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Estimation of protein by biuret method

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Protein concentration is a crucial factor in various research fields, including protein biology and molecular biology. Before proceeding to isolation, purification, and analysis, the concentration of protein samples must be estimated using one of several available methods. Each method has its own advantages and disadvantages, often relying on the levels of tryptophan, tyrosine, or other aromatic amino acids. When selecting a method, three key factors should be considered: sensitivity (ability to detect proteins at low concentrations), specificity (efficiency in detecting protein specifically amidst interfering substances), and time (duration for assay completion and result interpretation). Three main methods are commonly used: 1. **Biuret Method**: While not the most sensitive method, it is simple and can measure all protein samples with accuracy. However, it requires high protein levels and does not account for amino acid composition. The reaction involves copper sulfate, sodium hydroxide, and potassium sodium tartrate. 2. **UV Absorption Method**: This method has moderate sensitivity, detecting proteins in the range of 50-100 µg without needing reagents. It can be completed quickly within 10 minutes using devices like Nanodrop or pico drop that require only a small volume of protein sample (1 µl). 3. **BCA Assay and Lowry Assay**: Both methods are highly sensitive, detecting proteins at very low concentrations. The BCA assay involves copper ions binding to nitrogen in the protein, causing a color change proportional to concentration. This method can be affected by certain substances but is faster than others. The Lowry assay is also sensitive and gives accurate results, working with protein levels as low as 2-5 µg. These methods vary significantly in terms of sensitivity, specificity, time required for completion, and the potential interference from various substances. Agents and a Modified Lowry Assay for samples in detergents are available for use. A popular method is the Bradford assay, developed by Marion M. Bradford in 1976, which is highly sensitive and simple to perform. This dye-binding assay uses Coomassie brilliant blue-250 dye that binds with negatively charged protein molecules, and its color changes based on protein concentrations. The absorption can be measured at 595nm, making it relatively quick compared to other methods, with results obtainable within 10-15 minutes. A standard curve allows for the estimation of protein concentration without delay. However, this method has a drawback in being less specific to interfering substances like SDS and Triton x-100. A Bradford assay kit that includes reagents for cleaning samples prior to assaying is also available. Besides Coomassie blue 250 dye, other dyes such as Pyrogallol red and Bromocresol green are used for protein estimation. The Bradford assay stands out among its counterparts due to its speed and sensitivity, making it a preferred choice in many scientific applications. Sample preparation for the Biuret Test involves diluting unknowns to an estimated 1-10 mg/ml with buffer. A reference tube should contain 1 ml of buffer, and a standard curve should be prepared using absorbance versus micrograms protein. The actual concentration cannot always be estimated, so a range of dilutions is recommended. Add 9 ml Biuret reagent to each assay tube, then vortex immediately and let stand for 20 minutes before reading at 550 nm. Determine the amount from the curve and the concentrations of original samples from the amount protein, volume/sample, and dilution factor if any. The color remains stable but should be read within a 10-minute window to maintain accuracy. Proteins with high or low percentages of amino acids with aromatic side groups may give high or low readings respectively. The Biuret reaction forms a violet-colored complex proportional to protein concentration. This chemical reaction involves copper ions from the Biuret reagent and peptide bonds in proteins. A representation of this reaction includes copper ions, peptide bonds, and hydroxide ions, resulting in a violet-colored product with electron reduction. The color intensity is directly related to protein concentration. To perform the Biuret Test for Protein, prepare a 1% $CuSO_4$ solution by dissolving 1 gram in 100 ml of distilled water and adding 10 ml of 10% NaOH solution. Mix and dilute the solution to achieve a final volume of 100 ml. Add 2 ml of the sample and 2 ml of Biuret reagent to a test tube, mix gently, and incubate at room temperature for 10 minutes. Measure the absorbance at 540 nm using a spectrophotometer and compare it to a standard curve to determine protein concentration. The Biuret Test has applications in medical diagnosis, food analysis, biochemistry, biotechnology, and research, including diagnosing liver disease and detecting proteins in body fluids and recombinant protein production. The Biuret Test is a widely used method to determine the protein content of various food products, detect adulteration, and analyze proteins in biochemical and biotechnological research. The test utilizes a blue-coloured alkaline solution called the Biuret reagent, which reacts with peptide bonds in proteins to form a violet-coloured complex. This reaction's intensity is directly proportional to the protein concentration in the sample. The Biuret Test has several applications, including medical diagnosis, food analysis, and research. It is a simple, rapid, and inexpensive method for protein detection and quantification. However, it cannot distinguish between different types of proteins and may produce rare false-positive results due to interference from substances like amino acids or detergents. The test's sensitivity allows it to detect low concentrations of proteins as 1mg/mL, making it a valuable tool in various fields. The Biuret Test is based on the principle that copper ions react with peptide bonds to form a complex, and its procedure is straightforward, allowing for easy interpretation of results.