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Total Organic Carbon Analysis

Measurement of total nitrogen (TN) for protein estimation in pharmaceutical vaccines on the Shimadzu TOC-L with TNM-L

This article introduces the application of Total Nitrogen Measurement (TNM) as a powerful analytical technique for protein estimation in biopharmaceutical products such as vaccines. The study was performed at the Serum Institute of India Pvt. Ltd., Pune.

Nitrogen is a fundamental component of amino acids, which are the building blocks of proteins. Measurement of nitrogen content is widely being used for the estimation of proteins and its metabolites in pharmaceuticals, foods, beverages, and plant materials. Nitrogen commonly occurs in two forms-organic and inorganic. The inorganic forms include nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+).

In pharmaceutical vaccine production, the quantity of the antigen needs to be controlled at the initial-, the intermediate-, and the final stages of the production cycle. Thus, viral vaccines or bacterial vaccines are tested for the quantity of respective attenuated or inactivated viruses or bacteria. Since these antigens typically consist of proteins, analytical quantification of total protein becomes crucial. Diphtheria and tetanus toxoids combined with pertussis antigens are used as a combination DTP vaccine. It confers immunity in children against diphtheria, tetanus, and pertussis.

A conventional Kjeldahl method is used for protein nitrogen estimation in vaccines. Its major limitation is the need of different correction factors for different proteins as proteins have variable amino acid sequences. In addition, it is time-consuming and laborious. Further, the use of concentrated sulfuric acid at high temperatures can pose a considerable hazard to safety and health.

In this application, high temperature combustion coupled with chemiluminescence detection technique was explored for the estimation of organic nitrogen and inorganic nitrogen. This method provides a fast and an efficient way to monitor nitrogen loading by Total Nitrogen (TN) analysis [1]. The results were compared with both experimental values (Kjeldahl method) and theoretical values.

TN Determination

Samples containing nitrogen compounds decompose to nitrogen monoxide (NO) at 720°C by combustion. The thermoelectrically cooled and dehumidified NO gas passes through chemiluminescence analyzer, where NO reacts with ozone and forms a combination of nitrous oxide (NO_2) and excited nitrous oxide (NO_2*). As the NO_2* returns to the ground state, it emits radiation, which is measured photo-electrically. The detector signal generates a peak that is proportional to the nitrogen concentration in the sample. The reaction process of TN analysis has been described in Figure 1.

A comparative study of TN measurement of the DPT vaccine by the conventional Kjeldahl method and the TNM method has been presented in this application.

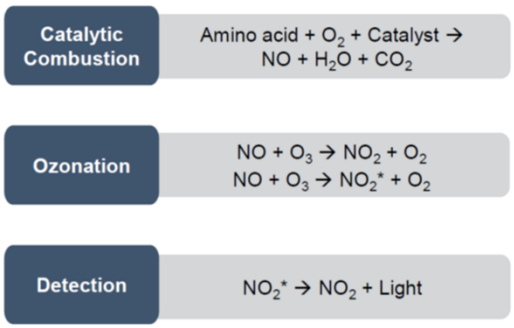

Figure 1. TN Reaction Process

Figure 2. TOC-L analyzer with TNM-L

Experimental Methods

Three vaccine batches each of Tetanus and Diphtheria toxoids, Pertussis Toxin & FHA (Filamentous Haemagglutinin) Antigen were selected for a comparative study of the Kjeldahl method and the TOC/TN method by the Serum Institute of India Pvt. Ltd., Pune. TN analysis was performed using the TOC-L_{CPH} analyzer with TNM-L.

TOC technique for nitrogen estimation was evaluated successfully by analyzing samples with standard amino acids on the TOC-L analyzer with TNM-L as shown in Figure 2. Both the TOC/TN technique and the Kjeldahl technique were compared and the results obtained have been shown in Table 1.

Calibration of the TOC/TN system was carried out by using a standard solution of aqueous potassium nitrate. Representative amino acids L-Glutamic acid and L-Histidine at the concentrations of 1000 mg/L and 500 mg/L, respectively, were used for the recovery study.

Table 1. TN measurement results for amino acids

Samples	Kjeldahl nitrogen content (mg/L)	TOC/TN nitrogen content(mg/L)	%Recovery
L-Glutamic acid (1000 mg/L)	95.2	91.5	96.11
L-Histidine (500 mg/L)	101.6	100.3	98.72

Results and Discussion

The results obtained by the comparative study of the Kjeldahl method and the TOC/TN method for vaccines have been shown in Tables 2 and 3.

Calibration curve of potassium nitrate showed good linear regression with a correlation coefficient (r) ≥ 0.9997 as shown in Figure 3. A representative measurement data obtained for amino acids has been shown in Figure 4.

The results of the total nitrogen estimation obtained for amino acids by the TOC/TN method and the Kjeldahl method were comparable. Percentage recovery was found to be within 100±10%.

Table 2. Results of the TOC/TN method and the Kjeldahl method for Purified Tetanus and Purified Diphtheria toxoids

Sample	Batch	TOC/TN nitrogen content (mg/L)	Kjeldahl nitrogen content (mg/L)
Purified Tetanus toxoid	Batch 1	1.496	1.546
Purified Tetanus toxoid	Batch 2	2.160	2.205
Purified Tetanus toxoid	Batch 3	1.935	1.991
Purified Diphtheria toxoid	Batch 1	1.481	1.537
Purified Diphtheria toxoid	Batch 2	1.523	1.559
Purified Diphtheria toxoid	Batch 3	1.544	1.587

Table 3. Results of the TOC/TN method and the Kjeldahl method for Purified Pertussis Toxin and FHA Antigen

Sample	Batch	TOC/TN nitrogen content (mg/L)	Kjeldahl nitrogen content (mg/L)
Purified pertussis toxin	Batch 1	71.590	69.100
Purified pertussis toxin	Batch 2	79.220	74.500
Purified pertussis toxin	Batch 3	83.260	76.500
Purified FHA Antigen	Batch 1	86.080	81.600
Purified FHA Antigen	Batch 2	35.540	32.900
Purified FHA Antigen	Batch 3	94.790	96.600

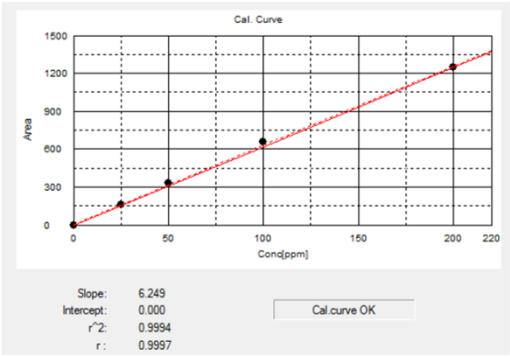

Figure 3. TN calibration curve

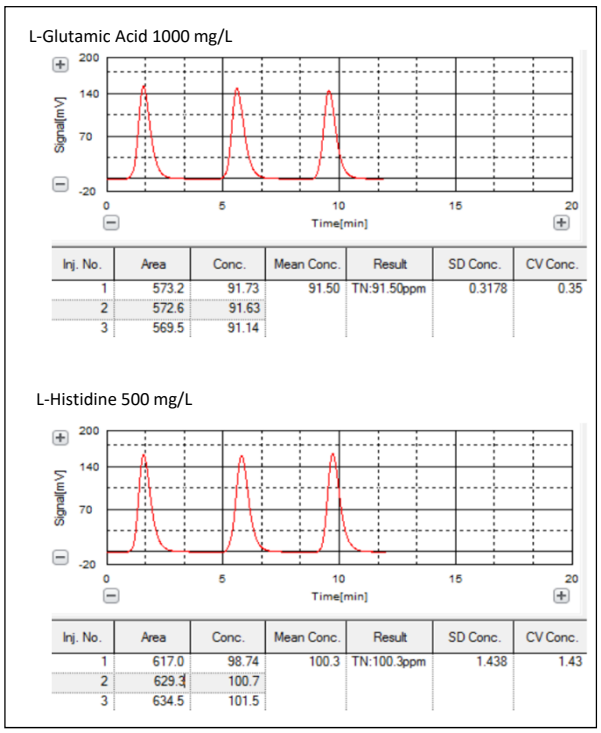

Figure 4. Results of TN measurement of amino acids

Conclusion

An alternate and a more effective method of total nitrogen estimation in proteins was developed by using the TOC-L analyzer equipped with TNM-L. The results obtained were comparable to those obtained by the conventional Kjeldahl method.

The TOC analyzer with TNM-L offers a time saving and a safe method for protein estimation. Thus, based on the generated data for samples under study, this application can be explored for various nitrogen-containing pharmaceutical and biopharmaceutical products.

Acknowledgements

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Reference

[1] ASTM D8-83-16: A New Method for Total Nitrogen (TN) & Total Kjeldahl Nitrogen (TKN)

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