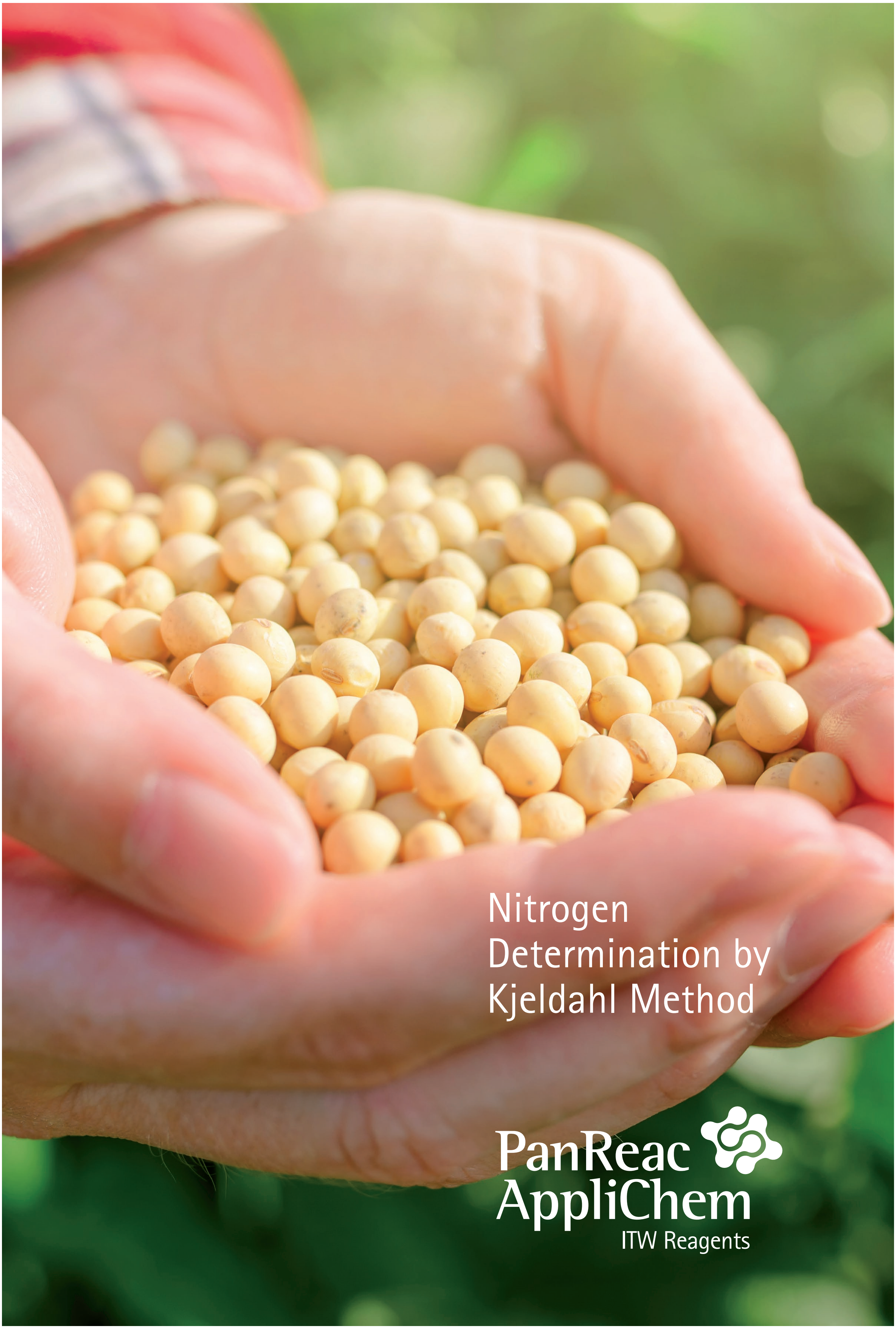

Nitrogen Determination by Kjeldahl Method

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Nitrogen Determination by Kjeldahl Method

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

For longer 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:

Digestion	Distillation	Titration
Organic nitrogen is converted into NH_4^+	NH_3 is distilled and retained in a receiver vessel	Nitrogen is determined

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 ºC**. The higher the temperature used, the faster digestion can be obtained. The speed of the digestion can be greatly improved by the addition of salt and catalysts. **Potassium sulfate** is 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.

$$\underbrace{\text{Protein }(-N)}_{\text{Sample}} + H_2SO_4 \xrightarrow{\text{Catalyst}} (NH_4)_2SO_4 + CO_2 + H_2O$$

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

2. Distillation

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. The ammonia is quantitatively captured by the boric acid solution forming solvated ammonium ions.

$$B(OH)_3 + NH_3 + H_2O \rightleftharpoons NH_4^+ + B(OH)_4^-$$

- Also other acids can be used as precisely dosed volume of sulfuric acid or hydrochloric acid that captures the ammonia forming solvated ammonium ions.

$$H_2SO_4\,(total) + 2NH_3 \longrightarrow SO_4^{2-} + 2\,NH_4^+$$

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 0.01N to 0.5N are used. 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. This titration is called **back titration**.

$$H_2SO_4\,(total) + 2NH_3 \longrightarrow SO_4^{2-} + 2NH_4^+$$

Process scheme

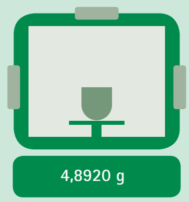
The optimal sample amounts (from 0.01 to 5 g) depend on the expected nitrogen contents but also affect the choice of titrant concentration. The limit of sample amounts normally needs to be found experimentally. It should contains 30 – 140 mg N. Ideally the particle size should be < 1 mm. The sample must be homogeneous and it should be milled if necessary.

The volume of sulfuric acid 98% used is a function of the expected consumption of sulfuric acid in the redox reaction converting sulfuric acid to sulfur dioxide. By the end of the digestion a surplus of acid has to be present in a sufficient amount in order to keep the non-volatile ammonium ions in solution and prevent the loss of volatile ammonia. Typically for 1 g sample two Kjeldahl tablets of 5 g are used together with 20 mL of 98% sulfuric acid and digestion times of 90 minutes are applied. A good ratio is 1 g of Kjeldahl catalyst mixture to 2 mL of 98% sulfuric acid.

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

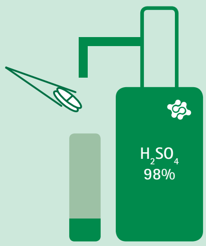
As an **example**, in the following figures we show the processes of digestion, distillation and titration for a **sample of milk**.

1. DIGESTION

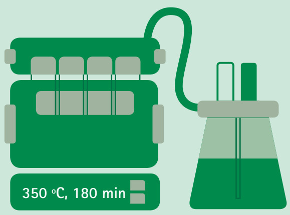


Balance

· Shake the milk sample carefully so that it does not foam.
· Weigh approx. 5 g of the homogeneous sample.



· Place the sample into a digestion flask.
· Add 2 Kjeldahl tablets of 5 g of the Missouri catalyst.
· Add 20 ml Sulfuric Acid 98%.
· Carefully suspend the sample by gently swirling the tube.



Heating block *Scrubber*

· Bring the digestion tube/flask and mixture into the digestion unit and into a heating block.
· Heat the mixture (350 – 380 °C) until white fumes can be seen.
· Continue the heating for about 180 minutes.
· The vapours of water and sulfuric acid are bubbled through a solution of sodium hydroxide (scrubber) to neutralize them.
· The digestion is finished when the sample will be totally transparent with a slightly blue color due to the Cu from the catalyst.
· The sample is allowed to cool to room temperature and cautiously approx. 100 ml of water is added.
· Then the content of the glass tube is transferred to the distillation unit.

2. DISTILLATION

1

Sample already digested with sulfuric acid 98%.

2

50 ml of sodium hydroxide 50% solution is added to the sample to neutralize the pH and to convert NH_4^+ into NH_3.

3

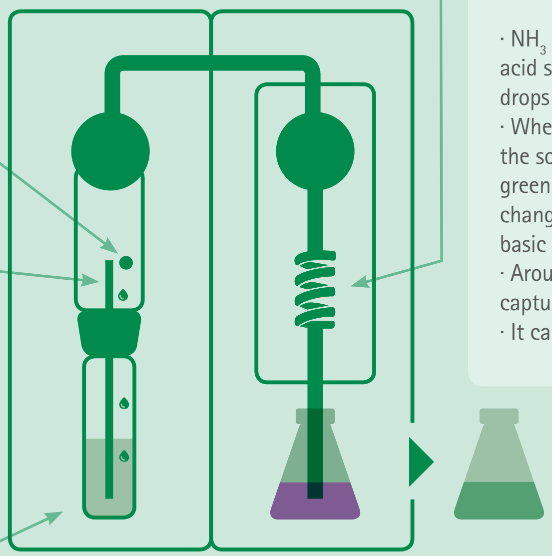
A stream of water vapor is bubbled into the sample to entrain the NH_3 formed.

4

NH_3 is condensed.

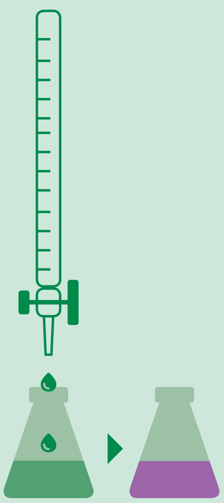
5

· NH_3 is captured in a 50 ml of boric acid solution 4% that contains 6 - 7 drops of Tashiro's indicator.
· When NH_3 reacts with boric acid the solution turns from red violet to green (pH 4.4-5.8) due to the color change of the indicator from acid to basic medium.
· Around 150 ml of condensate is captured in the boric acid solution.
· It can take approx. 5 minutes.



Distillation unit

3. TITRATION



· Titrate with HCl 0.25 mol/l until the solution has a slightly violet color.
· With the volume and concentration of HCl needed we can calculate the number of mol of nitrogen atoms in the sample and then the % of protein in the milk sample.

Reagents used in Kjeldahl analysis

1. Digestion

1.1. Kjeldahl Catalysts

The 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.

In water containing samples, e.g. Total Kjeldahl Nitrogen (TKN) determinations, strong foam formation and sputtering often is caused by Kjeldahl tablets. In such a situation a catalyst mixture in powder form and the use of boiling rods is appropriate.
Besides, digestion times depend on the type of sample, the volume of sulfuric acid, the ratio of acid to salt and the type of catalyst. For example, fat, oil and heterocyclic aromatic compounds are more easily digested if the catalyst contains selenium.

The use of copper as catalyst is becoming more common, as it is recognized to be more environmentally friendly. Today selenium or copper are used as catalysts in more than 90% of the Kjeldahl digestions being performed all over the world.

Product	Code	Tablet weight	Packaging	Composition					Recommendation
				Na_2SO_4	K_2SO_4	$CuSO_4.5H_2O$	Se	TiO_2	
Kjeldahl Catalyst (Cu) (0.3% in $CuSO_4.5H_2O$) tablets	173350.1213	3.5 g	3.5 kg		3.489 g	0.010 g			Missouri catalyst. Environmental compatibility due to the low content of copper, but the digestion takes longer.
	173350.1214	5 g	5 kg		4.985 g	0.015 g			
Kjeldahl Catalyst (Cu) (1.96 % in $CuSO_4.5H_2O$) tablets	177033.1214	5 g	5 kg		4.902 g	0.098 g			
Kjeldahl Catalyst (Cu) (6.25% in $CuSO_4.5H_2O$) tablets	174428.1211	1 g	1000 g		0.938 g	0.0625 g			
	174428.1246	4 g	4 kg		3.75 g	0.25 g			
Kjeldahl Catalyst (Cu) (9% $CuSO_4.5H_2O$) tablets	175639.12111	1.65 g	1650 g		1.501 g	0.148 g			Universal tablet. 1.5 g tablet is recommended for micro Kjeldahl applications. Good performance and low impact on the environment.
	175639.1214	5 g	5 kg		4.55 g	0.45 g			
Kjeldahl Catalyst (Cu) (10.26% in $CuSO_4.5H_2O$) tablets	177040.1246	4 g	4 kg		3.589 g	0.410 g			
Kjeldahl Catalyst (Cu-Se) (1.5% CuSO4.5H2O + 2% Se) powder	172429.1211	-	1000 g		0.965 g	0.015 g	0.02 g		Wieninger catalyst. Appropriate for water containing samples.
Kjeldahl Catalyst (Cu-Se) (1.5% $CuSO_4.5H_2O$ + 2% Se) tablets	172926.1211	1 g	1000 g		0.965 g	0.015 g	0.02 g		Wieninger catalyst
	172926.1213	3.5 g	3.5 kg		3.377 g	0.052 g	0.07 g		
	172926.1214	5 g	5 kg		4.825 g	0.075 g	0.1 g		
Kjeldahl Catalyst (Cu-Se) (9% $CuSO_4.5H_2O$ + 0.9% Se) tablets	175570.1246	4 g	4 kg		3.60 g	0.36 g	0.036 g		
Kjeldahl Catalyst (Cu-TiO_2) tablets	173349.1296	3.71 g	3.71 kg	1.75 g	1.75 g	0.104 g		0.104 g	Perfect balance between environment and fast digestion.
	173349.1214	5 g	5 kg	2.358 g	2.358 g	0.1415 g		0.1415 g	
Kjeldahl Catalyst (Se) tablets	173348.1213	3.5 g	3.5 kg		3.49 g		0.003 g		Fast digestion but not optimal for the environment.
	173348.1214	5 g	5 kg		4.99 g		0.005 g		

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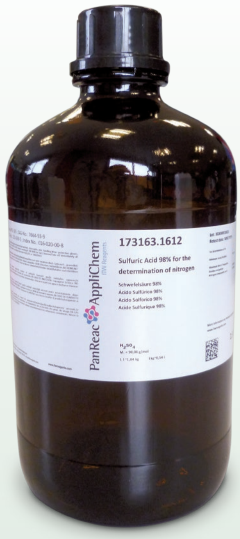
Reagents used in Kjeldahl analysis



1. Digestion

1.2. Acid and oxidant for digestion

In general food and feed applications, 98% sulfuric acid is used for digestions. Special applications may however call for modifications in the concentration of sulfuric acid or mixtures of acids could be envisaged. As an example, protein determinations of milk and cream are often carried out using a 69% sulfuric acid in order to reduce the risk of foaming.

Oxidizing agents can also be added to improve the speed even further. Hydrogen peroxide has the widest usage as accelerates the decomposition of organic material and also has an antifoaming action to control foaming during the digestion. Nevertheless this is extremely reactive and the risk for nitrogen losses is quite high. If foaming is the only problem 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	Code	Packaging
Sulfuric Acid 98% for the determination of nitrogen	173163.1611	1000 ml
	173163.1612	2.5 L
	173163.0716	25 L
Hydrogen Peroxide 30% w/v (100 vol.) for analysis	121076.1211	1000 ml
	121076.1214	5 L
Silicone antifoaming liquid (ORG)	211628.1208	100 ml
	211628.1209	250 ml
	211628.1210	500 ml
Water for analysis, ACS	131074.1211	1000 ml
	131074.1212	2.5 L
	131074.1214	5 L
	131074.1315	10 L

2. Distillation

2.1 Alkalis for neutralization and liberation of ammonia

The acidic sample is neutralized by means of concentrated sodium hydroxide solution. Usually 50% NaOH is added slowly down the neck of the flask. Being heavier, it forms a layer underneath the diluted acid digestion mixture. Generally, for each 5 ml of concentrated sulfuric acid used in the digestion, 20 ml of 50% sodium hydroxide is required to make the digest strongly alkaline (pH of >11). The ammonium ions are converted into ammonia which is transferred into the receiver vessel by means of steam distillation.

A distillation should last long enough such that more than 99.5% of the ammonia is recovered in the receiver vessel. A typical distillation time is 4 minutes at a steam power setting of 100%.

Product	Concentration	Code	Packaging
Sodium Hydroxide	Pellets	131687.1210	500 g
		131687.1211	1000 g
		131687.1214	5 kg
		131687.0416	25 kg
	50 % w/v	141571.1214	5 L
	40 % w/w	171220.1211	1000 ml
		171220.1214	5 L
		171220.1315	10 L
		171220.0715	10 L
		171220.0716	25 L
	32 % w/v	122666.1211	1000 ml
		122666.1214	5 L

2.2 Receiving solutions to capture the ammonia

The receiving vessel for the distillate is filled with an absorbing solution in order to capture the dissolved ammonia gas. Depending on the volume of the digestion mixture and the method being followed, 15 to 150 ml of condensate should be collected in the receiving flask to ensure complete recovery of nitrogen.
The receiving solutions can be boric acid, sulfuric acid or hydrochloric acid. The boric acid is being the method of choice because it allows automatization.

Product	Concentration	Code	Packaging
Boric Acid	1 %. Contains 0.00075% Methyl Red and 0.001% Bromocresol Green as indicators. For automatic analysis.	283334.1214	5 L
		283334.0716	25 L
	3 %	282928.1211	1000 ml
	4 %	282222.1211	1000 ml
		282222.1214	5 L
Hydrochloric Acid	0.1 mol/l	181023.1211	1000 ml
		181023.1212	2.5 L
		181023.1214	5 L
		181023.0715	10 L
		181023.1315	10 L
	0.5 mol/l	181022.1211	1000 ml
		181022.1214	5 L
		181022.1315	10 L
Sulfuric Acid	0.05 mol/l	181061.1211	1000 ml
		181061.1214	5 L
		181061.1315	10 L
	0.1 mol/l	182011.1211	1000 ml
	0.25 mol/l	181060.1211	1000 ml
		181060.1212	2.5 L
		181060.1315	10 L

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3. Titration

3.1 Volumetric solutions and Indicators

If the receiving solution is **boric acid**, the tetrahydroxyborate anions formed are titrated with a standard solution of a strong acid. This titration is called **Direct Titration**.

- The detection of the end point can be carried out manually or with a **colorimetric** titration and 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**. Then it is preferably to adjust the pH of the boric acid to 4.65 before distillation and use an end-point of pH 4.65 for the titration.

If the receiving solution is a **standardized hydrochloric acid or a standardized sulfuric acid**, the excess of acid solution is exactly neutralized by a carefully measured standardized alkaline base solution such as sodium hydroxide. The end-point is detected using a **color indicator**. Methyl orange is usually the preferred indicator. This titration is called **Back Titration**.

Product	Concentration	Code	Packaging
Direct Titration			
Hydrochloric Acid	0.1 mo/l	181023.1211	1000 ml
		181023.1212	2.5 L
		181023.1214	5 L
		181023.0715	10 L
		181023.1315	10 L
Sulfuric Acid	0.05 mol/l	181061.1211	1000 ml
		181061.1214	5 L
		181061.1315	10 L
Indicator 4.8, Mixed (Methyl Red-Bromocresol Green) Color-change: from pink violet to emerald green (pH 4.8-5.5)		283303.1609	250 ml
Indicator 4.4, Mixed (Methyl Red-Methylene Blue) (Tashiro's indicator) Color-change: from red violet to green (pH 4.4-5.8)		282430.1609	250 ml
Back Titration			
Sodium Hydroxide	0.1 mol/l	181693.1211	1000 ml
		181693.1214	5 L
		181693.1315	10 L
Methyl Red solution 0.1% Color-change: from red to yellow (pH 4.2-6.2)		281618.1208	100 ml

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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:

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

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

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

If it is desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor, the magnitude of the factor depending on the sample matrix. Many protein factors have been developed for use with various types of samples.

Here you can see the % Nitrogen, the Protein factor and the % Protein for different types of food:

Food		% Nitrogen	Factor	% Protein
Cereals, pasta				
	Brown Rice	1.3	6.25	7.9
	Wheat flour, whole-grain	2.4	5.7	13.7
	Macaroni, spaghetti	1.9	5.7	11.0
Pulses, nuts and seeds				
	Red beans	3.4	6.25	21.2
	Soy and soy products	6.3	5.71	36.0
	Almonds	4.9	5.18	25.3
	Peanuts	4.8	5.46	26.0
	Nuts	2.9	5.3	15.2
	Sunflower seeds	3.2	5.3	17.2
Dairy products				
	Milk, whole	0.5	6.38	3.3
	Cheese (i.e. Cheddar)	3.9	6.38	24.9
	Butter	0.3	6.38	2.0
	Yogurt	0.8	6.38	5.3
Meat, poultry, fish				
	Beef	3.0	6.25	18.5
	Chicken, breast meat	3.7	6.25	23.1
	Ham	2.8	6.25	17.6
	Egg, whole	2.0	6.25	12.5
	Fish	2.6	6.25	16.0

A173,EN;201707

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