

# Protein precipitation and isolation of casein from milk



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## Protein Precipitation Methods and Isolation of Casein from Milk

### Introduction

Protein is one of the major constituent of all living organisms on earth which are made up of in a sequence of amino acid. They are linear polymers of amino acids with high molecular weights which are known as macromolecules. Protein contain carbon, hydrogen, nitrogen and also oxygen. But sometimes in protein, sulphur might also can be observed (Ferrier, 2014).

Proteins are made up of smaller units called amino acids - the monomer form which the polymer proteins are made. Each of cells in all living organisms contain thousands of different proteins. All these proteins are made up of from twenty different amino acids. One fact, each amino acid has the same basic structure but different only from alkyl group which attaches into it. The simplest amino acid is glycine with an R group of a single Hydrogen atom (Vasudevan, 2013).

There are different shapes allows protein to fold into and they perform many different roles in chemistry of living things. When two amino acids are bonded, it is the C and N atoms join up in peptide bond. Amino acid can bond to each other one at a time where forming a long chain called a polypeptide is seen. They are made on the ribosomes in the cell (80s and 70s, mitochondria and chloroplast).

Proteins normally comprise one polypeptide. Most proteins are large molecules, and contain a hundred or so amino acids. There are four main structure of proteins as: primary, secondary, tertiary and quaternary.

Primary structure is a sequence of amino acids available in the polypeptide. Secondary structure is a helical or pleated sheet structure. Tertiary structure is bending, folding of polypeptide produce globular shape. Different types of bonds stabilizing tertiary structure. Quaternary structure aggregate of 2 or more polypeptides to form a complex structure (Gromiha, 2011).

Denaturation of protein may occur after exceeding 60 °C leading to the breakage of H bonds, peptide bonds and ionic bonds. This denaturation causes precipitation of proteins (Chawla, 2014). The following mentioned processes explains the procedure of protein precipitation.

1. Precipitation by salt
2. Isoelectric precipitation
3. Precipitation by organic solvent
4. Precipitation by acidic agents
5. Precipitation by heavy metals
6. Precipitation by heat and acid

### Objectives

The main objectives of this practical is to:

- Demonstrate the basic laboratory techniques on protein precipitation methods
- Demonstrate the biuret test for proteins
- Differentiate casein from non-fat milk powder

## Materials

- Beakers
- Bunsen burner
- Test tubes
- Filter papers
- Funnel
- Flask
- Albumin
- Ammonium sulphate solution
- Ammonium sulphate solid
- Acetone
- Alcohol (ethanol)
- Sulphosalicylic acid
- Lead nitrate
- Non-fat Milk powder
- Tissue papers
- Stirrer
- Chemical balance
- Petri dishes

## Methodology

### Protein Precipitation methods

#### Precipitation by salt

1. Half saturation with ammonium sulphate 3ml of albumin was taken into a test tube and equal amount saturated ammonium sulphate solution

was added into the test tube. Then the test tube was shaken to mix the solution well and allowed to stand it for 5 minutes. Then filtered the solution and biuret test was carried out.

2. Full saturation with ammonium sulphate solid 3ml of albumin was taken into a test tube and equal amount saturated ammonium sulphate solid was added into the test tube. Then the test tube was shaken to mix well and allowed to stand it for 5 minutes. Then filtered the solution and biuret test was carried out.

#### Precipitation of organic solvents procedure

2ml of albumin was taken into a test tube and 4ml of 95% ethanol was mixed together to observe a cloudy white color precipitate.

#### Precipitation by acidic agents

1ml of albumin was added to an equal amount of sulpho salicylic acid and mixed well to observe a thick white color precipitate.

#### Precipitation by heavy metals

1ml of albumin was taken into a test tube and 8 drops of lead nitrate solution was added into it and the observation of white color precipitate was observed.

#### Precipitation by heat and acid

Take 10ml of albumin was taken into a test tube and hold the upper position of the test tube into the flame and heated the solution to observe the cloudy

precipitate. After that 1% acetic acids was introduced to the same test tube and coagulation and albumin precipitation was observed.

#### Isolation of casein from milk

17. 50g of non-fat milk powder was mixed with 62. 50ml of water and allowed to heat in a 400ml beaker for 45 °C. Then 10% of acetic acid was added into the beaker and mixed until the liquid color change into milky to clear solution. Then 1. 50g of Powdered Calcium carbonate was added into the beaker and mixed it well with a stirrer. Then the casein was separated by filtering the solution. Separated casein was mixed with acetone acid and allowed get dried. After drying the casein by using tissue papers, it was measured by using a chemical balance and observed the readings.

#### Results and Observations

1. Test	Observation	Inference/conclusion
Precipitation by Salts		
2. Half saturation with ammonium sulphate solution	Filtrate : clamp was observed	Globulin can be precipitated by half saturation
Biuret reagent with the filtrate	Protein was present in the	
Purple color was observed.	solution	
Precipitation by Salts	Filterat: clamp was observed	Albumin and globulin

3. Full saturation with  
ammonium sulphate  
solids

precipitated by full s

Biuret regant with filterate      Protein was not present in the  
Purple color was not observed      solution

Precipitation by Organic      White color precipitate was      Acetone and methan  
Solvent      observed      precipitate protein

Precipitation by Acidic Agents      Thick white color precipitate      Sulpho salicylic acid  
was observed      precipitate protein

Precipitation by heavy metals      White color precipitate was      Lead nitrate can be  
observed      precipitate protein

Precipitation by heat and acid      Albumin precipitate was  
observed and coagulation was      Protein can be precip  
observed      heat and acid

Isolation of casein from milk      9. 62g casein was measured      17. 5g of non-fat mil  
contain 9. 62g of cas

### Calculation

Calculation of the percentage of casein was carried out after isolating the casein from non-fat milk powder.

$$9.62\text{g} / 17.50\text{g} = 0.54$$

$$0.54 \times 100 = 54\%$$

It is confirmed that 17.5g of milk powder contain 54% of casein in it.

### Discussion

The obtained results shows, the albumin which can be precipitated by organic solvents, heavy metals, acidic reagents. Heat and acid method, by half saturation and also the full precipitation. The albumin and globulin will be precipitated where the casein can be isolated by the non-fat milk. The casein which was obtained by the experiment was with water. And therefore to dry the casein, acetone used to remove water molecules from casein by evaporating them. Initial mass of milk powder was 17.5g, but it was found that 54% of casein in mass was included in that sample finally.

Results can be deviated, if the experiment was done with contamination errors by using same pipette for different purposes. So care should be taken when handling chemicals to ensure that one chemical will not get contact or mix with another chemical unless if required.

Mass of casein could be wrong if there are external disturbances when measuring the mass by using chemical balance. Since chemical balance shows very close values.

When casein is separating from the milk, it should be taken fully without keeping any residue in the beaker. If not deviation of the mass of the casein may vary.

### Conclusion

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Finally, the precipitation methods of protein was identified as:

1. Precipitation by salts
2. Isoelectric precipitation
3. Precipitation by organic solvents
4. Precipitation by acetic agents
5. Precipitation by heavy metals
6. Precipitation by heat and acid

Casein was the protein which is present in milk powder and it is the main protein component present in milk. As shown in the above calculation, It is confirmed that 17.5g of milk powder contain 54% of casein in it.

## References

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