C. Gerhardt compendium

NITROGEN ANALYSIS
THE JOHAN KJELDAHL METHOD

Evolution

From a historical perspective, the official analytical methods used to determine nitrogen are certainly old. Johan Kjeldahl (Fig. 1) published his "New method for the determination of nitrogen in organic substances" in 1883, revolutionising nitrogen analysis [1]. The versatility of the method and simplicity of performing the analysis, combined with its high precision, have made the method a standard in the food and animal feed sector. The method also continues to be very important in analyses of soil and water, and in fact wherever there is a need to determine bound nitrogen.

Fig. 1: Johan Kjeldahl

While Johan Kjeldahl was determinedly investigating the protein content of beer wort in his laboratory in the Carlsberg Brewery in Denmark, over in Paris, Jean Dumas was interested in combustion analysis of natural materials to identify their carbon and hydrogen content. But both researchers where acting on the same underlying idea: A sample has to be completely decomposed so that the nitrogen can be extracted and determined as a measurand. Kjeldahl accomplished this decomposition, or digestion, using highly concentrated sulphuric acid, whereas Jean Dumas combusted the sample with pure oxygen. Although both basic ideas appear at first glance both simple and logically consistent, implementing them in practical laboratory conditions involved many critical phases and a great deal of attention from trained laboratory staff.

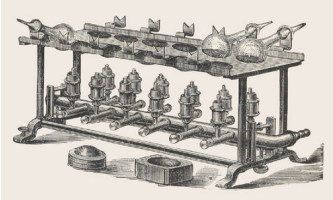

Fig. 2: Classic digestion apparatus in accordance with J. Kjeldahl, with gas burners and tilted Kjeldahl wide-necked round-bottom flask [2]

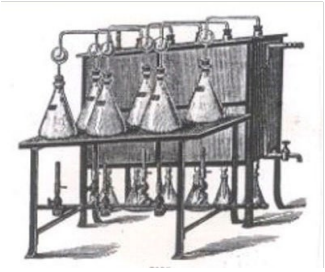

Fig. 3: Gas burner apparatus for distillation of digestion solutions

Analysis steps in Kjeldahl method

The analysis essentially consists of the following work steps:

- Digestion of samples with sulphuric acid
- Distillation of digestion solution with water steam
- Titration of the distillate and calculation of results

Acid digestion

Digestion according to Kjeldahl's method is based on the principle that the sample is oxidatively decomposed with concentrated, boiling sulphuric acid. The bound nitrogen is dissolved out of its bond matrix without any losses and is completely converted into inorganic ammonia nitrogen (NH4+-N). At the end of the digestion reaction, all the sample's nitrogen should be present as ammonia nitrogen.

$$(CHNO)\ (s) + H_2SO_4\ (l) \rightarrow CO_2\ (g) + SO_2\ (g) + H_2O\ (g) + (NH_4)2SO_4(aq) + H_2SO_4\ (l)$$

This is only technically possible in sufficiently large round-bottom flasks and with powerful gas heaters. Figure 2 shows a classic digestion apparatus with six units and gas burners – an early attempt at serial processing of acid digestion. Digestion of this kind in boiling sulphuric acid lasted about 3 to 5 hours and was suitable for sample quantities of up to 10 g – making it a very universal method.

Today, block or infrared digestion systems are used to meet contemporary safety needs in the laboratory. Heat is no longer transferred directly through the open flame of the gas burner, but rather through cast heating elements or contact-free infrared radiation. Regardless of which heat source is used, the boiling sulphuric acid should condense almost fully on the glass walls and runs back into the digestion solution. Figure 4 shows an idealised situation with digestion solution (green), condensing acid fumes and an exhaust manifold placed on top for the corrosive digestion gases.

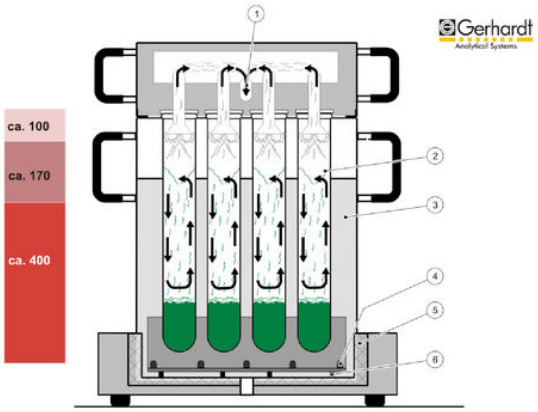

Fig. 4: Modern digestion apparatus according to Kjeldahl with cast heating elements in block arrangement, using the KJELDATHERM model from C. Gerhardt as an example.
On the left in red: the temperature range in the digestion tube
1-extraction, 2-return line and condensation zone,
3-insert rack, 4-tubular heating element, 5+6 insulation

To prevent corrosive gases from entering the laboratory air, the digestion apparatus is generally connected to a powerful gas scrubber. This condenses the escaping acid gases and, in a second step, neutralises the remaining acid gases with sodium hydroxide.

Figure 5 shows an example of a gas scrubber with one washing flask for condensing and one for neutralising acid fumes, as well as a powerful circulation pump for generating an adjustable, slight negative pressure.

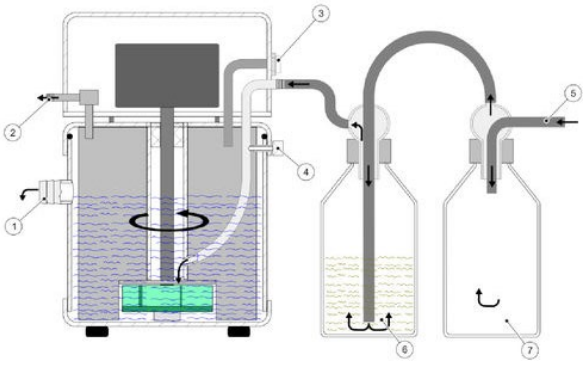

Fig. 5: C. Gerhardt TURBOSOG gas scrubber for suction and neutralisation of acid fumes.
1-water outlet, 2-gas outlet, 3-water inlet, 4-regulation of suction effect, 5-digestion gases input, 6-neutralisation flask with potassium hydroxide solution, 7-condensation flask

Quantity of acid for digestion

The quantity of sulphuric acid is determined by three factors:

- Consumption for oxidation of the organic sample matrix
- Evaporation losses during the boiling process
- Conversion of potassium sulphate into potassium hydrogen sulphate

While the latter two factors are constant under the same digestion conditions, the quantity of sulphuric acid needed for oxidation depends on the sample weight and the composition of the sample. For example, two to three times as much sulphuric acid is needed to oxidise fat and protein as carbohydrates. Table 1 shows calculated sulphuric acid quantities per gram of sample for different substrates.

Sample material	Consumption of sulphuric acid [g] per 1 g sample
Cane sugar	8.36
Cereal flour	6.27
Gelatine	17.64
Oleic acid	19.87

Tab. 1: Quantities of acid for digestion

Loss from evaporation should be kept to minimum. At the end of digestion, there should ideally still be an excess of unconsumed sulphuric acid. To simplify routine laboratory work, a standardised quantity of sulphuric acid is used, amounting to about 20 ml for 1 – 5 g of sample weight.

Digestion temperature

Only concentrated sulphuric acid is used for digestion according to Kjeldahl. It boils azeotropically at 338 °C (98%). To enable quicker conversion, a sulphate salt is added to raise the boiling point of the sulphuric acid, in accordance with van´t Hoff's rule.

Raising the reaction temperature accelerates oxidation and therefore the conversion to ammonium sulphate as per the Arrhenius equation for the speed constant k of the digestion reaction. The digestion temperature T therefore has a direct influence on speed constant k of the reaction because both variables are linked by an exponential function. Raising the temperature by 10 °C increases the reaction speed by a factor of 2, if the activation energy is E_A = 60 kJ/mol. Acceleration by a factor of 25 would result from an activation energy of E_A = 250 kJ/mol. Figure 6 shows typical dependencies for different organic sample matrices.

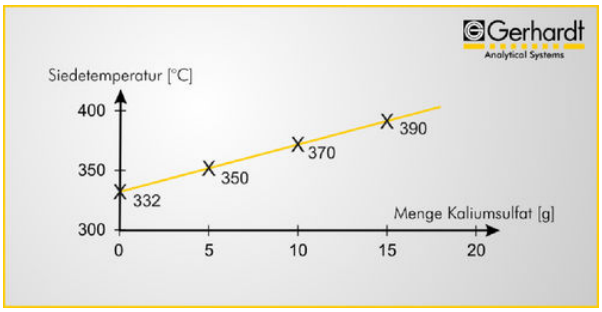

Fig. 6: Dependencies between the boiling temperature of 20 ml of concentrated sulphuric acid and the quantities of sulphate salt added, in grams.

If 5 g of potassium sulphate are added, the boiling point is raised by approximately 20 °C for 20 ml of concentrated sulphuric acid. Commonly, 10 g of sulphate salts are added for this quantity of sulphuric acid, enabling a higher digestion temperature without significant evaporation losses. The standard conditions are 400 °C adjusted temperature for simultaneous digestion of up to 20 samples in a 250 ml digestion vessel. The heavy metal sulphates that were once used here are no longer used, for environmental reasons; it is now accepted that a slightly longer digestion time is a price worth paying for less hazardous catalysts [3]. The use of conditioned catalyst mixtures in tablet form has proven practical. There are available commercially in the most commonly used compositions and are added to concentrated sulphuric acid in a proportion of 2 tablets for each 20 ml of acid.

Order nr.	Composition
12-0328	5.0 g K_2SO_4 + 0.5 g $CuSO_4$ x 5 H_2O
12-0329	5.0 g K_2SO_4 + 0.15 g $CuSO_4$ x 5 H_2O + 0.15 g TiO_2

If a target temperature of 400 °C is set in the digestion system, the concentrated sulphuric acid boils and generates a return line/condensation line on the wall of the digestion tube. The effect of this is for any parts of the sample that are boiled up are washed back into the solution and digestion is complete after about 1 to 2 hours, at which point the digestion solution is green, due to the copper salts (Figure 7).

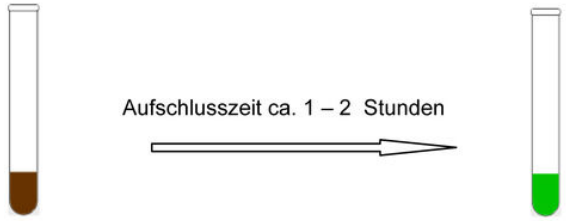

Fig. 7: Change in digestion solution from brown to green when copper salts are used in acid digestion according to the Kjeldahl method

Distillation of the digestion solution

To continue the nitrogen determination process, first ammonia is released quantitatively from the sulphuric digestion solution by adding concentrated base (33% NaOH solution) and the ammonia is then distilled from this solution.

$(NH_4)_2SO_{4(aq)} + 2\ NaOH_{(aq)} \rightarrow$

$Na_2SO_{4(aq)} + 2\ NH_{3(g)} + 2\ H_2O_{(l)}$

In the classic method, distillation of this type was accomplished using gas burner equipment (Fig. 3). The gas burner heats up the Erlenmeyer flask and the distillate passes through the Claisen bridge into the collecting vessel (lower down, behind the condenser). Before distillation, an underlayer of concentrate sodium hydroxide had to be applied very carefully to the sulphuric digestion solution and then water added to dilute it. Only then could the heating for distillation begin, causing the three liquids (sulphuric acid, base and water) to mix in a strongly exothermic reaction, with some impressive results. The distillate is collected in an Erlenmeyer vessel filled with approximately 70 ml of boric acid on the rear of the apparatus. This is later titrated with acid as a titration solution.

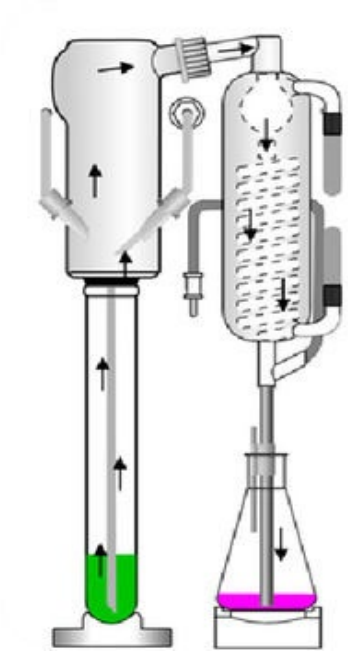

Fig. 8: Water steam distillation with VAPODEST from C. Gerhardt.

The distillation step can be shortened to less than four minutes by using water steam as actuator (Fig. 8).
After sodium hydroxide is added, applied water steam extracts the volatile component ammonia from the digestion solution (green) and transports the ammonia through the distribution head and coiled-tube condenser into the collection solution with boric acid (pink). Here, ammonia and boric acid react stoichiometrically to form ammonium borate, which prevents the ammonia from escaping. At the end, the residual boric acid is titrated with base, which provides quantitative conclusions about the nitrogen content in the original sample.

Collecting the ammonia in boric acid:

$$NH_{3\,(g)} + B(OH)_{4\,(aq)}^{-} + H_{(aq)}^{+} \longrightarrow$$

$$NH_{4\,(aq)}^{+} + B(OH)_{4\,(aq)}^{-}$$

Calculating the nitrogen content

The consumption of titration solution (H^+) during titration of the excess boric acid can be used to simply calculate the percentage nitrogen content in the initial sample.

The following formula applies here:

$$\%\ N = (c_{eq} * (V - V_{BL}) * M * 100\ \%) / E$$

where:

c_{eq}	equivalent concentration of the titration solution [mol/l]
V	consumption of titration solution sample [l]
V_{BL}	consumption of titration solution at blank point [l]
M	molar mass of nitrogen [g/mol]
E	sample weight of sample [g]

The following illustration shows a summary of a complete system for nitrogen analysis according to Kjeldahl:

Fig. 9: Complete system for nitrogen analysis according to Kjeldahl, consisting of 20-unit KTL-20 digestion system (centre) with TURBOSOG gas scrubber (right) and VAPODEST 500 water steam distillation unit with implemented titration (left).

Summary

Nitrogen/protein analysis according to Kjeldahl represents a complex analysis method consisting of several stages. It is used to determine protein in food and animal feed and can be used universally, thanks to its large sample weight. Over the years, it has become the established reference method in food analytics. Despite an increase in alternative methods today, such as the combustion method according to Dumas mentioned here, Kjeldahl analysis remains the dominant method. And this is not only because of its great flexibility and universal applicability for non-homogenous sample material – it is also because of its all-round high precision and reliability.

[1] Zeitschrift für Analytische Chemie, published by Dr. C. Remigius Fresenius. 22nd year, C.W. Kreidels Verlag 1883. Page 366-382 J. Kjeldahl, "Neue Methode zur Bestimmung des Stickstoffs in organischen Körpern".

[2] Illustration from C. Gerhardt catalogue from 1914.

[3] Dr. H. Hadorn, Ch. Obrist, Systematische Versuche mit verschiedenen Katalysatoren für den Kjeldahl-Aufschluß, Deutsche Lebensmittel Rundschau, Volume 3, 1973 page 109/114.

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