogenesis.

Human Diseases Associated with Protein Misfolding: Khan and Khan (2022) have reviewed in detail diseases that are associated with protein misfolding. Their study outlined several important neurodegenerative diseases that were linked to intrinsic protein aggregation, as it happens in Alzheimer's, Parkinson's, and amyotrophic lateral sclerosis. The authors explained that protein misfolding is normally initiated by the alteration of their native structure because of environmental stress or genetic mutations. The denatured proteins can be made of toxic aggregates that interfere with the cell's operation. As was previously mentioned in the paper, it is necessary to be aware of the problems that relate to the stability of proteins, including variation in temperature that may lead to denaturation and aggregation of proteins.

Spectroscopic Approaches to Studying Protein Aggregation: Bashir et al. (2024) investigated the recent technologies of spectroscopy, which assist in studying protein aggregation and neurodegenerative disease. Their study led to the revelation of certain methods of this detection of protein structural changes that encompassed Fluorescence spectroscopy, Raman spectroscopy, and absorbance methods. These authors offered an observation that spectroscopic measurements can come in handy, as they will allow the researcher to view the actual protein unfolding and aggregation. Changes in protein conformation can be observed with changes in either absorbance intensity or spectral change patterns. All these findings support the view that colorimetry is applicable in experimental studies to determine the egg albumin denaturation process by measuring the absorbances of the solution at different temperatures.

Molecular Mechanisms of Protein Aggregation: Johnson, Awosiminiala, and Anumudu (2025) talked about protein aggregation molecular pathways in neurological diseases. Their study rationalized the exposure of the hydrophobic regions of destabilized proteins, which are hidden in the folded form. After exposure, such regions of the proteins interact with other unfolded proteins in a way that they produce insoluble aggregates. The authors indicated that the environmental stress factors, like heat variations, could lead to such process stimulation. This study succeeds in supporting the need to explore the concept of temperature-induced denaturation to have a deeper understanding of how insoluble proteins are involved in the onset of a disease.

Protein Aggregation in Neurodegenerative Disorders: In this research, Hu, Lin, Wang, and Zhang (2025) inquired about the current information related to protein aggregation in neurodegenerative disorders. Their study highlighted the role of abnormal protein-folding in the manifestation of poison aggregates that go on to damage the neuronal cells. These authors have emphasized that structural destabilization of individual proteins triggers the protein aggregation process. Environmental factors such as heat stress that disrupt the hydrogen bonds and hydrophobic interactions between the protein structure might facilitate these disruptive occurrences. Their findings indicate that the denaturation of simple proteins, e.g., egg albumin, is a good sample to study the initial processes that lead to the aggregation of proteins in complex biological systems.

Research Gap

Although the mechanisms of protein folding, aggregation, and relation to neurodegenerative diseases have all been comprehensively covered in the literature, areas of gaps in experimental research on environmental interactions influencing protein denaturation have been identified. Many studies are performed using computational simulations to learn more about protein unfolding in the presence of temperature stress, whereas more complex biochemical analyses are considered in a few studies, and even more complex spectroscopic techniques, such as Raman spectroscopy (Davari et al., 2023). Despite essential theoretical and molecular data produced by these techniques, they are normally conducted using complex equipment or computer programs that may not necessarily be transferable to simple experimental research. In addition, there are studies where the stability of albumin is investigated in the presence of chemical or pH change (Del Giudice et al., 2025; Espinosa et al., 2025), although fewer studies take into account the direct quantitative dependence of temperature and protein denaturation under the influence of less complicated laboratory methods that may be readily utilized in research.

3 Material and Methods

3.1 Materials

Fresh chicken eggs were the source of egg albumin protein. The eggs were also obtained locally and eaten throughout the day to retain their freshness and minimize the degradation of the proteins. Egg albumin was chosen because it is a well-defined protein model that exhibits well-defined structural changes to external parameters such as pH. The protein solution was prepared and diluted using distilled water to prevent the interference of salts or ions that were likely to destroy the protein stability or absorbance values. To attain the intended regulation of the pH levels during the experiment, PH 2, 4, 6, 8, and 10 buffer solutions were made such that changes in the structure of proteins are indeed caused by the PH conditions of choice (Obianwuna et al., 2022). Test tubes, beakers, micropipettes, glass stirring rods, cuvettes, and a test tube rack were made of laboratory materials. Micropipettes and absorbance, which is a measure of solution turbidity as a result of protein unfolding and aggregation, were used to measure liquid and colorimeter (spectrophotometer), respectively. To ensure that the values of absorbance would be attributed to the aggregation of protein only, the calibration of the colorimeter was done in the presence of a blank of distilled water. Reagents were of analytical grade.

3.2 Preparation of Egg Albumin Solution

Chicken eggs were kept fresh by placing the egg whites in a beaker, and the egg yolk membrane was not broken. The yolk did not get contaminated because lipids in the yolk can affect absorbance values. A 5% (w/v) solution of egg albumin in distilled water was prepared, after which the dissolved egg white was added (Rao et al., 2020). A glass rod was used to thoroughly mix the mixture. Dilution was necessary to prevent excessive turbidity, which would have hampered the exact determination of the spectrophotometric values. In all the experimental treatments, the stock solution was taken as the stock solution since it ensured that the concentration of the protein was the same in all samples.

3.3 Experimental Design and Variables

Absorbance of the egg albumin solution was considered the dependent variable measured with the help of a colorimeter. The changes in absorbance were the result of the changes in turbidity that was caused by the unfolding and aggregation of proteins (Ho et al., 2021). Some of the factors were unchanged: 5 mL of egg albumin solution was pushed in each of them and 5 mL of the buffer was pushed into each sample; the exposure time was kept at 10 minutes, and the samples were kept at room temperature. The entire experiment was performed with the help of the stock solution and cuvettes.

3.4 pH Treatment of Egg Albumin

There were five test tubes that were labelled with the pH levels. A micropipette was used to pour the egg albumin solution into the respective tubes (5 mL) and 5 ml of the respective buffer was added. The mixtures

were stirred at a low intensity which was done to bring about homogeneity and allowed the mix to rest at the room temperature of 10 minutes in order to give enough time to allow the pH to initiate changes in the structure.

3.5 Measurement of Protein Denaturation

All the solutions were placed in clean cuvettes at the end of the exposure time to measure the absorbance. After the calibration of the colorimeter with the distilled water, the measurement was performed. The absorbance was measured at a fixed wavelength, and three repetitions with each pH treatment procedure were undertaken to improve reliability (Dinç et al., 2020). The average reading was calculated out of these three readings to obtain the average absorbance of each level of pH that was to be used in analyzing the data.

4 Results and Discussion

4.1 Absorbance Measurements

Absorbance values of egg albumin solutions that were subjected to different pH conditions were obtained using the colorimeter. Measurement was made at five levels of pH: 2, 4, 6, 8, and 10. The absorbance value of each of the treatments has been measured thrice, and the means of the absorbance values were obtained to compare the results (Li et al., 2025).

Table 1: Absorbance of Egg Albumin at Different pH Levels

pH	Trial 1	Trial 2	Trial 3	Mean Absorbance
2	0.66	0.65	0.64	0.65
4	0.41	0.40	0.42	0.41
6	0.21	0.20	0.19	0.20
8	0.37	0.36	0.38	0.37
10	0.63	0.61	0.62	0.62

The averages of the absorbance obtained were maintained within 0.20 and 0.65. The maximum absorbance was determined as 0.20 and the mean was 0.20 at PH 6. This fact suggests that the solution was rather evident at near-neutral conditions. More direct resolutions refer to the fact that there would hardly have been any significant change in the albumin molecules during the period of exposure.

Quite to the contrary, pH 2 had the largest absorbance of 0.65, and the average pH was 0.65. The very high pH of absorbance was PH 10 (0.62). These were intermediate values at pHs (4 0.41) and pH 8 (0.37). The increase in absorbance is an indicator of increased turbidity in the solution. The turbidity incidents happen when unfolded protein molecules bind together, and as a result, aggregates are formed in large amounts and will scatter incident light (Bagiyal et al., 2026). The lower end of the absorbance, thus, represents less

aggregation, whereas the higher end represents less protein denaturation and aggregation in acidic or alkaline pH.

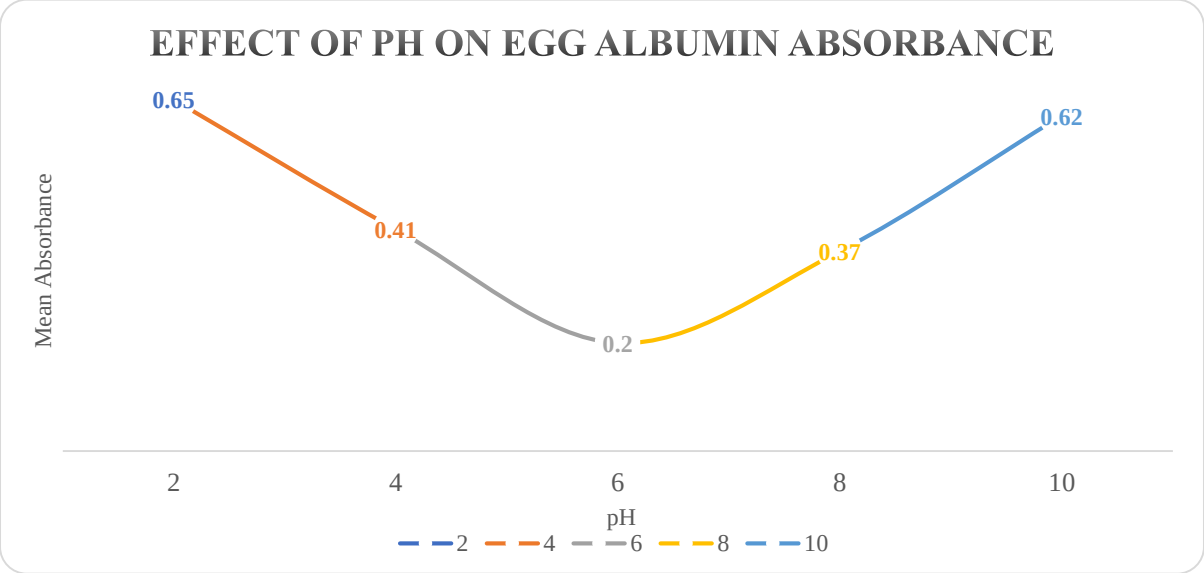

Figure 1: Effect of pH on egg albumin absorbance (self-made)

The graph indicates the pH has an impact on the absorbance. The maximum reading of the absorbance is at pH 6, which shows the low turbidity and stability of the proteins. The absorbance at acidic (pH 2, 4) and alkaline (pH 8, 10) increases. The higher the absorbance, the higher the amount of denaturation and aggregation of proteins and that proves that extreme pH values result into unstable proteins.

4.2 Variation in Absorbance Across pH Conditions

The notable variations in the absorbance were apparent in the state of the experimentally adjusted pH. The lowest mean absorbance was achieved at pH C, where the solution was observed to be clearer than in other treatments. This demonstrates that the egg albumin molecules had not unfolded under almost neutral conditions. When the structure of the protein is maintained, it decreases the amount of aggregates formed in the solution, resulting in the decrease of turbidity and light scattering, which the colorimeter measures. In contrast, the absorbance values were high at acidic pH and at alkaline pH. The peak absorbance was at pH 2, although the absorbance was also quite high at PH 10. The higher the absorbance, the higher the turbidity of the solution (Law & Leaver, 2020). This turbidity is produced due to the loss of the natural structure of the molecules of the proteins and subsequent association with other molecules of the proteins to form aggregates that scatter light. It is therefore pointed out in the outcomes that extreme acidic and extreme alkaline conditions promote protein denaturation.

In between PH 4 and PH 8, it was found that intermediate values were recorded in the absorbance. They were less than the value at PH 2 and PH 10, but at least, they were also less than the absorbance at PH 6. The result of this tendency is that even moderate variations of the neutral pH could influence the stability of the protein, though it is not as intense as the extreme conditions of pH. The tendency is that, in the neutral state, the egg albumin is the most unstable, and the more the acidity or concentration of the alkalinity

of the surroundings, the greater the structural instability (Tseng et al., 2025). The pH change impacts the concentration of the solution's hydrogen ions. These ions have the role of influencing the distribution of charge of the side chains of amino acids in the protein molecule. Electrostatic interaction is the energy that holds the structure of the protein steady and can be weak in case of a difference in this charge balance. These stabilizing forces are disrupted, and the protein begins to unfold, leading to the formation of aggregates that cause the principle of turbidity and the increase of the absorbance value.

4.3 Discussion

Effect of pH on Protein Structure

Proteins have several stabilizing interactions that are used to maintain the three-dimensional structure of proteins. One of these interactions is useful in the maintenance of the folded shape of a protein (Rehman et al., 2022). The pH is changeable in these interactions. The number of hydrogen ions present in the surrounding also varies with the PH of a solution. These amino acids have ionizable groups and react with these hydrogen ions. This results in the charge distribution of the protein molecule possibly changing. This reversal of charge also affects the ionic bonds, also referred to as salt bridges, that would usually help in maintaining the structure of the proteins. This causes the destabilization or disruption of such electrostatic interactions, which cause the protein structure to be weak.

At very acidic pH, e.g., pH 2, the hydrogen ions can protonate a large percentage of the negative charges of the amino acids. This disruption of ionic interactions and repulsion between similar charged locations of the protein. Under alkaline conditions of the solution, e.g., pH 10, the hydroxide ions might remove protons of certain amino acid groups, and change the charge state as well (Vener et al., 2020). The acidic and alkaline environments disrupt the balance of the electrostatic forces that hold the structure of the proteins together.

The experiment results support this molecular explanation. Quite on the contrary, a high turbidity value of 2 and 10 was located. Should be, enhanced in transparency is a symptom of aggregation of disaggregated proteins. These results indicate that extreme acidic and alkaline conditions are favorable to the process of protein denaturation, in which the forces that hold the folded form of the egg albumin together are disrupted.

Protein Aggregation and Light Scattering

Democracy of protein native structure leads to the exposure of internal components of the protein to the solution. These open spaces contain a good amount of hydrophobic amino acids that are normally concealed in the folded protein structure. It is because the exposure of these hydrophobic regions results in the interaction of these hydrophobic regions with the corresponding ones on the other unfolded protein molecules (Rahban et al., 2023). The interactions cause the unfolded proteins to bind to one another,

causing larger aggregates. The aggregates of protein molecules are created by further aggregation in the solution. Otherwise, these aggregates may result in visible precipitates.

Protein aggregation increases the solution turbidity. The turbidity can be explained by the fact that the aggregates scatter the incident light passing through the sample. There is more light scattering, and the absorbance number of the colorimeter is large. In this way, the correlation of the protein unfolding directly with the absorbance exists. Aggregation is more likely to occur during the denaturation of proteins and hence increases the turbidity. This turbidity causes the light scattering to increase, and the absorbance value is high at the time of measurements.

Biological Relevance to Protein Misfolding and Disease

Normal biological functioning requires protein folding. Proteins could also be lost in the three-dimensional form and thus they are misfolded and become nonfunctional. Protein misfolding is likely to build up in most biological systems and assume the form of aggregates. Not only do such plaques damage communication between neurons, but they also cause progressive impairment in cognition (Kamatham et al., 2024). When an individual has Parkinson's disease, there is increased accumulation of the misfolded alpha-synuclein proteins that enter the nerve cells and interfere with the normal functioning of the cells. Prion diseases are also caused by proteins that alter their shape to an abnormal configuration, resulting in a significant neurological defect that causes the misfolding of other proteins.

The results of the experiment with egg albumin provide a simplified model of the understanding of such processes. The experimental findings indicated that the high temperature of extreme pH caused the albumin proteins to unfold and form aggregates to make the solution turbid. Egg albumin cannot cause any disease in human beings, but the aggregation process shown in the experiment is comparable to the aggregation pattern of maladjusted disease-related proteins.

5 Conclusion

In conclusion, one of the aspects that improve the stability of the egg albumin protein structure is pH. It was observed that, the absorbance levels were not the same in the different levels of pH observed and this means that, there are variations in pH that affect the degree of denaturation of the protein. The minimal absorbance was found at pH 6 and this enabled determination that the protein structure was not very unstable under circumstances that were approaching to neutral. The lower turbidity at this pH indicates that there were fewer aggregates of proteins in the solution that had formed. The absorbance of a high value was obtained in an extremely acidic and alkaline environment. The highest turbidity was determined at pH 2 and pH 10, which suggests that the severe pH conditions prefer protein denaturation and aggregation. These findings suggest that the interactions that give the protein structure its integrity are disrupted by the high concentrations of the hydrogen ions and the hydroxide ions. The experiment found that pH change

varies the structural stability of egg albumin. These structural changes are quantitatively determined by the absorbance, and it provides an effective method of evaluating the denaturation of protein.

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