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Estimation of protein in human albumin solution (for infusion) by Kjeldahl method

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ABSTRACT

A few reports are available on human serum protein determination by Kjeldahl method but there is no report on estimation of total protein in human albumin solution 20% (for infusion). This is a first report on estimation of total protein in human albumin solution (for infusion) by Kjeldahl method and is based on the data of 20 specimens of human albumin solution. The method has delivered good results with a sample standard deviation of 0.35. The mean value of 19.82 has confirmed the reliability and consistency of the method. All results were within the criteria of acceptance.

KEYWORDS

Human albumin;
Albumin;
Human serum protein.

INTRODUCTION

The Kjeldahl method for protein estimation is basically a method developed by John Kjeldahl in 1883 for determination of total nitrogen in organic substances[1]. Since then, the Kjeldahl method has been the official worldwide standard for the determination of total nitrogen or protein in all kinds of food samples[2]. The Kjeldahl digestion converts nitrogenous compounds (proteins, amines, organic compounds etc.) into ammonia compounds and these compounds release ammonia upon addition of caustics during distillation. The same basic principle approach is still being used today, although a number of modifications and improvements have been made to speed up the process and to obtain accurate results. It is usually considered as the standard method of estimation of protein concentration. The protein content in an organic substance is not directly measured by Kjeldahl method but it measures nitrogen and a conversion factor is used to convert the measured nitrogen to a protein concentration[3]. For many proteinaceous compounds a conversion factor of 6.25 which is equivalent to 0.16 g nitrogen per gram of protein, is used[4]. However, this is only an average value and each protein may require a different conversion factor depending on its composition. Proteins are polymers of amino acids and they differ from each other according to the type, number and sequence of amino acids present in their polypeptide backbone. Proteins are also the major structural components of human tissues. Human serum albumin is the most abundant protein in human blood and constitutes about 50% of the blood serum protein. Being a part of connective tissue albumin transports hormones and fatty acids. It is also responsible for maintaining pH and osmotic pressure of the blood. The concentration of albumin in a healthy

adult individual ranges from 35 - 50 g/L and has a serum half-life of approximately 20 days. Human albumin is generally infused to restore blood volume in trauma, burns and surgery patients. It is frequently infused in conditions where loss of albumin is a major problem, such as liver disease with ascites. The infusion of albumin is also recommended in chronic dehydration or hypoalbuminemia. The concentrations of human albumin solution generally available for medical use is 5-25% and mostly 20% solution is used for infusion. Therefore, the accurate determination of concentration is must before its infusion to the body.

Our institute is a quality control institute for biologicals including albumin and as one of the parameter the estimation of protein in human albumin solution is done by modified Kjeldahl method.

EXPERIMENTAL

Sample

Twenty specimens of Human albumin solution (20% as per label) for infusion and positive control (Albumin, M/s Sigma Aldrich).

Determination of albumin concentration

The Kjeldahl method with modifications was followed for all specimens, blank and positive control. This method was carried out in three steps:

Digestion - 200 µl of albumin solution was diluted six times and digested at 380 °C for 2 hours with 2 ml conc. H_2SO_4, 100 mg of Copper (II) sulfate and 900 mg of Potassium sulfate to liberate the reduced nitrogen as ammonium sulfate. The clear and colourless content got on completion of chemical decomposition, was cooled to room temperature.

Distillation - The decomposed content was dissolved in 20 ml distilled water and distilled with 5M NaOH (final conc.) to collect 65 ml of the distillate by dipping the tip of condenser in a mixture of 10 ml saturated Boric acid, 10 ml water and 60 µl indicator mixture (0.1% Methyl red and 0.05%).

Titration - The distillate was titrated with 0.02 N HCl to get end point (from green to pink color).

Calculation of total protein

The net volume of 0.02 N HCl consumed was calculated by subtracting volume of 0.02 N HCl consumed by blank. This net volume of 0.02 N HCl was used in the following formulae to calculate total protein.

$$\text{Total protein} = \text{Net volume of 0.02 N HCl consumed} \times 0.2802 \times \frac{6.25 \times \text{actual normality of HCl}}{\text{Normality of HCl}}$$

Note: One ml of 0.02 N HCl is equivalent to 0.2802 mg of nitrogen and 6.25 (100/16) is the factor to convert nitrogen into protein because most of the proteins contain 16% nitrogen.

The Standard deviation was calculated using http://www.calculator.net/standard-deviation-calculator.html.

RESULTS AND DISCUSSION

The most commonly used procedures for total protein estimation are folin phenol[5], biuret[6], UV absorption[7], coomassie blue dye binding[8] and classical Kjeldahl[2,9]. The Kjeldahl method is used widely at international level, and is still the standard method for comparison against all other methods. Its universal use, high precision and good reproducibility have made it the major method for the estimation of protein.

This is first study on estimation of total protein in human albumin solution (for infusion) by Kjeldahl method. Being a regulatory institute, this institute receives albumin solution for quality testing. The estimation of total protein in human albumin solution was carried out following guidelines of pharmacopoeia. This report is based on analysis of twenty (20) albumin solution specimens (TABLE 1).

The sample standard deviation (s) was 0.35 and sample standard variance (s^2) was 0.12. The mean concentration determined was 19.82. Population standard deviation (σ) and population standard variance (σ^2) were 0.34 and 0.11 respectively. As per Indian pharmacopoeia, the acceptance criteria for albumin 20% solution is 19-21%. The deviations were small and fell within the margin of acceptance criteria and experimental variation of the method. The protein content is conventionally estimated from the nitrogen content determined by the Kjeldahl technique. A number of modifications of the original procedure have been proposed[10]. In our study, the conversion factor of 6.25 worked well for albumin. While this factor has been widely used for many plant tissues. Peterson[11], examined various methods

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TABLE 1 : Concentrations of protein in albumin solution specimens.

Specimen no.	Conc. As per label	Conc. by Kjeldahl method
1	20 %	19.89 %
2	20 %	20.07 %
3	20 %	20.30 %
4	20 %	20.28 %
5	20 %	19.43 %
6	20 %	19.07 %
7	20 %	19.25 %
8	20 %	19.80 %
9	20 %	20.00 %
10	20 %	20.00 %
11	20 %	20.28 %
12	20 %	19.63 %
13	20 %	20.18 %
14	20 %	19.25 %
15	20 %	19.77 %
16	20 %	19.94 %
17	20 %	19.90 %
18	20 %	19.79 %
19	20 %	19.80 %
20	20 %	19. 96 %

of protein determination based on their relative sensitivity for bovine serum albumin and a range of proteins. He concluded that all spectroscopic methods are similar in sensitivity. He also reported that these simple procedures usually give relative rather than absolute protein quantitation[11]. Agusti and Beltran[12], showed that Lowry and Bradford methods measure higher amounts of proteins than by using the Kjeldahl method[12]. Biuret reagent test provides results with a positive bias for total protein in serum analysis[13]. In view of results obtained, the author is inclined to pronounce that the Kjeldahl method is satisfactory in estimation of protein in albumin solution.

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CONFLICT OF INTEREST

None declared

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