Quick and reliable determination of complete proteins in pharmaceutical products by way of a TOC/TN_b Analyser

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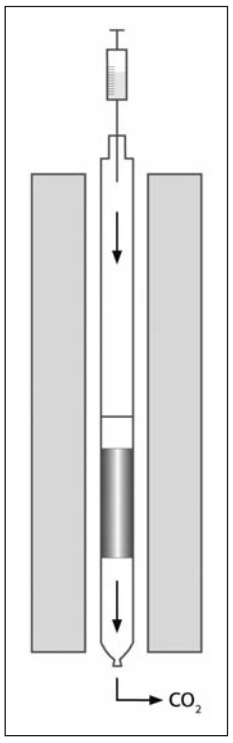
In the pharmaceutical industry, the protein contents in culture media (for example, in vaccines) often have to be determined within the context of quality controls of raw materials, intermediate and finished products. Today a broad range of methods is available for this purpose. The European Pharmacopeia (Ph.Eur. 2.5.33) alone describes seven different methods that are based on different reaction and detection principles. The largest group of such methods consists in protein tests according to Lowry, Bradford, BCA, and Biuret which are all based on different colouring reactions with certain amino acid rests, followed by an absorption measurement at certain wavelengths in the visible spectral range. In addition, the pharmacopeia contains descriptions of the direct UV absorption method, a fluorescence analytical method as well as two methods based on the nitrogen content measurement.

At this point it would be of advantage to add a brief note on protein chemistry. Proteins are macromolecules consisting of amino acids and therefore belong to the basic modules of all animal and plant cells. Proteins consist of different amino acids, and amino acid chains with a length of less than 100 amino acids also are called peptides. Many proteins like viruses or antibodies (immunoglobulins) form so-called protein complexes and consist of several single proteins, which are connected with each other by hydrogen bridges, salt bridges, or disulfide bridges.

All these connections, however, have in common that they contain nitrogen atoms to a large extent and therefore can be analysed very well together with a complete nitrogen method.

On one hand this can be done by a sulphuric acid pulping according to the Kjeldahl method or, in a far more elegant way, by the catalytic high temperature incineration in the oxygen flow, which is described in DIN EN 12260 and treated more thoroughly within the context of this article.

The measuring method



For determining the nitrogen content in solutions or suspensions containing proteins, only a measuring device with the possibility to inject directly a sample aliquote can be used as the available sample quantities often are very small. With injection volumes from 50 to 100µl, a few 100µl of sample already are sufficient to conduct a multiple determination.

According to DIN EN 12260, existing now for about 15 years and regarding the determination of the complete bound nitrogen (TN_b) in water samples, the combustion temperatures have to be guaranteed higher than 700°C for sample digestion in presence of a catalyst. When this so-called thermocatalytic digestion of the sample occurs, the nitrogen connections, which are contained in the sample mainly form nitrogen monoxide and, to a smaller extent, higher oxidised nitrogen connections. The generated NO is transported to a chemiluminescence detector (CLD) via the adjacent carrier gas flow. There the NO molecules first react with the ozone generated in the CDL that leads to the creation of highly charged NO_2 molecules. According to the reaction scheme explained above, these charged NO_2 molecules will be transformed again to NO_2 in its basic state by discharging luminescence radiation, which is detected by a photomultiplier.

$$NO + O_3 \longrightarrow NO_2^* + O_2$$

$$NO_2^* \longrightarrow NO_2 + h\bullet v$$

The presented method is appropriate for detecting all connections containing nitrogen but not for detecting molecular nitrogen (N_2). This, however, means that the presence of anorganic nitrogen connections as well as of organic non-proteinogenic nitrogen connections may lead to interferences when using this method for determining the protein content. The complete nitrogen method though convinces by eliminating the necessity of preparing any samples and by extremely short measuring times of only 3-5 minutes per sample injection.

TN_b measurements in vaccines with the multi N/C 2100 S

The multi N/C 2100S from Analytik Jena AG can determine protein contents in all liquid matrices efficiently and automatically. In addition, further parameters as TOC, TIC, or TC can be measured.

The multi N/C 2100S is a TOC/TN_b analyser, which follows the principle of injecting the liquid sample directly into a quartz glass combustion tube filled with catalyser. The sample supply at the multi N/C 2100S can be adjusted optimally to the application requirements by using different injection syringes of the sizes 100, 250, or 500µl in connection with different autosampler racks for 2ml vials or even 8ml vials. Even suspensions containing particles can be measured in a representative way by using canulas with a large interior diameter and by the standard sample homogenisation with a magnetic stirrer at the sampler. Due to the septum-free injection principle, this analysing device reaches highly reproducible measuring results independently from the canula diameter of the used syringe. Generally, during the analysing procedure the combustion temperature is at 800°C in presence of a platinum catalyst and using synthetic air as carrier gas.

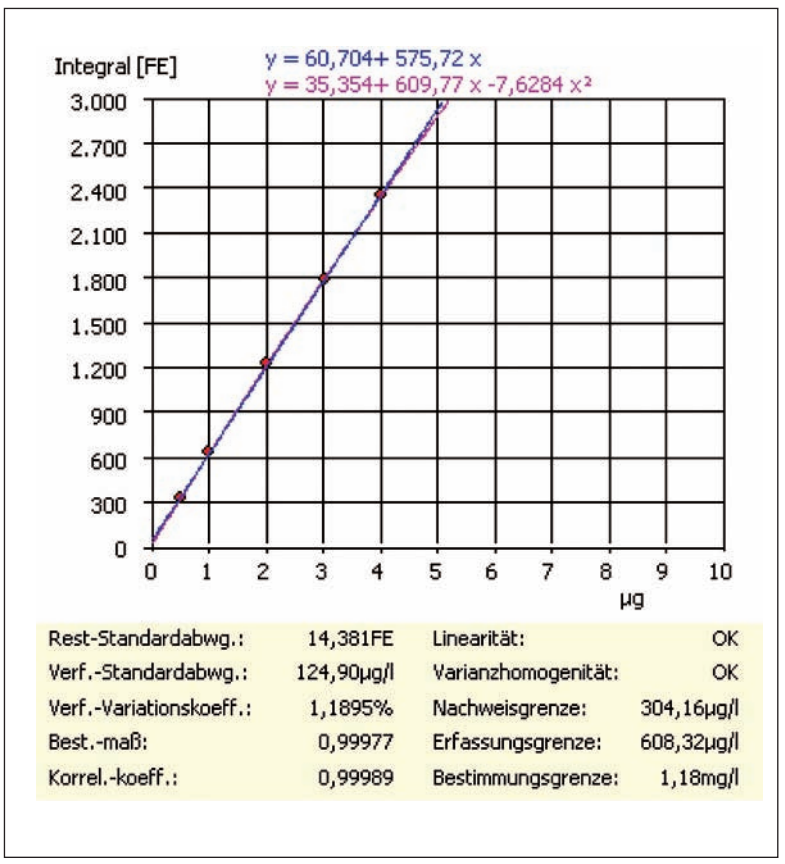
With a measuring range of 0.1 up to 200 µg/ml TN, the analyser offers a measurement sensitivity that is comparable to the most sensitive protein test of the BCA method (0.2 – 50 µg/ml protein).

The main point of this measuring method for determining proteins is the calibration of the method as well as the conversion of the received results in the form of the complete nitrogen content into the complete protein content of the sample. Generally, it is recommended to calibrate a method close to the matrix, i.e. when determining TN in solutions containing proteins a protein standard should be used also for calibration. This deviates from the ordinary method in accordance with the norm which describes a calibration with an ammonium / nitrate mixed standard.

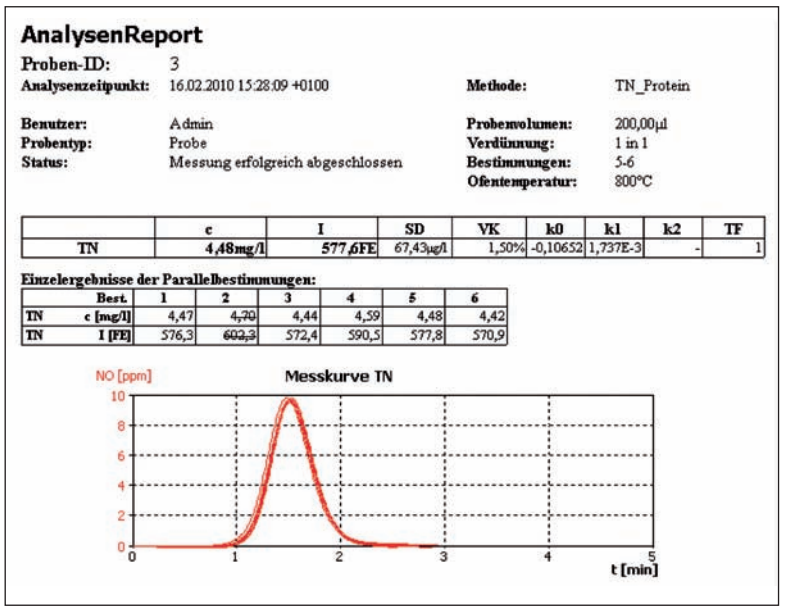
The nitrogen measurements in vaccines as described here have been carried out using the Bovine serum albumin - BSA (Sigma Art.No. A-7906) with a specified nitrogen content of 15.87% and a purity exceeding 98%. For this BSA standard a nitrogen conversion factor of 6.30 has been calculated.

The conversion factor often to be found in reference literature for converting the N concentration within the protein concentration is at 6.25.

protein content [mg/ml] = nitrogen factor (6.25) * TN content [mg/ml]



Multiple point calibration with a BSA standard of 2.5 to 20mg/l TN_b



AnalysenReport

Proben-ID: 3
Analysenzeitpunkt: 16.02.2010 15:28:09 +0100
Methode: TN_Protein

Benutzer: Admin
Probentyp: Probe
Status: Messung erfolgreich abgeschlossen
Probenvolumen: 200,00µl
Verdünnung: 1 in 1
Bestimmungen: 5-6
Ofentemperatur: 800°C

	c	I	SD	VK	k0	k1	k2	TF
TN	4,48mg/l	577,6FE	67,43µg/l	1,50%	-0,10652	1,737E-3	-	1

Einzelergebnisse der Parallelbestimmungen:

Best.		1	2	3	4	5	6
TN	c [mg/l]	4,47	~~4,70~~	4,44	4,59	4,48	4,42
TN	I [FE]	576,3	~~602,3~~	572,4	590,5	577,8	570,9

TN measuring curve of a vaccine measurement.

Next to BSA solutions as a control standard also an aqueous solution of the hyperimmune antiserum 1-3, rabbit 37 with a specified IgG concentration of 8.3mg/ml has been used as reference material during the measurement series. After conversion with the N factor for proteins of 6.25 this corresponds to a N content of 1.328mg/ml. A corresponding dilution of this master solution has been measured as independent control standard.

The following table sums up the measuring results for several analysed vaccines. These results have been received by 5-fold injection of 200µl undiluted sample.

Sample name	TN concentration [µg/ml]	RSD [%]	Protein content calculated with N factor 6.25 [µg/ml]
BSA standard (10 mg/l)	10,23	0,86	63,9
Vaccine 1	9,32	1,23	58,3
Vaccine 2	6,83	1,81	42,7
Vaccine 3	4,48	1,50	28,0
Hyperimmune antiserum 1-3 (5 mg/l)	5.03	1,39	31,4

Summary

By using a TN_b analyser in accordance with DIN EN 12260, for example, the multi N/C 2100S from Analytik Jena AG, for analysing proteins, you can replace the conventional protein test methods which often are work-intensive, hardly reproducible and interference-sensitive by a quick, reliable and automatable instrumental analytical method. This method is characterised by a large linear measuring range, high sensitivity and selectivity, and unnecessary sample preparation steps are eliminated. Therefore the impact of the human factor on the analysing results is remarkably minimised. The method is described in the European pharmacopeia and can be validated relatively easily. Interferences like anorganic nitrogen salts or non-proteinogenic organic nitrogen connections that have a disturbing effect on many protein tests can be excluded here, if necessary, by protein precipitation and separation. Altogether the determination of the complete nitrogen content with a TOC/TN_b analyser offers great potential for making work easier and increasing the quality during the pharmaceutical quality control for a whole range of products.

When will you begin to use this advanced measuring method for analysing devices in your laboratory?

New Generation of Possibilities in Guiding Decisions for Drug Discovery

Optibrium™ launched the next generation of StarDrop™ featuring the ground breaking Nova™ module at the American Chemical Society (ACS) National Exposition in March. This intuitive platform helps to guide scientists' decisions to quickly identify compounds with a high chance of success for their drug discovery project. Nova is a completely new module within StarDrop 5, helping pharmaceutical and biotech companies to search for high quality drugs by generating new chemistry ideas, prioritised against specific property profile requirements when making critical decisions in drug discovery. Fast identification of high quality compounds with the best balance of multiple property requirements is a major challenge in making effective drug discovery decisions. Complex, often conflicting requirements, combined with uncertain data, make it difficult to decide with confidence which lines of enquiry to pursue and which compounds to prioritise. So, it's no surprise that most research in drug discovery results in expensive failure.

The revolutionary Nova module stimulates the search for high quality compounds by generating new chemistry ideas, prioritised against defined property profile requirements. It facilitates the search for prospective drug candidates by expanding the chemistry around an initial compound based on medicinal chemistry transformation rules, ensuring that they are chemically feasible. Independent tests proved that more than 94% of generated compound structures were acceptable to medicinal chemists. In addition to an initial library of more than 200 transformations derived from literature and provided with Nova, chemistry knowledge can be transferred between medicinal chemists by defining their own transformation rules, flagging favourites and grouping rules tailored to specific goals. Nova provides the user with complete control of which transformations they wish to apply and enables a rigorous exploration of possibilities while searching for compounds with improved properties. Nova ensures a broad exploration for new chemical ideas and, combined with StarDrop's existing probabilistic scoring, chemical space visualisation, Glowing Molecule™ and predictive modelling capabilities, helps to quickly focus on high quality chemistry and speed up hit-to-lead and lead optimisation. Example applications include rigorously exploring the chemistry around new hits to identify those most likely to provide access to good drug-like properties; searching for new chemical strategies to overcoming issues in lead optimisation, including lead hopping; and identifying opportunities for patent busting.