

FACULTY OF
HUMANATIES & SCIENCE

Module Name	Introduction to Biology
Module Code	SC1143
Title of Experiment	Determine the proteins solution of unknown samples
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**negative biuret
test results**

**positive biuret
test results**

Objective:- To identify forms of proteins

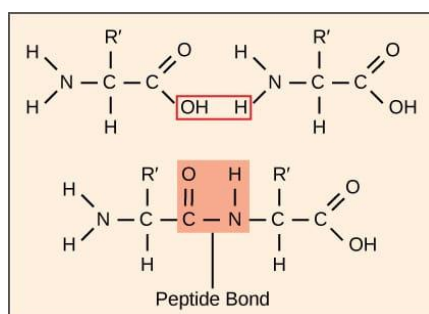
To detect the protein in the given solution

To demonstrate the presence of the peptide bond

To learn how that to do biuret test for proteins

Introduction:-

proteins are naturally occurring, extremely complex substance that consists of amino acid residues joined by peptide bonds. Proteins are present in all living organisms and include many essential biological compounds such as enzymes, hormones, and antibodies and proteins are very important. proteins differ from carbohydrates and lipids by containing nitrogen and sometimes phosphorus and Sulphur. Proteins are among the most abundant organic molecules in most living systems they make up about 50 percent dry weight. The protein is built up by large number of amino acids. Amino acid is building block of protein.



Protein has got multiple functions. Protein acts as a growth material for the organism, Structures of living materials are composed of different types of protein molecules, It also acts as a part of fuel of the organism, all the pituitary hormones, hypothalamic-releasing factors, certain placental hormones, pancreatic hormones, are proteins in nature, Similarly all enzymes are proteins in nature.

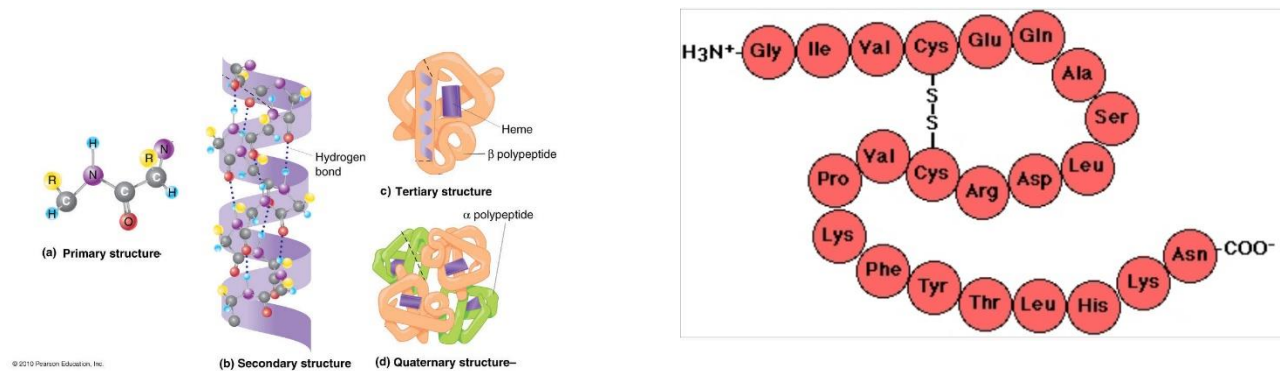
It is convenient to describe protein structure in terms of 4 different aspects of covalent structure and folding patterns. The different levels of protein structure are known as primary, secondary, tertiary, and quaternary structure.

The primary structure of a protein is its unique sequence of amino acids. Secondary structure, found in most proteins, consists of coils and folds in the polypeptide chain. Tertiary structure is determined by interactions among various side chains (R groups). Quaternary structure results when a protein consists of multiple polypeptide chains. The coils and folds of secondary structure result from hydrogen bonds between repeating constituents of the polypeptide backbone and typical secondary structures are a coil called an alpha helix and a folded structure called a beta pleated sheet.

Collagen is a fibrous protein consisting of three polypeptides coiled like a rope. Hemoglobin is a globular protein consisting of four polypeptides two alpha and two beta chains.

Sickle-cell disease, an inherited blood disorder, results from a single amino acid substitution in the protein hemoglobin.

The biuret test measures peptide bonds in a sample. proteins are made up of amino acids connected together with peptide bonds. In an alkaline solution, copper II is able to form a complex with the peptide bonds. Once this complex has been formed, the solution turns from a blue color to a purple color



Material and methods:_

Materials:-

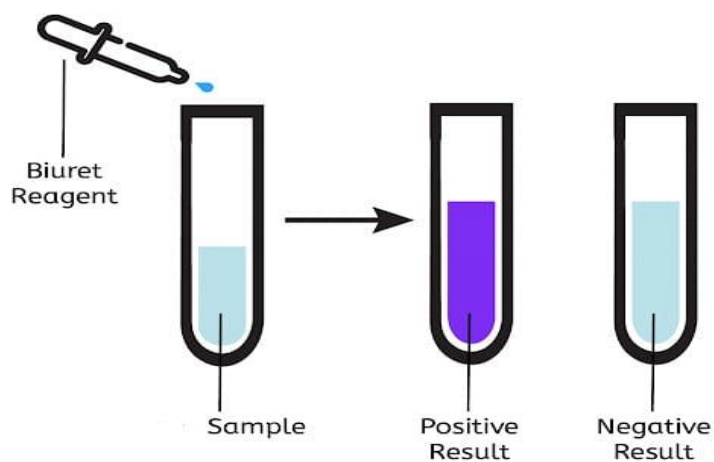
- seven test tubes
- 1% CuSO₄ solution
- NaOH solution
- eyedropper
- Test tube holder
- distilled water
- egg albumin solution
- honey solution
- protein solution
- amino acid solution
- bean solution
- dry fish solution

Methods/ procedure:-

Firstly seven test tubes were taken and numbered them since 1 to 7. Then each was added the materials to be tested. There were distilled water, egg albumin solution, honey solution, protein solution, amino acid solution, bean solution, dry fish solution. Then NaOH solution was added about 2ml to each tubes and after 1% CuSO₄ solution about 3 drops were added them and mixed. Test tubes were observed color changes in during time. Finally test tubes' color recorded in a table.

Results:-

solution	Biuret's color reaction
Distilled water	Pale blue color
Egg albumin solution	Purple color
Honey solution	No color
Protein solution	Violet color
Amino acid solution	No color
Bean solution	Little purple color
Dry fish solution	Light purple color



Discussion:-

A Biuret test is a chemical test used to determine the presence of a peptide bond in a substance. It is based on the biuret reaction in which a peptide structure containing at least two peptide links produces a violet color when treated with alkaline copper sulfate.

Biuret solution was added any solution when if it has protein or peptides bonds it gives purple color and if it has not proteins or peptides it gives blue color. Purple color is positive result and blue color is negative result.

CuSO₄ solution was used here because it also gives a positive reaction to the peptide-like bonds in the biuret molecule. The copper (II) binds with nitrogen present in the peptides of proteins. In a secondary reaction, the copper (II) is reduced to copper(I).

As a strong alkali, NaOH can induce the denaturation of proteins when added to fresh egg white. In the presence of NaOH, the secondary structure of the denatured proteins was destroyed, hydrogen bonds were broken, and the number of hydrophilic groups increased.

When biuret solution was added into the distilled water, pale blue color changes was given because there are not present proteins or peptide bonds. Water gives a blue color because CuSO₄ solution is blue color, and it dissolves in water.

When biuret solution was added into the egg albumin solution, purple color was given because there are present protein and peptide bonds. When biuret solution was added into the honey solution, color change was not given because there are not proteins. When biuret solution was added into the protein solution, violet color change was given due to it contains abundance of protein or peptide bonds. So, it was given high positive result. When biuret solution was added into the amino acid solution, no color change, because there are not peptides bonds.

All peptides and protein give the test positive. But histidine is the only amino acid that give biuret test positive When biuret solution was added into the bean solution, little purple color was given and difficult to seen, because there are little of proteins and peptide bonds. When biuret solution was added into the dry fish solution, light purple color was given because there are somewhat proteins and peptide bonds.

Adding too much biuret's reagent or test solution may give false results. 3. If too much CuSO₄ solution was added can give false results.

Conclusion:-

Biuret reagent in the detection of protein applications, impact detection reagents and calibrators will test result, during the test than when it is necessary to detect deviation detection reagents and calibrators due to be considered.

Reference:-

Shrestha B (2002). Practical biochemistry and biotechnology. First edition. 99933-665-1-X.

Allen, L.H., E.A. Oddoye, and S. Margen. 1979. Protein-induced hypercalciuria: A longer term study. Am. J. Clin. Nutr. 32:741-749. [PubMed]

Anderson, J.W., B.M. Johnstone, and M.E. Cooke-Newell. 1995. Meta-analysis of the effects of soy protein intake on serum lipids. N. Engl. J. Med. 333(5): 276-282.

<https://www.sciencedirect.com/topics/medicine-and-dentistry/biuret>

<https://peptidesciences.com/information/peptide-bonds/>

<https://www.slideshare.net/jammalavamsikrishna/amino-acids-71579203>