Determination of isoelectric point of protein (casein) essay sample

Introduction:

Casein is a globular colloidal protein. Globular proteins are hydrophobic proteins which in certain external condition are soluble in eater.

The ph at which the protein is electrically neutral is known as the isoelectric point. A globular protein such as a casein becomes increasingly insoluble as it approaches its isoelectric point.

Objectives

The object of this experiment is to determine the isoelectric point of casein (protein), which can be precipitated from the solution.

Apparatus

9 test tubes

pipettes

– 1ml

– 5ml

– 10ml

colorimeter

Materials

Distilled water

Acetic acid

– 0. 01 M

– 0. 1M

– 1. 0 M

casein – 0. 5g/1 in 0. 1 M sodium acetate

Method

1. The calorimeter is switched ON to allow it to “ warm up”.

2. In order to distinguish between the different acidity levels contained in each test tube, the 9 test tubes were labelled from 1-9. This is important because all solution are a similar colour.

3. Following the designated volumes required on table 1, the volumes of distilled water was then pipetted into each test tube. The acetic acid was then pipetted into each test tube according to the values in the table 1.

4. In order to reduce the chances of contamination , the designated amounts of 0. 01M acetic acid was pipetted first to test tubes 1 and 2 because the 0. 01 M acetic acid is the least concentrated acid of the 3 acids. If the process was carries out the other way round, the higher concentration of the 1. 0 M acid would raise the acidity level of the 0/01 acid.

5. Second, the designated amounts of 1. 0 M acetic acid according to table 1 was added to test tubes 3, 4, 5, 6, 7 and 8.

6. Third, the designated amount of 1. 0 acetic acid according to table 1 was added to test tube 9.

7. Fourth, 1ml of casein 0. 5g/1 in 0. 1M sodium acetate was pipetted into each test tube. The pipette was then quickly blown out each time as much of the casein enters the test tube each time. It is important to ensure as accurate a measurement as possible by reading off the bottom of the meniscus precisely each time because the volume and concentration of casein added to each test tube has to remain constant.

8. Then covering each test tube with a different finger for each solution in order to avoid contamination, the solution contained in each test tube were shaken together.

9. After 30 min, the degree of turbidity was recorded on a scale of 0 to 3+ and recorded in a table 1.

10. In order to measure the turbidity of each solution by using the calorimeter, the 9 samples from each of the test tubes were poured into 9 curvettes destined for the calorimeter and labelled accordingly.

11. One curvette was filled with distilled water which acts as a marker for zero turbidity.

12. The curvette containing the distilled water was placed into the calorimeter and the value of turbidity (light absorbency) was recorded for each sample. The calorimeter was set to zero according to the clarity of the distilled water.

13. The remaining 9 curvettes were each placed individually into the calorimeter and the value of turbidity (light absorbency) was recorded for each sample. The calorimeter was reset to zero using the curvette containing the distilled water for each sample to maximise accuracy.

Observations and Conclusion

When acetic acid was added to the distilled water, the solution became acidic because because the concentration of hydrogen ions became greater than the concentration of hydroxide ions. Therefore, the level of energy within the solution has changed. There is a potential difference. The level of the potential difference in the solution can be controlled by alterations of the level of hydrogen ions in relation to the concentration of hydroxide corresponding to the pH scale, which is equal to the expression, where the aqueous hydrogen ion concentration is in mol dm.

The level of pH in the solution was controlled by varying the volume of acetic acid that was added to each test tube, which maintaining a constant overall volume of 9mls.

1ml. of casein 0. 5/1 in 0. 1M sodium acetate was added to each solution and exposed to each solution for 30 minutes for signs of turbidity. The range of the acidity level of acidity for this experiment was (5. 9 – 3. 5) pH.

Casein is a protein. Its structure is composed of amino acids held together by electrostatic bonds. Amino acids are amphoteric. The molecular inherent structure of amino acids uniquely determines it chemical properties. Amino

acids are composed of an amino group, NH2, which is basic, and a carboxyl group, COOH, which is acidic.

The basic group tends to accept hydrogen ions from the acidic group. The acidic group tends to donate hydrogen ions to the basic group. The specific structure of the functional groups, directly affect the behaviour of the amino acids. The chemical behaviour of amino acid will change relatively, according to changes in the functions of state.

According to the results, value for turbidity continuously increased form zero to 0. 4 as pH continuously from 3. 5 to 4. 7.

The value for turbidity reached its maximum at pH 4. 7. Beyond pH 4. 7. the value of turbidity continued to decrease to zero.

The maximum value of turbidity was 0. 4, which occurred in the solution of pH 4. 7, where the hydrogen ion concentration is equal.

This value corresponds to the position of the isoelectric point of the amino acid, because the level of turbidity began to decrease in the examples of casein that are exposed to pH levels greater than pH 4. 7. The isoelectric point corresponds to the position of the energy level at which , the amino acid becomes dipolar, where its overall electrostatic charge is neutral, when the charge expressed by the basic amino group of the amino acid is equal to the charge expressed by the acidic carboxyl group.

Once the protein becomes dipolar, the overall electrostatic charge becomes zero.

The protein becomes hydrophilic and insoluble in water because water can only dissolve molecules that are polarised. The water molecules release the amino acids from their hydrogen bonds.

By observation, the experience is such that when casein amino acids are in this particular stage, where the electrical charge of the amino groups equal the charge of the carboxyl group, the casein amino acids appear to cluster together in to the precipitate, which can be extracted from the solution. The shape of the cluster is related to the fundamental geometric arrangement of the functional group that determines the precise molecular structure of casein.

If the isoelectric point of casein is approximately pH 4. 7, it suggests that the side chain of the amino acids of casein contains a greater amount of acidic functional groups because a greater concentration of hydrogen ions has to be added to the distilled water in order to neutralise the charge emitted from the functional groups.

Altering the level of pH in relation to its specific isoelectric point has a simultaneous effect on the way electrical energy is expressed by the two functional groups of an amino acid.

As the pH level decreases beyond the isoelectric point, the charge expressed by ionisation of the amino group increases, but the charge expressed by the carboxyl group experiences an increasing suppressive effect.

As the pH level increases beyond the isoelectric point, the charge expressed by ionisation of the carboxyl group increases, but the charge expressed by the amino group experiences an increasing suppressive effect.

Thus, the level of electrical energy of the molecule is activated by deviations in the level of pH in the solution from the isoelectric point of the amino acids in which the amino acids are contained.

At extreme levels of acidity, where the level of pH exceeds the operating range of the amino acid, the hydrogen ions combine with COO_ groups of the amino acid form COOH. If the force of the hydrogen ions becomes greater than that of the force of the hydrogen bonds, the bonds are broken and the 3 – D tertiary structure of the protein can no longer be maintained.

However, an excessive reduction in the concentration of hydrogen ions causes the amino group, to lose hydrogen ions.

In either direction, the 3-D tertiary structure of the protein which is held together by electrostatic hydrogen bond and ionic bonds are hence broken and consequently, the function of the casein is altered.

When the results corresponding to turbidity in relation to pH was drawn graphically, the isoelectric point of casein corresponds to the peak of the curve according to the two variables pH and turbidity.

The shape of the graph follows a relatively smooth curve, which represents a definitive relationship between the two variables. The peak of the curve corresponds to approximately 4. 7 on the pH axis, although the precise value is actually pH 4. 75.

Evaluation

Possible errors which could have caused the inaccuracy of the final determination could possibly be located back to the procedures used to carry out the experiment during the pipette stage where relatively low volumes (fractions of 1ml, for example, in the case of test tube 1 where 0. 62 mls) of acetic acid were added to the distilled water were probably not dispensed accurately enough. Since the operating threshold of biological molecules is relatively fine, the finest of differences causes substantially greater differences in the readings for the levels of turbidity in the solutions.

For example, the mouth of the 1ml pipette gauge did not fit tightly into the dispenser which continuously caused air bubbles to enter the solution, which would affect the actual volume of solution dispensed into the test tubes, particularly wherever the volume required was less than 1 ml.