Frequently Asked Questions

1. What are proteins? Give some different methods of analysis of proteins?

Ans) Proteins are complex nitrogenous organic substances.These might be of plant or animal origin. Twenty different types of amino acids occur naturally in proteins. Proteins differ from each other according to the type, number and sequence of amino acids that make up the polypeptide backbone. Various methods for anlaysis of proteins are kjeldahl method, combustion method, biuret, Lowrys method and Dumas method

2. What is Kjeldahls method and gives its significance?

Ans) It is the method used to estimate the nitrogen content in the samples through which its protein content can be estimated. In this method ,a sample (food) is digested with a strong acid so that it releases nitrogen which can be determined by a suitable titration technique. The amount of protein present is then calculated from the nitrogen present in the sample. It helps to find the amount of protein in biological fluids and foods.

3. Explain secondary and tertiary structure of proteins?

Ans) Secondary structure: Protein secondary structures are regular structures formed by hydrogen bonds between amide planes. The secondary structure of protein involves folding the primary structure. Hydrogen bonds between amide nitrogen and carbonyl oxygen are the major stabilising forces. The two main types of secondary structure are α-helix, and the β - pleated sheets. The α-helix is stabilized by intra molecular hydrogen bonds, the sheet structure by intermolecular hydrogen bonds.

Tertiary structu**re: The** tertiary structure of a protein is its three-dimensional structure. Fibrous proteins have secondary structure elements arranged parallel to a single axis. Examples include a-keratin and collagen.

4. Why do we use blank solution in Lowrys Method?

Ans) A blank solution is used to be able to subtract the influence of reagents and light that is scattered on the surfaces of the cuvette.

5. Explain the primary structure of proteins?

Ans) The sequence of amino acids in a polypeptide chain is known as the primary structure of proteins. The primary structure of proteins is responsible not only for the final shape of the protein but also for its biological function. Proteins are made up of about twenty-two amino acids, which are linked by the peptide bond, an amide linkage involving the amino group and carboxyl group. The formation of peptide bond results in the loss of an amino group and a carboxyl group of each amino acid, the remaning portion of the molecules (the residues) being the major components in the structure of protein.

6. Why Specific Jones factor for nitrogen content of the sample being analysed is used?

Ans) A Specific Jones factor for nitrogen content of the sample being analysed should be used to convert nitrogen content present. This is because a single factor of 6.25 is based on the assumptions: that dietary carbohydrates and fats do not contain nitrogen and nearly all of the nitrogen in the diet is present as amino acid in proteins. On these basis, the nitrogen content of proteins was found to be 16% leading to the calculation N x 6.25 (1/0.16 = 6.25). However these assumptions are counteracted by two considerations. First, not all nitrogen in foods is found in proteins where it is referred to as non protein nitrogen (NPN). Secondly, the niotrogen content of specific amino acid varies according to the molecular weight of the amino acid and the number of nitrogen atoms it contains. Based on these facts, the nitrogen content of the proteins actually varies from about 13 to 19%, This requires nitrogen conversion factors ranging from 5.26 (1/0.19) to 7.69(1/0.13). Hence Jones suggested replacement of “N x 6.25” by “N x a factor specific” for the food in question. Also, use of general factor for the individual foods that are major sources of protein in the diet introduces an error in protein content that is relative to the specific factors and ranges from -2 percent to +9%.

7. Why biuret test is positive for proteins?

Ans) Biuret test is positive for protein and polypeptides because a substance called biuret formed by heating urea, gives a postive test for detection of proteins.

8. Explain isoelectric point of a protein?

Ans) The *isoelectric point* (pI) of a protein is the pH where the net charge on the protein is zero. Proteins tend to aggregate and precipitate at their pI because there is no electrostatic repulsion that may keep them apart. Proteins have different isoelectric points because of their different amino acid sequences (*i.e.,* relative numbers of anionic and cationic groups), and thus they can be separated by adjusting the pH of a solution. When the pH is adjusted to the pI of a particular protein it precipitates leaving the other proteins in solution.

9. Write a note on ion exchange Chromatography?

Ans) Ion exchange chromatography relies on the reversible adsorption-desorption of ions in solution to a charged solid matrix or polymer network. This technique is the most commonly used chromatographic technique for protein separation. A positively charged matrix is called an *anion-exchanger* because it binds negatively charged ions (anions). A negatively charged matrix is called a cat*ion-exchanger* because it binds positively charged ions (cations). The buffer conditions (pH and ionic strength) are adjusted to favor maximum binding of the protein of interest to the ion-exchange column. Contaminating proteins bind less strongly and therefore pass more rapidly through the column. The protein of interest is then eluted using another buffer solution which favors its desorption from the column (*e.g.,* different pH or ionic strength).

10. What is electrophoresis?

Ans) Electrophoresis is often used to determine the protein composition of food products. The protein is extracted from the food into solution, which is then separated using electrophoresis. Sodium dodecyl sulphate-Polyacrylamide gel electrophoresis (SDS-PAGE) is used to determine the molecular weight of a protein by measuring Relative mobility(*R*m), and then comparing it with a calibration curve produced using proteins of known molecular weight. A plot of log (molecular weight) against relative mobility is usually linear. Denaturing electrophoresis is more useful for determining molecular weights than non-denaturing electrophoresis, because the friction to movement does not depend on the shape or original charge of the protein molecules.