DRAFT REVISIONS FOR COMMENTS

This draft revision contains revised general chapter text for inclusion in the Indian Pharmacopoeia (IP). The content of this draft document is not final, and the text may be subject to further revisions prior to publication in the IP. This draft does not necessarily represent the decisions or the stated policy of the IP or Indian Pharmacopoeia Commission (IPC).

Manufacturers, regulatory authorities, health authorities, researchers, and other stakeholders are invited to provide their feedback and comments on this draft proposal. Comments received after the last date will not be considered by the IPC before finalizing the monograph.

Please send any comments you may have on this draft document to lab.ipc@gov.in before the last date for comments.

Document History and Schedule for the Adoption Process

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Further follow-up action as required.	

1. **General chapter- (2.3.30) Nitrogen; IP 2022 Pg. 179**
   Method G
   Para 2

   Change **from:**

   To 1 g of catalyst……For Blank, take 1ml of distilled water instead of substance under examination.

   to:

   To 1 g of catalyst……For Blank, take 1ml of distilled water instead of substance under examination. For those products having nitrogen containing excipients in the formulation, take 1 ml of the placebo or appropriate blank instead of water as blank.

2. **General chapter- (2.3.49) Protein; IP2022 Pg. 199**

   Change **from: Method A**

   to: Method A-I

   Insert following before method B:

   Method A-II (Biuret Method for Blood and blood related Products)

   This method is based on the interaction of cupric (Cu^{2+}) ion with protein in alkaline solution and resultant development of absorbance at 545 nm. This test shows minimal difference between equivalent IgG and albumin samples. Addition of the sodium hydroxide and the biuret reagent as a combined reagent, insufficient mixing after the addition of the sodium hydroxide, or an extended time between the addition of the sodium hydroxide solution and the addition of the biuret reagent will give IgG samples a higher response than albumin samples. The trichloroacetic acid method used to minimise the effects of interfering substances also can be used to determine the protein content in test samples at concentrations below 500 µg/ml.

   Use distilled water to prepare all buffers and reagents used for this method.

   Test solution: Dissolve a suitable quantity of the substance to be examined in 0.9% solution of sodium chloride to obtain 0.5% solution or having a concentration within the range of the concentrations of the reference solutions.

   Reference solutions: Dissolve the reference substance (Bovine Serum Albumin/ Human Serum Albumin) for the protein to be determined in 0.9% solution of sodium chloride. Dilute portions of this solution with 0.9% solution of sodium chloride to obtain not fewer than three reference solutions having protein concentrations evenly spaced over a suitable range situated between 0.5 mg/ml and 10 mg/ml (0.05% to 1.0%).

   Blank: Use a 0.9% solution of sodium chloride.

   Biuret reagent: Dissolve 3.46 g of copper sulfate pentahydrate in 10 ml of hot distilled water, and allow to cool (Solution A). Dissolve 34.6 g of sodium citrate and 20.0 g of anhydrous sodium carbonate in 80 ml of hot distilled water, and allow to cool (Solution B). Mix solutions A and B and

dilute to 200 ml with distilled water. Use within 6 months. Do not use the reagent if it develops turbidity or contains any precipitate.

Procedure: To one volume of the test solution add an equal volume of 6% solution of sodium hydroxide and mix. Immediately add biuret reagent equivalent to 0.4 volumes of the test solution and mix rapidly. Allow to stand at a temperature between 15° and 25° for not less than 15 min. Within 30 min of addition of the biuret reagent, determine the absorbances (2.4.7) of the reference solutions and test solution at the maximum at 545nm using the blank as compensation liquid. Any solution that develops turbidity or a precipitate is not acceptable for calculation of protein concentration.

Calculations: The relationship of absorbance to protein concentration is approximately linear within the indicated range of protein concentrations for the reference solutions. Plot the absorbances of the reference solutions against protein concentrations and use linear regression to establish the standard curve. Calculate the correlation coefficient for the standard curve. A suitable system is one that yields a line having a correlation coefficient not less than 0.99. From the standard curve and the absorbance of the test solution, determine the concentration of protein in the test solution.

Interfering substances: To minimise the effect of interfering substances, the protein can be precipitated from the test sample as follows: add 0.1 volumes of a 50% solution of trichloroacetic acid to 1 volume of a solution of the test sample, withdraw the supernatant layer and dissolve the precipitate in a small volume of 0.5 M sodium hydroxide. Use the solution obtained to prepare the test solution.