Nitrogen determination by means of the Kjeldahl method

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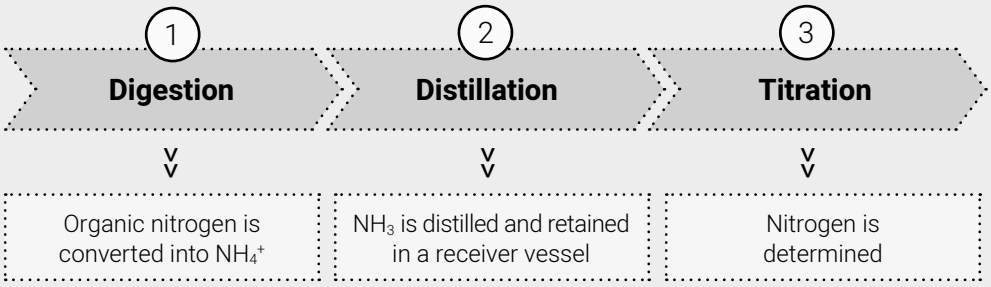
The Kjeldahl method is used to determine the nitrogen content in organic and inorganic samples.

For more than 100 years the Kjeldahl method has been used for the determination of nitrogen in a wide range of samples. The determination of Kjeldahl nitrogen is made in foods and drinks, meat, feeds, cereals and forages for the calculation of the protein content. Also the Kjeldahl method is used for the nitrogen determination in wastewaters, soils and other samples.

It is an official method and it is described in different normatives such as **AOAC, USEPA, ISO, DIN, Pharmacopeias and different European Directives.***

The Kjeldahl procedure involves three major steps:



What are the limitations of the Kjeldahl method? This method measures only nitrogen bound to organic components (proteins, amino acids, nucleic acids) and ammonium in the sample. **This method is not suitable for compounds containing nitrogen in azo and nitro groups or in rings** (quinoline, pyridine, nitrate, nitrite, etc).

1. Digestion

The aim of the digestion procedure is to break all nitrogen bonds in the sample and convert all of the organically bonded nitrogen into **ammonium ions (NH_4^+).**

Organic carbon and hydrogen form carbon dioxide and water. In this process the organic material carbonizes which can be visualized by the transformation of the sample into black foam. During the digestion the foam decomposes and finally a clear liquid indicates the completion of the chemical reaction. For this purpose, the sample is mixed with **sulfuric acid** at temperatures between **350 and 380 °C**. The higher the temperature used, the faster digestion can be obtained. The speed of the digestion can be largely improved by the addition of salts and catalysts. **Sodium** and/or **potassium sulfate** are added in order to increase the boiling point of sulfuric acid and catalysts are added in order to increase the speed and efficiency of the digestion procedure. Oxidizing agents can also be added to improve the speed even further.

The digestion time depends on the chemical structure of the sample, the temperature, the amounts of sulfate salt and the catalyst.

Sample

$$\underset{\substack{\text{Organic Nitrogen compound}\\ \text{(Protein, amino acid, peptide, amine, amide, etc.)}}}{CHNO} + H_2SO_4 \xrightarrow{\textbf{Catalyst}} (NH_4)_2SO_4 + CO_2 + H_2O$$

After digestion is completed the sample is allowed to cool to room temperature, then diluted with water and transferred to the distillation unit.

** Some examples of these official procedures are:*
- *Analysis of Milk: Determination of nitrogen content: EN ISO 8968, AOAC 991.20, Total Nitrogen in Milk; AOAC 991.22 and 991.23, Protein Nitrogen Content of Milk; European Commission Regulation (EC) No 273/2008, Methods for the analysis and quality evaluation of milk and milk products.*
- *Analysis of Water: USEPA Method 351.2, Determination of Total Kjeldahl Nitrogen in water.*
- *Analysis of Feed: European Commission Regulation (EC) No 152/2009, Methods of sampling and analysis for the official control of feed - Determination of the content of Crude Protein.*
- *Analysis of Pharmaceutical Products: European Pharmacopoeia (Ph. Eur.) method 2.5.9., Pharmacopoeia of the United States (USP), method <461>. Nitrogen determination.*

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Kjeldahl Catalysts

The Kjeldahl catalysts are composed of more than 97% of a salt which increases the boiling temperature of the sulfuric acid and 1–3% of one type of catalyst or a mixture of catalysts in order to increase the speed and efficiency of the digestion procedure. Typical catalysts are selenium or metal salts of copper or titanium.

The selection of a particular catalyst depends on ecological and toxic aspects or more practical reasons as the reaction time or foaming and sputtering. For example, selenium-containing catalyst reacts fastest but it is toxic while a copper-containing catalyst is considerably **safer for both humans and the environment** but gives a slower digestion process. An ideal compromise is the mixed catalyst consisting of copper and titanium sulfate.

Product number	Product name	Tablet weight	Pack size	Composition					Recommendation
				Na_2SO_4	K_2SO_4	$CuSO_4 \cdot 5H_2O$	Se	TiO_2	
173350.0413	Kjeldahl Catalyst (Cu) (0.3% $CuSO_4 \cdot 5H_2O$) tablets	3.5 g	3.5 kg / 1000 tablets		3.489 g	0.010 g			Missouri catalyst. Environmental compatibility due to the low content of copper, butthe digestion takes longer.
173350.0414		5 g	5 kg / 1000 tablets		4.985 g	0.015 g			
174428.1211	Kjeldahl Catalyst (Cu) (6.25% $CuSO_4 \cdot 5H_2O$) tablets	1 g	1000 g / 1000 tablets		0.938 g	0.0625 g			
174428.0446		4 g	4 kg / 1000 tablets		3.75 g	0.25 g			
175639.0414	Kjeldahl Catalyst (Cu) (9% $CuSO_4 \cdot 5H_2O$) tablets	5 g	5 kg / 1000 tablets		4.55 g	0.45 g			Universal tablet. 1.5 g tablet (approx.) is recommended for micro Kjeldahl applications. Good performance and low impact on the environment.
177040.0446	Kjeldahl Catalyst (Cu) (10.26% $CuSO_4 \cdot 5H_2O$) tablets	4 g	4 kg / 1000 tablets		3.589 g	0.410 g			
172926.1211	Kjeldahl Catalyst (Cu-Se) (1.5% $CuSO_4 \cdot 5H_2O$ + 2% Se) tablets	1 g	1000 g / 1000 tablets		0.965 g	0.015 g	0.02 g		Wieninger catalyst
172926.0413		3.5 g	3.5 kg / 1000 tablets		3.377 g	0.052 g	0.07 g		
172926.0414		5 g	5 kg / 1000 tablets		4.825 g	0.075 g	0.1 g		
175570.0446	Kjeldahl Catalyst (Cu-Se) (9% $CuSO_4 \cdot 5H_2O$ + 0.9% Se) tablets	4 g	4 kg / 1000 tablets		3.60 g	0.36 g	0.036 g		
173349.0496	Kjeldahl Catalyst (Cu-TiO_2) tablets	3.71 g	3.71 kg / 1000 tablets	1.75 g	1.75 g	0.104 g		0.104 g	Perfect balance between environment and fast digestion.
173349.0414		5 g	5 kg / 1000 tablets	2.358 g	2.358 g	0.1415 g		0.1415 g	
173348.0413	Kjeldahl Catalyst (Se) tablets	3.5 g	3.5 kg / 1000 tablets		3.49 g		0.003 g		Fast digestion but not optimal for the environment.
173348.0414		5 g	5 kg / 1000 tablets		4.99 g		0.005 g		
177768.0414	Kjeldahl Catalyst (Se) tablets	5 g	5 kg / 1000 tablets		4,95 g		0,05 g		Fast digestion but not optimal for the environment.
177010.0431	Kjeldahl Catalyst (Cu) (0,3% $CuSO_4 \cdot 5H_2O$) tablets	6 g	3 kg / 500 tablet s		6 g	0,025 g			Catalyst environmentally friendly

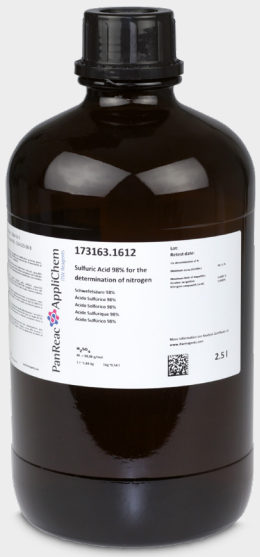
Acid and oxidant for digestion

In general food and feed applications, 98% sulfuric acid is used for digestions.

Oxidizing agents can also be added to improve the speed even further. Hydrogen peroxide is the most widely used, as it accelerates the decomposition of organic material and also has an antifoaming action to control foaming during the digestion, particularly advantageous when the sample contains fat or carbohydrates. However, the use of hydrogen peroxide, which is highly reactive in the presence of sulfuric acid, can cause the loss of nitrogen as N_2 gas. Therefore, hydrogen peroxide is only recommended when there is an appreciable improvement in digestion time and it should be added to the sample gradually. If foaming is the only challenge it is better to use 1-3 drops of a proprietary antifoam emulsion.

After the digestion and before the neutralization of sulfuric acid by adding concentrated sodium hydroxide, the sample is allowed to cool to room temperature and diluted with distilled water. This is done to avoid splashing of the sample due to boiling induced by the heat of reaction dissipated when the concentrated acid and base are mixed. Moreover, if samples are diluted with 10-20 mL of water just after cooling, crystallization can be avoided.

Product number	Product name	CAS number	Pack size
173163.1611	**Sulfuric Acid 98% for the determination of nitrogen**	7664-93-9	1 L
173163.1612			2.5 L
173163.0716			25 L
131077.1211	**Hydrogen Peroxide 33% w/v (110 vol.) (Reag. USP) for analysis, ACS, ISO***	7722-84-1	1 L
211628.1210	**Silicone antifoaming liquid (ORG) technical grade**		500 mL
131074.1211	**Water for analysis, ACS**	7732-18-5	1 L
131074.1212			2.5 L
131074.1214			5 L
131074.1315			10 L
131074.0716			25 L



**The concentration of hydrogen peroxide expressed in volumes means the volume of oxygen gas released by the decomposition of one volume of hydrogen peroxide (1 mL of a 100-volume solution generates 100 mL of oxygen gas when completely decomposed).*

2. Distillation

The acidic sample is neutralized by means of concentrated sodium hydroxide solution. During the distillation step the ammonium ions (NH_4^+) are converted into ammonia (NH_3) by adding alkali (NaOH). The ammonia (NH_3) is transferred into the receiver vessel by means of steam distillation.

$$(NH_4)_2SO_4 + 2NaOH \rightleftharpoons 2NH_3\,(gas) + Na_2SO_4 + 2H_2O$$

The receiving vessel for the distillate is filled with an absorbing solution in order to capture the dissolved ammonia gas. Common absorbing solutions involve aqueous boric acid [$B(OH)_3$] of 2-4% concentration. Other acids, precisely dosed, such as sulfuric acid or hydrochloric acid, can also be used to capture ammonia, in the form of solvated ammonium ions.

The boric acid is being the method of choice because it allows automatization.

Alkalis for neutralization and liberation of ammonia

Product number	Product name	CAS number	Pack size
131687.1210	**Sodium Hydroxide pellets**	1310-73-2	500 g
131687.1211			1 kg
131687.1214			5 kg
131687.0416			25 kg
141571.1214	**Sodium Hydroxide solution 50% w/v**	1310-73-2	5 L
171220.1214	**Sodium Hydroxide solution 40% w/w**	1310-73-2	5 L
171220.1315			10 L
171220.0715			10 L
171220.0716			25 L
122666.1211	**Sodium Hydroxide solution 32% w/v**	1310-73-2	1 L
122666.1214			5 L
176682.1214	**Sodium Hydroxide solution 32% w/w**	1310-73-2	5 L
176682.0715			10 L
176682.0716			25 L

Receiving solutions to capture the ammonia

Product number	Product name	CAS number	Pack size
283334.1214	**Ammonia Fixative solution 1% (Boric Acid 1%*)**	10043-35-3	5 L
287096.1214	**Boric Acid solution 2%**	10043-35-3	5 L
287096.0716			25 L
282222.1211	**Boric Acid solution 4%**	10043-35-3	1 L
282222.1214			5 L
181023.1211	**Hydrochloric Acid 0.1 mol/L**	7647-01-0	1 L
181023.1212			2.5 L
181023.1214			5 L
181023.0715			10 L
181023.1315			10 L
181022.1211	**Hydrochloric Acid 0.5 mol/L**	7647-01-0	1 L
181022.1214			5 L
181022.1315			10 L
181061.1211	**Sulfuric Acid 0.05 mol/L (0.1N)**	7664-93-9	1 L
181061.1315			10 L
181061.0716			25 L
182011.1211	**Sulfuric Acid 0.1 mol/L (0.2N)**	7664-93-9	1 L
181060.1315	**Sulfuric Acid 0.25 mol/L (0.5N)**	7664-93-9	10 L
181059.1211	**Sulfuric Acid 0.5 mol/L (1N)**	7664-93-9	1 L
181059.1315			10 L

** Contains 0.00075% Methyl Red and 0.001% Bromocresol Green as indicators. For automatic analysis.*

3. Titration

The concentration of the captured ammonium ions can be determined using two types of titrations:

- When using the boric acid solution as absorbing solution, an acid-base titration is performed using standard solutions of sulfuric acid or hydrochloric acid and a mixture of indicators. Depending on the amount of ammonium ions present, concentrations in the range of 0.01 mol/L to 0.5 mol/L are used. The detection of the end point can be carried out manually, with a **colorimetric** titration, using a combination of indicators. The combination of methyl red and methylene blue indicators is frequently used in many methods. Alternatively the end point can be determined potentiometrically with a **pH-electrode**. This titration is called **direct titration**.

$$B(OH)_4^- + HX \rightleftharpoons X^- + B(OH)_3 + H_2O$$

HX = strong acid ($X = Cl^-$, etc.)

- When using sulfuric acid standard solution as absorbing solution, the residual sulfuric acid (the excess not reacted with NH_3) is titrated with sodium hydroxide standard solution and by difference the amount of ammonia is calculated. The end-point is detected using a color indicator. Methyl red is usually the preferred indicator. This titration is called **back titration**.

$$H_2SO_4 \text{ (residual)} + 2NaOH \longrightarrow SO_4^{2-} + 2Na^+ + 2H_2O$$

Product number	Product name	Concentration	Pack size
Direct Titration			
181023.1211	**Hydrochloric Acid**	0.1 mol/L	1 L
181023.1212			2.5 L
181023.1214			5 L
181023.0715			10 L
181023.1315			10 L
181061.1211	**Sulfuric Acid**	0.05 mol/L	1 L
181061.1315			10 L
181061.0716			25 L
283303.1609	**Indicator 4.8, Mixed (Methyl Red-Bromocresol Green)** Color change: from pink violet to emerald green (pH 4.8-5.5)		250 mL
282430.1609	**Indicator Tashiro 4.4. Mixed (Methyl Red-Methylene Blue)** Color change: from red violet to green (pH 4.4-5.8)		250 mL
Back Titration			
181693.1211	**Sodium Hydroxide**	0.1 mol/L	1 L
181693.1214			5 L
181693.1315			10 L
281618.1208	**Methyl Red solution 0.1%** Color change: from red to yellow (pH 4.2-6.2)		100 mL

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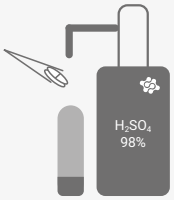
As an **example**, in the following figures the processes of digestion, distillation and titration for a **milk sample** are shown.

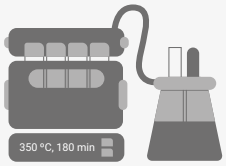
DIGESTION

Balance

- Weigh approx. 5 g of the homogenized sample.



- Place the sample into a digestion flask.
- Add 2 Kjeldahl tablets of 5 g of the Missouri catalyst, 20 mL Sulfuric Acid 98% and homogenize gently.



Heating block Scrubber

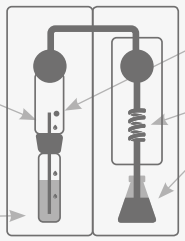
- Place the mixture into the digestion/heating block.
- Heat the mixture (350 – 380 °C) until white fumes and continue heating approx. 3 h.

- The vapors of water and sulfuric acid are bubbled through a solution of sodium hydroxide (scrubber) to neutralize them.
- The digestion is finished when the sample turns clear with a slightly blue color.
- Allow to cool and carefully add about 100 mL of water.
- Transfer the sample to the distillation unit.

DISTILLATION

3. A stream of water vapor is bubbled into the sample to entrain the NH_3 formed.

1. Sample already digested with sulfuric acid 98%.



Distillation unit

2. 50 mL of NaOH 50% are added in excess to neutralize the acidic digestion mixture and to convert NH_4^+ into NH_3 (alkaline solution).

4. NH_3 is condensed.

5. NH_3 is captured in a 50 mL of boric acid solution 4% with Tashiro's indicator. The solution will turn from red violet to green (pH 4.4-5.8).
Around 150 mL of condensate is collected in the boric acid solution.

TITRATION

- Titrate with HCl 0.25 mol/L until the solution becomes slightly violet.
- Use the volume and concentration of HCl consumed to calculate the nitrogen content and then the % of protein in the milk sample.

Calculations

The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process. In the equations below, "N" represents normality. "mL blank" refers to the millilitres of base needed to back titrate a reagent blank if standard acid is the receiving solution or refers to millilitres of standard acid needed to titrate a reagent blank if boric acid is the receiving solution.

- When boric acid is used as the receiving solution the equation is:

$$\% \text{ Nitrogen} = \frac{(\text{mL standard acid} - \text{mL blank}) \times \text{N of acid} \times 1.4007}{\text{weight of sample (g)}}$$

- When standard acid is used as the receiving solution, the equation is:

$$\% \text{ Nitrogen} = \frac{[(\text{mL standard acid} \times \text{N of acid}) - (\text{mL blank} \times \text{N of base})] - (\text{mL standard base} \times \text{N of base}) \times 1.4007}{\text{weight of sample (g)}}$$

If it is desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor that depends on the type of protein present in the sample, e.g. for eggs or meat the factor is 6.25, for dairy products it is 6.38, for wheat it is 5.70, soya and derivatives 5.71, etc.

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