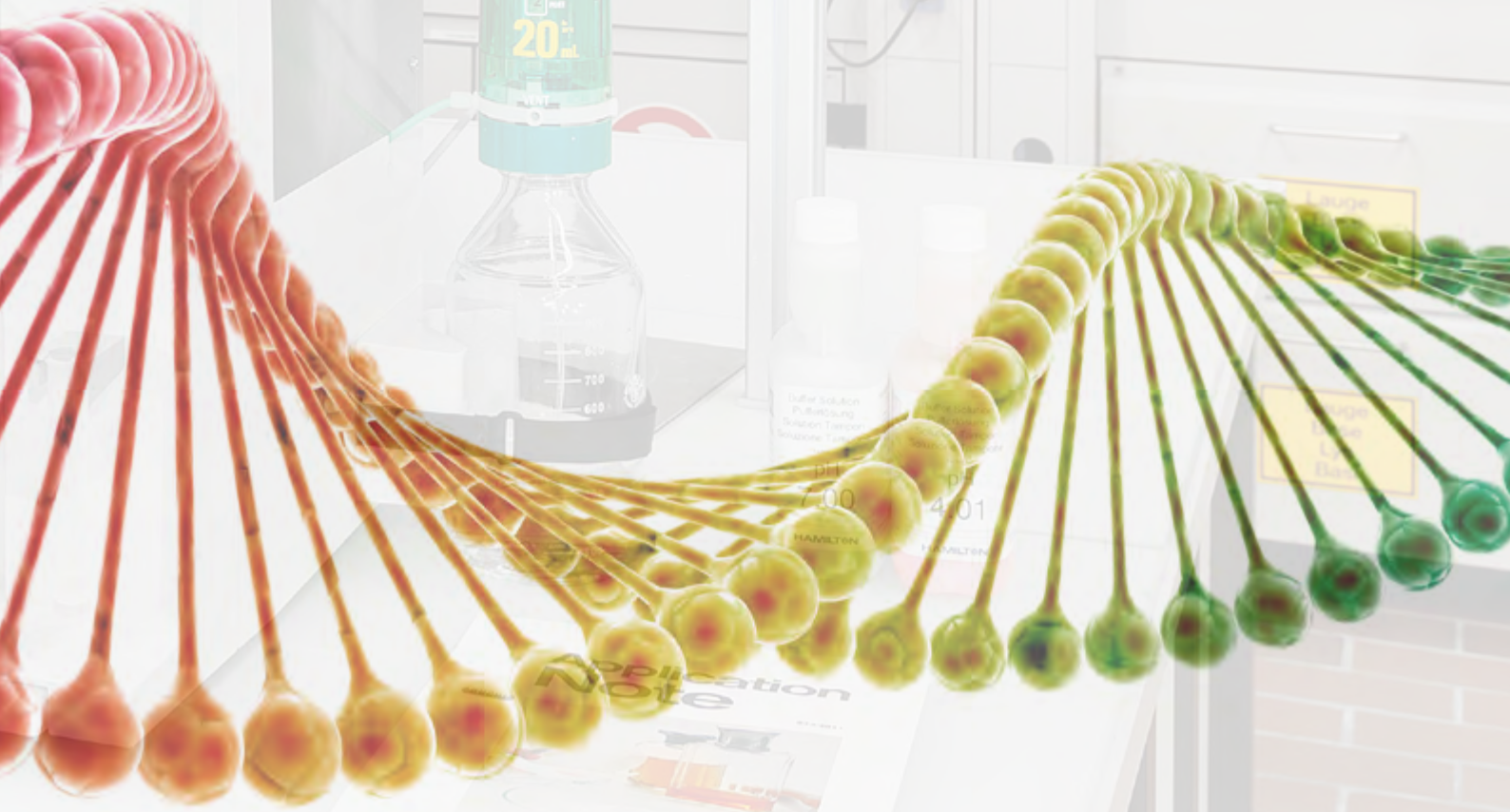
Kjeldahl Knowledge Base

Decode the mysteries of the nitrogen and protein determination according to Kjeldahl

1. Introduction
2. Procedure
3. Chemicals
4. Regulations



Impressum

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Reviewer	Andrea Mühleis, Melanie Hasler
Publisher	BÜCHI Labortechnik AG, CH-9230 Flawil, Switzerland
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Order Code	11595478

Comprehensive guide covering all aspects and know-how of the theoretical and practical nitrogen and protein determination according to Kjeldahl. The Kjeldahl Guide helps you to understand the method and enables you to set up experiments correctly.

Foreword

Over 50 years of experience developing Kjeldahl instrumentation and applications are summarized in this Knowledge Base. The aim of it is to share the BUCHI expertise in regards to the nitrogen and protein determination according to the Kjeldahl method. The deeper understanding of the procedure will help laboratory personnel, laboratory supervisors, students and teachers to optimize the individual method and increase the reliability of the results.

It is our intention to revive the basic knowledge needed to understand the chemical and physical background associated with nitrogen determinations according to Kjeldahl and provide clear instructions in a wide area of Kjeldahl applications. The mayor theoretical part of the Kjeldahl Knowledge Base contains basic knowledge, consolidation of the Kjeldahl know-how followed by an extensive list of regulations and actual BUCHI Application Notes describing successful nitrogen determinations.

With this Kjeldahl Knowledge Base BUCHI would like to live up to its company slogan
«Quality in your hands»

and support you in your daily work by not only providing high quality instrumentation but also offering comprehensible theoretical background information and showcase applications.

Yours sincerely,

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Introduction

BUCHI offers “Quality in your hands” since 1939

1. Introduction
2. Procedure
3. Chemicals
4. Regulations


BUCHI

1.1 History of the Kjeldahl method

For more than 130 years the determination of nitrogen by means of the method developed by the Danish chemist Johan Gustav Christoffer Thorsager Kjeldahl (1849 – 1900) has been an internationally accepted standard. The method was introduced in 1883 at a meeting of the Danish Chemical Society by Johan Kjeldahl as a means to determine nitrogen in barley and yeast[1]. The method named after its inventor has since found wide-spread application in life science and chemistry and has extended its scope to the determination of nitrogen and proteins in dairy products, meat products, beer, cereals and other food materials. Kjeldahl was a member of the innovative laboratory team at the Carlsberg brewery in Copenhagen, also famous in microbiology for isolating the famous beer yeast Saccharomyces Carlsbergensis which is still used today. As the head of the chemistry department at the Carlsberg brewery he was involved in a very modern problem: quality management and optimization of productivity. Kjeldahl intended to determine the protein content of grain in order to find out how the protein content influences quality and quantity of the brewed beer.

Johan Gustav Christoffer Thorsager Kjeldahl in his laboratory

- 1849: born in Jagerpris in Denmark
- 1867: graduated from university
- 1876: Scientist and head of lab at Carlsberg Breweries
    - Fermentation studies
    - Research on carbohydrate in barley and malt
    - Developing of method for exact determination of nitrogen in animal and vegetable material
- 1883: published the “Kjeldahl method” for protein determination
- 1900: died in Tisvilde

[1] J. Kjeldahl, Sur une nouvelle méthode de dosage de l’azote dans les substances organiques, Résumé du compte-rendu des travaux du Laboratoire de Carlsberg, Copenhague, en commission chez H. Hagerup, Imprimerie de Thiele 1983

Citation from Kjeldahl's original publication of 1883

Original citation

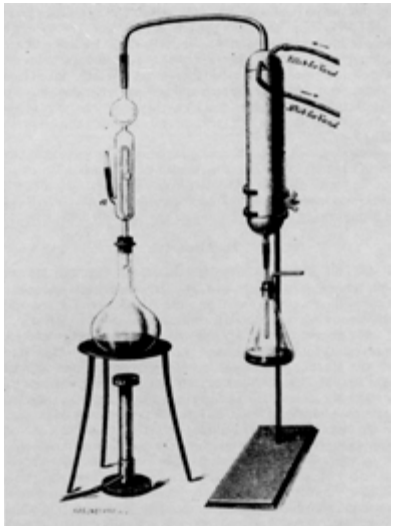
«Le principe de la nouvelle méthode consiste donc à chauffer pendant quelque temps la matière à analyser avec une forte proportion d'acide sulfurique concentré jusqu'à une température voisine du point d'ébullition de l'acide, et à oxyder la dissolution ainsi obtenue avec un excès d'hyperpermanganate de potasse sec en poudre. Dans ces conditions, l'azote des substances organiques, … , se transforme complètement en sulfate d'ammoniaque, qui, l'oxydation une fois terminée et après saturation avec la soude, peut être distillé et dosé par les méthodes ordinaires.»

Translation

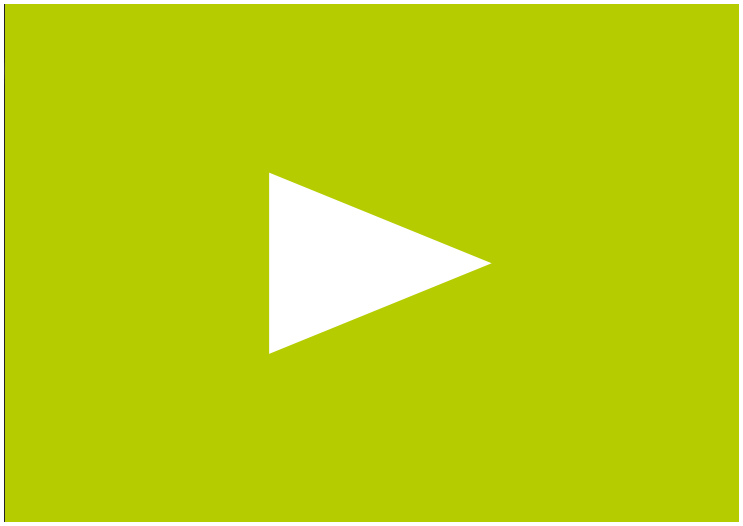
«The principle of the new method is to heat the test material for some time with a large quantity of concentrated sulfuric acid at a temperature close to the acid's boiling point and to oxidize the solution thus obtained with an excess of dry potassium permanganate powder. Under these conditions the nitrogen of the organic substances, … , is completely transformed into ammonium sulfate which, once the oxidation is completed and after saturation with caustic soda can be distilled and determined by ordinary methods.»

Although individual chemicals used in the Kjeldahl method have changed over the years it is possible to give a concise general definition:

The Kjeldahl Method consists in a procedure of catalytically supported mineralization of organic material in a boiling mixture of sulfuric acid and sulfate salts at boiling temperatures between 340 and 370 °C. In the digestion process the organically bonded nitrogen is converted into ammonium sulfate. Alkalizing the solution liberates ammonia which is quantitatively steam-distilled and determined by titration.



Original Kjeldahl apparatus from 1883



BUCHI Kjeldahl evolution

1960

Distillation

Steam generator

Nitrogen determination apperatus

1970

Distillation

Vapodest

Büchi 325 + 345

Nitrogen determination apperatus

B-320

Digestion

IH 4.2 / IH 2.1

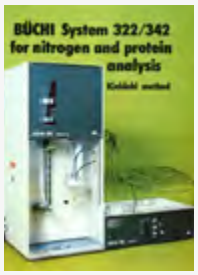
Büchi 425 / 430

Ventilator

1980

Distillation

B-322 + B-342 / B-343



B-321

B-315

Scrubbing

Scrubber B-412

1990

B-339

B-323

B-324

B-316

K-314

2000

AutoKjeldahl System
K-370 / K-371

KjelFlex K-360

Distillation Unit
K-350 / K-355

2010

KjelMaster K-375
KjelSampler K-376 / K-377

Rapid Digester
Büchi 420

B-424 / B-435 + B-436
K-424 / K-435 + B-436

Digest System K-437
Digest Automat K-438

Digest System K-431
Digest Automat K-432

KjelDigester
K-446 / K-449

SpeedDigester
K-425 / K-436 / K-439

Scrubber
B-414

Scrubber
K-415

1.2 Kjeldahl at BUCHI since 1961

At the time when Johan Kjeldahl published his method for the determination of nitrogen in 1883 the electric lamp was just patented and the technical age in its childhood. Seldom in human history has an invention remained basically unchanged for such a long time as Kjeldahl's method for nitrogen determination.

As in 1883 the Kjeldahl nitrogen determination starts with sample preparation, proceeds to the digestion followed by separation using steam distillation and subsequent volumetric determination of the amount of ammonia formed in the process. Kjeldahl's visionary idea of providing a simple method for nitrogen and protein determinations, which can also be carried out by non-academic lab personnel, has been put into practice by BUCHI's Kjeldahl systems since 1961.

With this guide BUCHI wants to support you in your daily work not only by providing high quality instrumentation but also offering theoretical background information for your daily routine.

1961: BUCHI Market Innovation

For more efficient distillations

Launch of the first automatic nitrogen distillation instrument with integrated steam generator.

2012: BUCHI Market Innovation

For highest productivity

Launch of the first nitrogen distillation instrument with integrated titration for potentiometric and colorimetric titration and sample changer for a fully automated process.

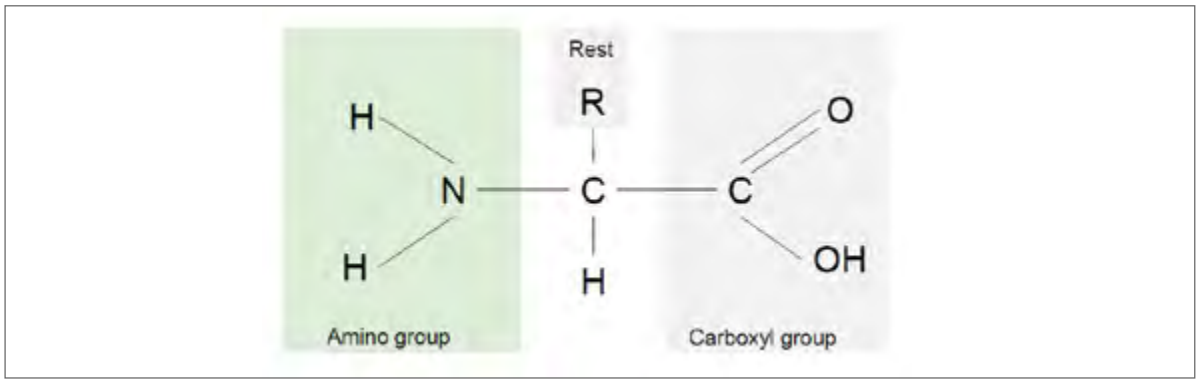
KjelOptimizer
To optimize your individual Kjeldahl method.
www.buchi.com/kjeloptimizer

KjelReports
For easy result calculation and reporting of Kjeldahl and SO_2 determinations.
www.buchi.com/kjelreports

Application Finder
Find detailed information for several sample matrices on our Application Database.
www.buchi.com/applications

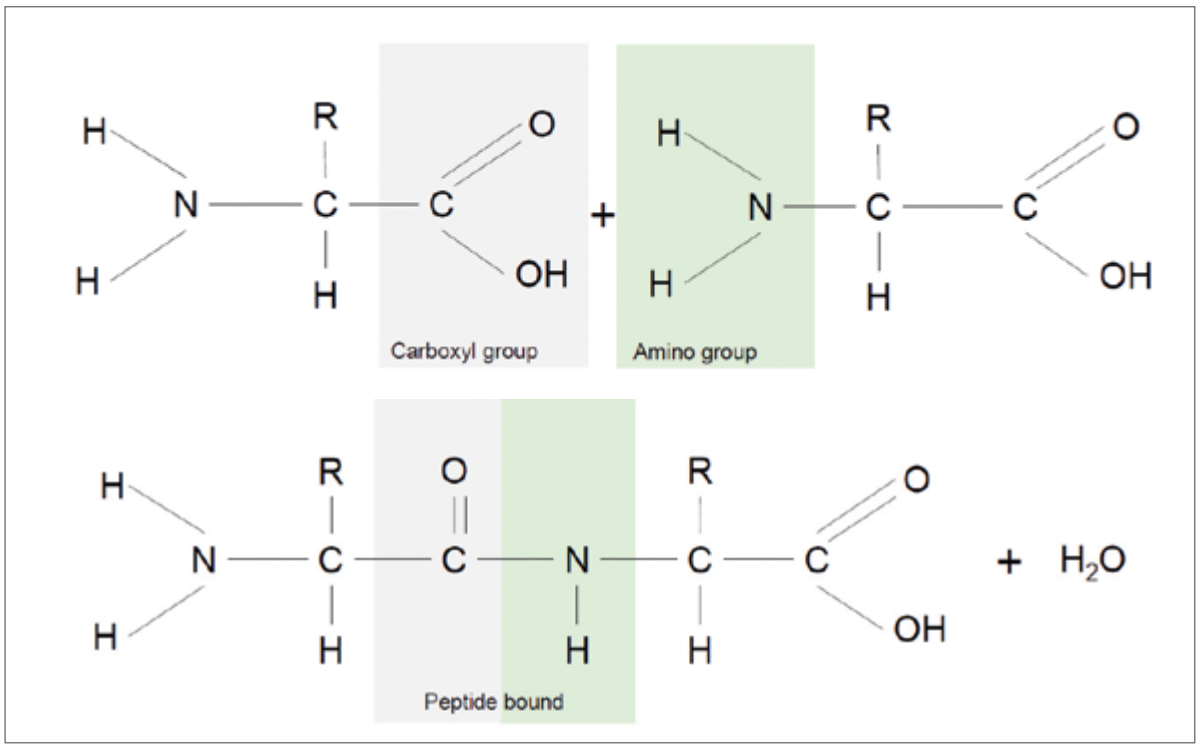
1.3 Chemistry of protein

Amino acids are the components of proteins. They consist of a carboxyl group (COOH), C-atom, side chain (R) and at least one amino group (NH_2). The amino group is bound at the same C-atom like the carboxyl group and the side chain (R).



General structure of an α-amino acid

The amino group of an amino acids can react with the carboxyl group of another amino acid by splitting off water (H_2O). The emerging C-N-connection is called peptide bound.



Formation of a peptide by splitting off water

In this manner long molecular chains with many amino acids can be built. If such a chain contain less than 100 amino acid components it is called peptide. When it contains more than 100 amino acid components it is called protein. Amino acids are basic components of the body and contain nitrogen (N). Only amino acids are able to build up body tissue, organs, muscles, hair and skin. Many hormones, enzymes and anti-bodies consist of amino acids.

1.4 The Kjeldahl method

Nutrition label

Protein is an essential nutrient for most species and consists of amino acids. There are about 200 amino acids found in nature. There are about 20 amino acids in a protein hydrolysate. The nitrogen content in protein is about 16 % which leads to a general protein factor of 6.25 to calculate the protein content.

The amount and composition of amino acids of the individual foodstuffs differs. As the nitrogen content is related to the type of amino acid, individual protein factors are used to calculate the protein content.

The Kjeldahl method allows the calculation of protein contents in various samples based on the determined nitrogen which is a general constituent of all proteins. Nowadays the scope of Kjeldahl nitrogen determinations also includes applications in the fields of environmental analysis, research and development, pharmaceutical, chemical and cosmetics industries and is also used in governmental and regulatory laboratories.

Why do we analyze the nitrogen and protein content?

Food and feed products are mainly composed of fat, protein, fiber, carbohydrates, ash (total mineral content) and moisture (water). Since the overall protein content plays an important role both for the payment of raw material (e.g. milk) delivered as well as for labeling of the vending package, it is subject to regular checks. Protein content serves also as signifier of basic nutrient, has an impact on the caloric value, is a quality parameter (e.g. milk, meat) and influences the production process. Proteins are of indispensable nutritional value for humans and animals and are contained in beverages, food and feed[2].

[2] Souci Fachmann Kraut, Food Composition and Nutrition Tables, 6th revised and completed edition (2000), compiled by Heimo Scherz und Friedrich Senser, medpharm Scientific Publishers, ISBN 3-88763-076-9

Nutrition facts of food

Protein contents of some foodstuffs

Food	Protein [%]	Food	Protein [%]
Apple, raw	0.3	Flour	11.0
Peach	0.8	Oats	12.6
Carrot	1.0	Chicken	19.9
Raspberry	1.3	Halibut	20.1
Potatoes	2.0	Red beans	21.2
Elderberry	2.5	Beef	22.0
Spinach	2.7	Lentils (dry)	22.9
Horse-radish	2.8	Cheddar cheese	24.7
Rose hip	3.6	Peanuts	24.7
Milk	3.2	Sunflower seed	26.5
Pea sprouts	5.1	Ostrich	35.5
Corn	9.2	Soybeans	37.6

For packed and processed food nutrition fact labels are in use almost everywhere in the world. The protein content is one of the most important parameters declared on nutrition fact labels.

Procedure

Kjeldahl nitrogen and protein determination

1. Introduction
2. Procedure
3. Chemicals
4. Regulations


BÜCHI Distillation Unit K-350
STOP

Kjeldahl Knowledge Base
Procedure

1. Preparation
2. Digestion
3. Distillation
4. Titration
5. Blanks
6. Calculation

The Kjeldahl procedure involves three major steps:

Step	Process	Description
Digestion	$(CHNO) + H_2SO_4 \rightarrow CO_2 + SO_2 + H_2O + NH_4^+$	Organic matter is destroyed by boiling in concentrated sulfuric acid. Kjeldahl Tablets (catalyst) raise the boiling point and accelerate the process.
Distillation	Neutralization/Alkalization: $H_2SO_4 + 2\ NaOH \rightarrow 2\ Na^+ + SO_4^{2-} + 2\ H_2O$ Distillation: $NH_4^+ + OH^- \rightleftharpoons NH_3\ (gas) + H_2O$	The digestion mixture is alkalized with NaOH prior to distillation to free up the ammonia. The ammonia is steam distilled into an acidic receiver solution.
Titration	Receiver: $B(OH)_3 + NH_3 + H_2O \rightleftharpoons NH_4^+ + B(OH)_4^-$ Titration: $B(OH)_4^- + HX \rightarrow X^- + B(OH)_3 + H_2O$	The pH in the acidic receiver solution rises upon addition of ammonia. The nitrogen and protein content is then determined by titration of the borate complex.

2.1 Sample preparation

Two critical points involved in sample preparation are the amount of sample and its homogeneity. A further aspect is the expected titrant consumption which, for reasons of accuracy, should be in a range of 3 to 17 mL for titrant concentrations of 0.01 to 0.5 N if a 20 mL burette is used. An immanent problem associated to Kjeldahl digestions is foam formation and, especially if large sample volumes are present, the risk of foaming over into the suction module. In such cases the use of antifoam agents can be of help. A common substance used as antifoam agent in Kjeldahl digestions is stearic acid of which a tip of a spatula is added to the sample. For easier handling and to allow same digestion conditions to all samples the use of the BUCHI Kjeldahl Tablet Antifoam[3] is recommended. Digestions of liquid samples, e.g. in TKN (Total Kjeldahl Nitrogen) determinations, may be affected by bumping caused by boiling delays. In older descriptions of such applications boiling stones or similar boiling aids were recommended. With modern distillation units the distillation process may be completed by an aspiration of the sample in which case the presence of boiling aids leads to blockages of the hoses. For this reason digestion rods[4] are recommended. The glass digestion rods remain in the sample tube during the distillation.

Digestion rods (length 18 cm) also do not impede the transfer of samples from the sample changer (e.g. KjelSampler K-376 / K-377) to the distillation unit (e.g. KjelMaster K-375). Powder, solids, viscous fluids or sticky samples can easily be transferred into the sample tubes for reliable nitrogen and protein analysis by means of the Kjeldahl weighing boats[5]

[3] BUCHI Kjeldahl Tablets Antifoam (11057984)
[4] BUCHI digestion rods (043087)
[5] BUCHI weighing boats (11060522)

BUCHI digestion rods (043087) prevent from boiling delays

BUCHI weighing boats (11060522)

2.1.1 Amount of sample and sample tube size selection

Depending on the nitrogen content per sample the terminology macro, semimicro- (standard-) and micro Kjeldahl is used[6] [7]:

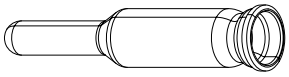
Terminology depending on the nitrogen content per sample

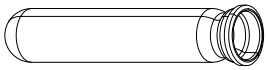
Terminology	N amount per sample tube	Sample tube size
Macro Kjeldahl	10 – 30 mg N	300 or 500 mL
Semimicro Kjeldahl	0.1 – 3 mg N	300 mL
Micro Kjeldahl	1 – 15 mg N	100 mL

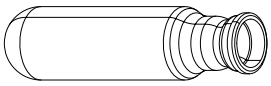
[6] Die Stickstoffbestimmung nach Kjeldahl, Die Umrechnung von Stickstoff zu Protein, Literaturstudie und Erfahrungsbericht, M. Ugrinovits, Büchi Laboratoriumstechnik GmbH, Göppingen

[7] R. Lange, R. Friebe und F. Linow (1979). Zur Anwendung der Methodenkombination Kjeldahl-Aufschluss/Bertholet-Reaktion bei der Stickstoffbestimmung in biologischen Materialien, 1. Mitt. Stand der Kenntnisse – Teil I, Die Nahrung 23, 3, 1979, 255-261 referring to Exley, D., Biochem. J. 63, 496 (1956)

Selection of sample tubes

Micro	
Sample weight	< 0.2 g
Sample volume	2 – 3 mL
H_2SO_4	2 – 5 mL
Kjeldahl Tablets	1 (Micro)
Recommendation	Homogeneous samples high in nitrogen/protein
Benefit	Reduction of chemicals by 80 % compared to 300 mL tubes

300 mL	
Sample weight	0.1 – 5 g
Sample volume	< 200 mL
H_2SO_4	5 – 30 mL
Kjeldahl Tablets	2
Recommendation	Standard tube for most applications
Benefit	All-round sample tube

500 mL	
Sample weight	> 4 g
Sample volume	< 400 mL
H_2SO_4	> 10 mL
Kjeldahl Tablets	2
Recommendation	Especially for high sample volumes or strongly foaming samples
Benefit	Problem-free digestion of strongly foaming samples

Micro Kjeldahl

With samples consisting predominantly of pure proteins like ovalbumine, lactalbumine or other globular proteins, amounts of 1 – 2 mg can be digested in 100 mL sample tubes. One Kjeldahl Tablet Titanium Micro of 1.59 g[8] or Copper Micro[9] in combination with 2 – 5 mL of 98 % sulfuric acid is sufficient to break up the organic matrix.

Standard Kjeldahl

Depending on the expected nitrogen content, optimal amounts of sample are usually in the range of 0.1 – 5 g. Most samples from food and feed containing between 6 – 30 % protein require sample amounts of 0.5 – 1 g. Such amounts of organic material can be digested using the 300 mL sample tubes with two Kjeldahl Tablets Titanium of 3.71 g[10] or Missouri of 5 g[11] and 15 – 20 mL of 98 % sulfuric acid.

Macro Kjeldahl

Dealing with low nitrogen contents on the other hand requires large sample amounts in order to produce a detectable amount of ammonia. Maximum sample amounts depend on composition and the size of the sample tube. With milk samples a typical sample amount is 3 – 5 mL in 300 mL tubes.

Low nitrogen wax samples however may require up to 10 g. The limit of sample amounts normally needs to be found experimentally and would be around 10 g in 500 mL sample tubes. For water samples a maximum volume of 200 mL in 300 mL sample tubes and a maximum of 400 mL in 500 mL sample tubes can be used.

For the weighing of samples analytical balances accurate to 0.1 mg should be used. The actual weight of a sample required for analysis also depends on its homogeneity. More sample is needed to achieve reproducible results for less homogeneous material.

As described above optimal sample amounts depend on the expected nitrogen contents but also affect the choice of titrant concentration. In the below weighing table recommended sample weights are correlated with the amounts of catalyst, the volume of 98 % sulfuric acid and suggested titrant concentrations.

See chapter 2.2.8
Ideal ratio of salt to sulfuric acid

[8] BUCHI Kjeldahl Tablet Titanium Micro (11057981)
[9] BUCHI Kjeldahl Tablet Copper Micro (11057985)
[10] BUCHI Kjeldahl Tablet Titanium (11057980)
[11] BUCHI Kjeldahl Tablet Missouri (11057982)

Weighing tables for solid samples

	Sample: weight [g]					Titrant concentration [N]			
	5	2	1	0.5	0.125	0.01	0.05	0.1	0.5
N [mg]	N [%]					Titrant consumption sample [mL]			
0.5	0.01	0.03	0.05	0.10	0.40	3.6			
2.0	0.04	0.10	0.20	0.40	1.60	14.3	2.9		
2.5	0.05	0.13	0.25	0.50	2.00		3.6	1.8	
7.0	0.14	0.35	0.70	1.40	5.60		10.0	5.0	
10.0	0.20	0.50	1.00	2.00	8.00		14.3	7.1	1.4
50.0	1.00	2.50	5.00	10.00	40.00				7.1
100.0	2.00	5.00	10.00	20.00	80.00				14.3

Procedure:
A: Select N % of sample
B: select titrant concentration
C: Choose weight in order that the titrant consumption can be expected between 3 and 17 mL

Depending on the expected nitrogen contents suitable sample volumes have to be chosen:

Weighing tables for liquid samples

Sample [mL]	N [%]	N mg/L	Titrant [N]
4	0.1 – 0.6		0.10
6	0.06 – 0.4		0.10
10		100 – 200	0.01
25		50 – 100	0.01
50		20 – 50	0.01
100		10 – 20	0.01
250		5 – 10	0.01
400		< 5	0.01

For more refined optimization of Kjeldahl parameters the BUCHI Kjeldahl Optimizer App can be downloaded from the app store for free (available for iOS, Android, Windows Phone and Windows Desktop PC).

KjelOptimizer
To optimize your individual Kjeldahl method.
www.buchi.com/kjeloptimizer

2.1.2 Mincing and homogenization

As a general rule of thumb particle sizes should not be larger than 1 mm. Inhomogeneous samples would lead to increased standard deviations in repeated determinations. A useful measure for inhomogeneity is the relative standard deviation (rsd) expressed in % of the mean value. Protein contents of 6 – 30 % of homogeneous samples show rsd values < 1 % and in turn rsd values > 1% are strong evidence for insufficient homogeneity or a nitrogen level close to the LOD. It is essential to homogenize solid samples.

For inhomogeneous samples high precision of the measurements cannot be obtained using small sample sizes. It is recommended to homogenize samples in order to be able to carry out the analysis with sample amounts as low as possible since homogeneous samples are less critical in this respect.

BUCHI Mixer B-400

2.1.3 Drying of samples

Drying of samples may be necessary if results referring to dry matter are required. Alternatively the water content of a sample may be determined by classical water analyses like Karl-Fischer titration or by gravimetric methods.

2.2 Digestion

Foam formation during digestion

Clear-green digestate

In the digestion step the organically bonded nitrogen is converted into ammonium ions. Organic carbon and hydrogen form carbon dioxide and water.

The oxidation process of organic material with sulfuric acid involves a first step of carbonization in which the formation of water can be observed when it condenses at cool parts of the glassware. Carbonization starts at room temperature and is enhanced by increased temperatures. At higher temperatures the decomposition of carbonized material to carbon dioxide commences and is reflected by foam formation expanding black sample material up in the Kjeldahl sample tube.

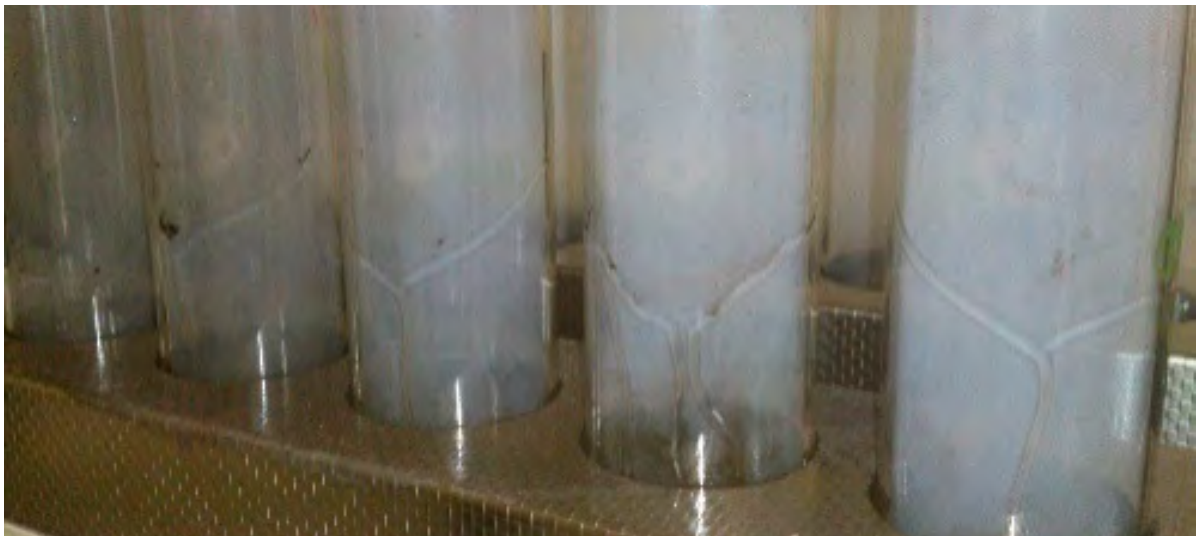
It is important to keep foam formation under control in order to avoid poor reflux of carbonized sample material attached to the glass wall. The choice of the ideal digestion parameters is a key for a successful digestion. During the digestion the foam decomposes and finally a clear liquid indicates the completion of the chemical reaction.

The generalized non-stoichiometric chemical equation shows how a general nitrogen containing starting material (CHNO) is mineralized to dissolved ammonium ions. Optimal digestion conditions are achieved when the condensation zone remains 5 cm below the constriction of the sample tube.

After the digestion has led to a clear liquid, an additional digestion time of about 30 minutes is usually added, in order to allow complete mineralization[12].

In the original procedure published by Kjeldahl the digestion was carried out in boiling sulfuric acid. The oxidation was supported by the addition of the strong oxidizing agent potassium permanganate. After its introduction by Kjeldahl, the digestion reaction was further improved and optimized. Examples were the addition of salts and the use of catalysts or hydrogen peroxide which allowed for shorter digestion time. The most common salt used historically was potassium sulfate and the catalysts were selenium and metal salts, particularly of mercury, copper or titanium.

[12] H. Hadorn, R. Jungkunz und K.W. Biefer, Über die Stickstoffbestimmung in Lebensmitteln nach Kjeldahl und den Einfluss des Katalysators im Besondern, (1953) Mitt. Gebiete Lebensm. Hygiene 44:14



The condenzation zone should remain 5 cm below the constriction of the sample tube

Two heating technologies are used to heat up the sample together with the reagents to boiling temperatures of 350 to 370 °C. One technology is infrared (IR) digestion and the other is block digestion.
For the digestion step working in a fume hood and the use of the Scrubber K-415 is highly recommended. The fume removal and neutralization by the Scrubber, provides additional safety to laboratory personnel and environment protect of the equipment against corrosion.

2.2.1 Heating technologies

Two different heating technologies are used to digest samples for the nitrogen determination according to Kjeldahl. One is referred to as an IR (infrared) digester and the other one is called a block digester. IR digesters are equipped with a heating element similar to those used in an oven or furnace. The sample is directly irradiated in a heating zone of approximately 9 cm high. With block digesters the sample tubes are embedded in a well of about 6 cm. The heat is transferred from the aluminum block through the glass wall to the sample.

IR- and block digesters are suitable for all applications provided, if correct parameters are chosen. Important decisive parameters are the automation, flexibility in tube size and applications, speed of heating up samples, the digestion temperature and the duration of the digestion. A major technical difference between IR- and block digestion consists in the temperatures generated in the oven and in the block respectively. In IR digestion a temperature of 580 °C can be reached in the oven and in block digesters the aluminium block can reach temperatures of up to 450 °C. With both techniques the heat transfer to the sample ensures that the Kjeldahl sample mixture reaches the required boiling temperatures of 350 – 370 °C and could be maintained during the digestion process.

A higher heating zone has a positive effect in foaming samples by allowing rising gas bubbles to burst such that the carbonizing material flows back into the sample tube and reduces the level of foam in the sample tube. In addition carbon deposits at the glass walls are refluxed more efficiently in IR digesters.

A further technical aspect associated with IR- and block digestion is the time needed to heat up the respective digester to the operation temperature. The SpeedDigester (IR) needs 10 – 15 minutes and the KjelDigester (block) approximately 20 minutes to heat up from room temperature to 400 °C. Other conventional block digesters need up to 60 minutes to reach the operating temperature.

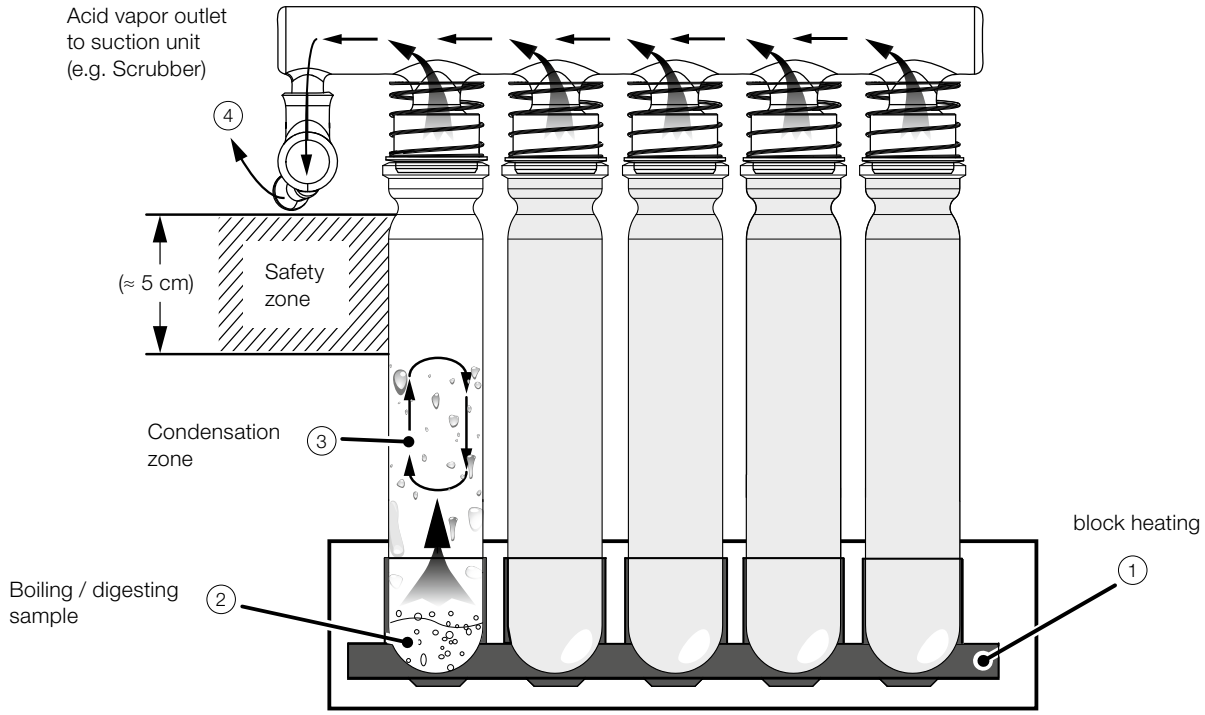
2.2.2 IR-digestion vs. Block-digestion

Due to direct physical contact of the block's metal surface with the glass the flux of heat energy is higher compared to the heat flow in an IR digester where convection and irradiation is the medium. Aiming at enhanced productivity measurable in terms of sample throughput, the advantage of block digesters has to be seen in the larger number of samples which can be digested at one time. With BUCHI's KjelDigester 20 samples can be placed into one rack. The largest number of samples to be digested with an IR digester is 12. The ultimate advantage of block digesters finally is identified in the suitability of the racks to be used in sample changers (KjelSampler) as realized in the KjelMaster System K-375 / K-376 and K-375 / K-377. The KjelDigester K-449 (fully automatic block digester) and the K-446 (manual block digester) fit into the scheme for automation of subsequent distillation and titration making use of the sample changer KjelSampler.

Functional principle of the KjelDigester (block)

The KjelDigester is used to convert organic bound nitrogen (e.g. in proteins) in a sample into ammonium sulfate. During the digestion process, acid fumes and reaction gases may emerge and are handled by the Scrubber K-415. The K-415 is connected to digester system for neutralizing acid fumes and adsorbing unpleasant odors.

- The aluminum block (1) generates high temperatures in the samples (2)
- The sample is digested in constantly boiling sulfuric acid
- Hot acid fumes rise into the condensation zone (3), and condense and rinse back down to the sample creating a constant reflux
- Residual fumes (4) which escape the condensation zone are highly corrosive and must be withdrawn and efficiently neutralized (e.g. with the Scrubber K-415)



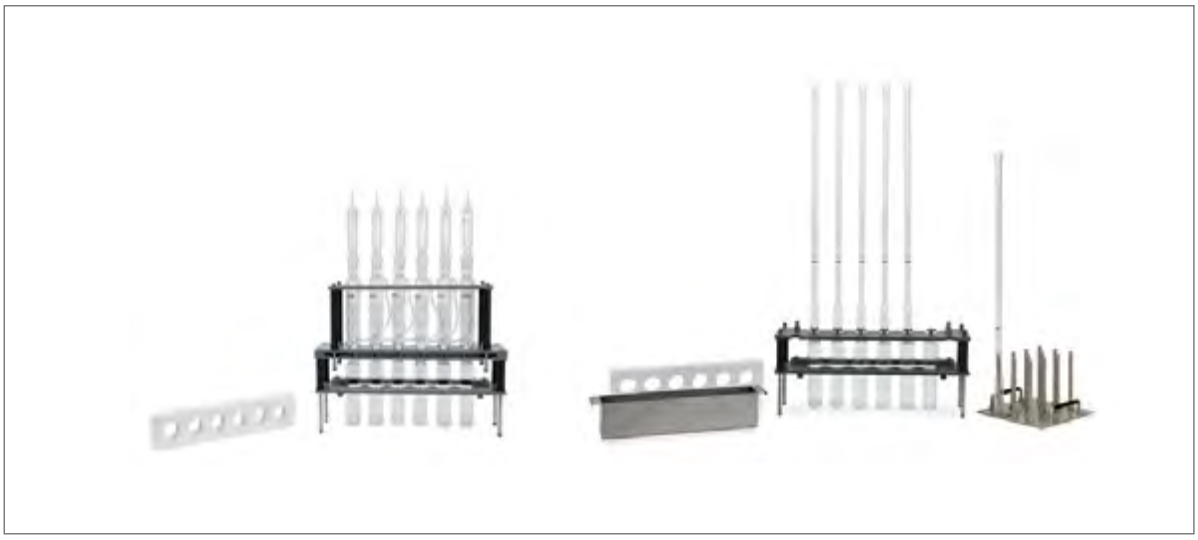
Kjeldahl Knowledge Base
Procedure

Scrubber K-415 with KjelDigester K-449

SpeedDigester K-425 / K-436 / K-439 with Kjeldahl setup

SpeedDigester K-439 with water reflux setup

SpeedDigester K-439 with air reflux setup



Accessories for water and air reflux digestion

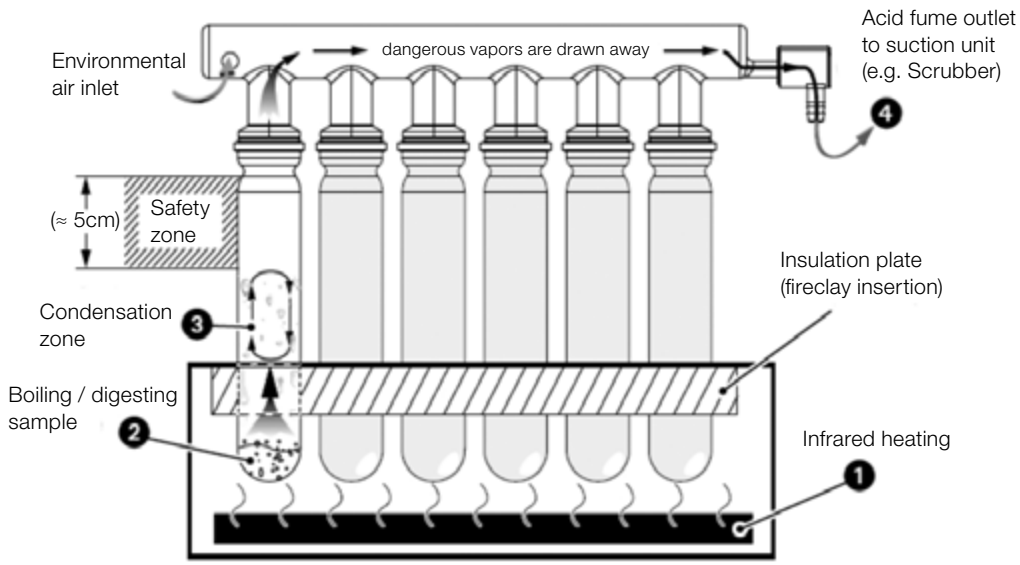
In contrast to the block the main advantage of the SpeedDigester is its flexibility. One SpeedDigester can be used for Kjeldahl applying different sample tube sizes (100 mL, 300 mL, 500 mL sample tubes), as well as for reflux digestion (water and air condensers) by only changing the accessory.

With the Reflux Digestion Setup more applications beside the classical Kjeldahl digestion are possible by using the same instrument but with a different accessory.

The water reflux setup extends the range of applications using aqua regia, HNO_3 or HCl, for heavy metal determination in soil, electronic waste, water, food, feed and textiles. The air reflux setup is used to determine the COD (chemical oxygen demand) in water to evaluate the water quality. The automatic SpeedDigester K-439 is the only infrared digester available on the market with precise temperature control and easy to read graphical display, which allows for an easy and automated digestion process and uniform, standardized sample processing.

Functional principle of the SpeedDigester (IR)

- The infrared heating (1) generates high temperatures in the sample (2).
- The sample (2) is digested in the constantly boiling acid.
- Hot acid fumes rise into the condensation zone (3), condense and rinse back down to the sample creating a constant reflux.
- Residual fumes which escapes the condensation zone is highly corrosive and must be withdrawn and efficiently neutralized (i.e. with the Scrubber K-415) (4).



Comparison of the BUCHI IR and block digester

	IR (SpeedDigester)	Block (KjelDigester)
Advantage	Fast and flexible	Automated for highest sample throughput
Heating principle	Radiation	Contact
Preheat time (400 °C)	10 min	20 min
Heat transfer to sample	Fast	Slow
Temperature control	Yes	Yes
Max. temperature	580 °C	450 °C
Programmable profiles	Yes (K-439)	Yes (K-449)
Lift	No	Yes (K-449)
Heating zone	9 cm	6 cm
Sample positions	6 / 12	20
Sample tube sizes Kjeldahl	100 / 300 / 500 mL	300 mL
Other sample tubes	Reflux sample tubes (e.g. COD) 3rd party tubes	None

2.2.3 Suction manifold and scrubbing

For both types of digestion units, the IR and the block digesters, specifically designed suction modules are used to conduct the digestion fumes into an absorbing media be it by means of a water jet pump or a scrubber. Dedicated suction modules for aqueous samples with a condensate trap or for accelerated digestion using H_2O_2 with capillary funnels are available. The intention of the suction manifold is to avoid a health hazard to laboratory personnel. The use of a water jet pump would at least serve this purpose but is not environmentally sound. A better solution consists in the use of a scrubber filled with a neutralization solution. BUCHI offers the Scrubber K-415 which is specially designed for Kjeldahl applications. A final remark to the strength of suction may be appropriate at this point. A suction of 200 to 400 mbar below atmospheric pressure will be sufficient to transport the toxic fumes but does not enhance the evaporation of sulfuric acid.

Condensate trap of a suction module

Suction module for accelerated hydrogen peroxide digestion using the SpeedDigester

2.2.4 Acid mixtures

In Kjeldahl 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. In modified Kjeldahl digestions mixtures of sulfuric and chromic acid[13] [14] are described. Oxidative destruction of organic matrices however is not limited to the nitrogen determination by the Kjeldahl method. Digestions aiming at the analysis of phosphates in waste water are performed in a mixture of sulfuric and nitric acid[15] and for the analysis of heavy metals hydrochloric and nitric acid (aqua regia) are described in the literature[16]. Mixtures of sulfuric and nitric acids bear a potential to nitrify organic materials for instance in electrophilic aromatic substitutions or if hydroxyl-groups are present. This could lead to the production of very hazardous explosives. It is very important to follow the published methods carefully and always assess the explosion potential when nitric acid is involved.

2.2.5 Acid consumption during digestion

The total required amount of sulfuric acid is given by:

1. Conversion of K_2SO_4 to $KHSO_4$

(K_2SO_4 is a component of Kjeldahl Tablets)
Consumption ca. 2 – 3 mL H_2SO_4

2. Consumption by organic matter

The volume of consumed concentrated sulfuric acid in a Kjeldahl digestion depends on the sample[17] and the used catalyst amount added to the sample.

[13] M. Jurecek, Einige analytische Aspekte der Oxydation organischer Stickstoffverbindungen mit Chromsäure, Mikrochim. Acta 926 – 938, 5 (1962)
[14] V. Novak, P. Kozak, P. Matousek und M. Jurecek, Analytische Aspekte der Oxydation organischer Stickstoffverbindungen mit Chromsäure. Bestimmung der Nitro- und Nitrosogruppen, Mikrochim. Acta 1101 – 1107, 6 (1962)
[15] Standard Methods for the examination of water and waste water, 19th edition 1995, 4 – 146, Inorganic Nonmetals (4000), 4500-P Phosphorus
[16] BUCHI Application Note No. 095/2012, Determination of Trace Metals by Aqua Regia Digestion according to ISO 11466
[17] R. Lange, R. Friebe und F. Linow (1979). Zur Anwendung der Methodenkombination Kjeldahl-Aufschluss/Bertholet-Reaktion bei der Stickstoffbestimmung in biologischen Materialien, 1. Mitt. Stand der Kenntnisse – Teil I, Die Nahrung 23, 3, 1979, 255 – 261 referring to Exley, D., Biochem. J. 63, 496 (1956)

Consumption of 98 % sulfuric acid by fat, protein and carbohydrates

Organic matter	Consumption: H_2SO_4 / g [mL]	Example: Salami	e.g. for 1.5 g Salami (weight · org. matter):
Fat	9.7	27.3 %	$1.5 \cdot 9.7 \cdot \frac{27.3}{100} = 3.97$ mL
Protein	4.9	20.6 %	$1.5 \cdot 4.9 \cdot \frac{20.6}{100} = 1.51$ mL
Carbohydrates	4.0	0.0 %	$1.5 \cdot 4.0 \cdot \frac{0.0}{100} = 0.0$ mL

Based on the acid consumption for the organic matter, the consumption of H_2SO_4 for individual samples can be calculated by adding the individual acid consumptions per constituent. As shown in the example of Salami above. An excess of sulfuric acid is needed because it ensures the formation of ammonium ions and nitrogen loss by evaporation of ammonia is avoided.

3. Losses due to evaporation

Losses ca. 1 mL / hour

4. Required remaining volume

Remaining volume = amount of used Kjeldahl Tablet (e.g. 10 g Kjeldahl Tablets = 10 mL H_2SO_4)

Example to calculate the required H_2SO_4 amount:

H_2SO_4 volume required
= conversion + (total consumption by org. matter) + evaporation + remaining volume
= 3 mL + (3.97 + 1.51 + 0.00) mL + 1 mL + 10 mL
= 18.48 mL ~ 18 mL

2.2.6 Catalyst used for digestion

Usually the Kjeldahl Tablets consist mainly of an inert salt (K_2SO_4 or Na_2SO_4) which increases the boiling point of sulfuric acid. A further component is 1 – 3 % of one or several metal catalysts which speed up the chemical reaction. Some samples additionally require additives like silicone to reduce the formation of foam at the beginning of the digestion.

Different reports show that a various number of catalysts help to accelerate the digestion process and to increase the recoveries[18] [19]. Typically mercury, selenium, titanium and copper are the catalysts of choice to help increase the efficiency and speed of the digestion process. However, mercury and selenium are highly toxic and should rather be avoided. It is therefore advisable to use titanium and copper which are of low toxicity but almost as effective[20].

[18] Rexroad, P.R.; Cathey, R.D; Gehrke. C.W.; The Kjeldahl nitrogen determination, aper presented at 88th Annual AOAC Meeting, (1974) Washington D.C.
[19] Hadorn, H.; Jungkreuz, R.; Biefer, K. W.; Über die Stickstoffbestimmung in Lebensmitteln nach Kjeldahl und den Einfluss des Katalysators im Besonderen, Mitt. Gebiete Lebensm. Hyg., Band 45, 14 – 29, (1955)
[20] Ugrinovits, M.; Kjeldahl nitrogen determination with various catalysts, Band 71, 124 – 139, (1980)

Advantages and disadvantages of the most common catalysts

Catalyst	Advantage	Disadvantage
Mercury	Very short digestion time at reproducible results	Toxic and expensive, waste must be collected for safe disposal
Selenium	Very short digestion time, even for fatty samples	Very toxic, N losses can occure when incorrect ratio, too long digestion times or too high temperatures are applied
Copper	Compliant to official regulations, low toxicity	Slower digestion process than selenium and mercury
Titanium	Low toicity and better recovery than copper	Slower digestion process than mercury

The rate speed of a chemical reaction also depends on the temperature[21]. A useful generalization implies that the reaction rate doubles with every 10 °C increase in temperature. It is however not possible to heat the digestion mixture above its boiling point by only increasing the heating temperature. To increase the boiling point of sulfuric acid (approx. 335 °C) it is necessary to add inert salts. Different types of inert salts (potassium vs. sodium sulfate) increase the boiling point as follows:

·

- 354 °C with H_2SO_4 + potassium sulfate
- 352 °C with H_2SO_4 + potassium and sodium sulfate
- 348 °C with H_2SO_4 + sodium sulfate

Potassium sulfate increases the boiling point of the digestion mixture by around 6 °C more than sodium sulfate. During the digestion process the sulfuric acid evaporates thereby changing the salt / acid ratio and leading to even higher temperatures. The boiling temperature should never exceed 380 °C as at a temperature of 390 °C or higher nitrogen losses can occur due to a possible transformation of ammonia into elemental nitrogen gas (N_2). Generally it can be said that potassium sulfate is the most adequate salt to be used for a Kjeldahl digestion. This is due to the fact that it achieves a higher boiling point, and, moreover to the circumstance that mixtures with sodium sulfate rather tend to crystallize. As mentioned above the optimal Kjeldahl Tablet offers an ideal balance between different criteria such as easy handling, environmental effects and digestion speed.

Due to its easy and unproblematic handling copper sulfate outmatches mercury and selenium as the ideal catalyst. Even when used in only small amounts it is the main component which help to speed up digestions

→ Due to its low toxicity but nevertheless very efficient catalytic effect, copper sulfate nowadays often replaces selenium and mercury. Copper sulfate is the most frequently used catalyst, and is also mentioned in modern regulations of AOAC and ISO.

Titanium dioxide also has catalytical effects and helps to increase the digestion speed. For samples with high organics titanium dioxide shows better effects than copper and it is also accepted by AOAC and ISO.

→ The shortest digestion times can be achieved using the catalysts titanium dioxide and copper sulfate in a mixing ration of 1:1.

Potassium and sodium sulfate increase the boiling point of sulfuric acid and therefore speed up the digestion

→ Potassium sulfate achieves a higher boiling point than sodium sulfate and moreover decreases the risk of crystallization. Therefore potassium sulfate is the most adequate inert salt for a Kjeldahl digestion.

[21] Arrhenius equation – IUPAC Goldbook definition

The ratio of sulfuric acid to salt and catalyst ideally is 2 mL H_2SO_4 to 1 g of salt and catalyst e.g. in the form of 2 Kjeldahl Tablets of 3.71 to 5 g.

2.2.7 Selecting the appropriate catalyst

The decisive factors for selecting a particular catalyst follow ecological, economical and practical aspects like for example reducing the formation of foam. While a toxic, selenium-containing catalyst reacts fastest, a copper-containing catalyst is considerably safer for both humans and the environment. An ideal compromise in this regard is the mixed catalyst consisting of copper- and titanium sulfate (Kjeldahl Tablet Titanium). In water containing samples, e.g. Total Kjeldahl Nitrogen (TKN) determinations, strong foam formation and sputtering could be found. In such a situation the digestion temperature should be reduced at the beginning and then increased slowly, additionally the use of boiling rods[22] is very important to prevent from boiling delays. If environmental aspects are of major importance or the to-be analyzed sample only contains very little organic material, the Kjeldahl Tablet ECO might be the ideal choice as it features the lowest content of copper.

Comparison of BUCHI Kjeldahl Tablets for standard Kjeldahl digestions

Tablet type	Tablet weight [g]	Recommended sample type	Benefit
Titanium #11057980	3.71	High in organic matter, high fat content	· Time saving · Mentioned in official regulations
Missouri #11057982	5.00	Middle or high in organic matter	· Compromise between Titanium and ECO · Mentioned in official regulations
ECO #11057983	4.00	Low in organic matter	· Most eco-friendly tablet, due to the very low copper content · Reduced H_2SO_4 amount (16 mL) due to the low tablet weight

Composition of the BUCHI Kjeldahl Tablets for standard Kjeldahl

Tablet type	Tablet weight [g]	Inert salt K_2SO_4 [g]	Metal catalyst $CuSO_4 \times 5\ H_2O$ [g]	TiO_2 [g]	H_2SO_4 amount (ratio 2:1) for 2 tabs [mL]
Titanium	3.71	3.5	0.105	0.105	15
Missouri	5.00	4.98	0.02	–	20
ECO	4.00	3.998	0.002	–	16

If the main incitement is to reduce the amount of chemicals used in the process, and therefore also the running costs, it is advisable to prefer the micro-Kjeldahl method and the corresponding micro Kjeldahl Tablets (Copper Micro or Titanium Micro).

Kjeldahl Tablet Cofigurator
Use the Configurator to select the Kjeldahl Tablet for digestion that suits your needs best.
www.buchi.com/tablet-configurator

best@buchi No. 65
Decode the mysteries of the Kjeldahl Tablets
www.buchi.com/media-center

[22] BUCHI boiling rods (043087)16

Composition of the BUCHI Kjeldahl Tablets for micro Kjeldahl

Tablet type	Tablet weight [g]	Inert salt K_2SO_4 [g]	Metal catalyst $CuSO_4 \times 5\ H_2O$ [g]	TiO_2 [g]	H_2SO_4 amount (ratio 2:1) for 2 tabs [mL]
Titanium Micro #11057981	1.59	1.5	0.045	0.045	3
Copper Micro #11057985	1.65	1.5	0.15	–	3

Use the Kjeldahl Tablet Configurator to select the Kjeldahl Tablet for digestion that suits your needs best. Just select the most suitable answer to each question of the Kjeldahl Tablet Configurator, to get information about the most adequate Tablet(s) for your needs. Alternatively or in addition to selenium and metal-salt catalysts hydrogen peroxide or sodium persulfate together with sulfuric acid are used with very good results.

2.2.8 Ideal ratio of salt to sulfuric acid

Pure sulfuric acid boils at approx. 290 °C and upon boiling it releases a surplus of SO_3 together with H_2O until a 98.3 % sulfuric acid is obtained finally boiling at 338 °C[23]. Commercially available sulfuric acid of 98 % for this reason shows a boiling temperature of 338 °C. With the addition of Kjeldahl Tablets the boiling temperature of the mixture in the sample tube is increased to a desired temperature of 350 to 370 °C. With time and duration of the digestion, the concentration of the sulfuric acid in the sample tube decreases, leading to even higher temperatures. The ratio of sulfuric acid to sulfate salts is crucial for the boiling temperature actually found. A good ratio is 1 g of Kjeldahl catalyst mixture to 2 mL of 98 % sulfuric acid. Typically for 1 g sample two Kjeldahl Tablets of 3.71 to 5 g are used together with 15 to 20 mL of 98 % sulfuric acid and digestion times of 90 to 120 minutes are applied.

The ideal ratio of sulfuric acid to sulfate salts by the end of a digestion should be such that the sample temperature does not exceed 380 °C. Above 390 °C the formation of elemental nitrogen gas (N_2) becomes a possibility, eventually leading to too low nitrogen results. Samples which exhibit final ratios close to the limits are prone to crystallization after cooling. This is not desirable especially when the sample changer (e.g. KjelSampler) is used, because then the dip tube would not be able to reach the bottom of the sample tube and sample transfer would be hampered.

Temperature behavior caused by amount of catalyst in 20 mL sulfuric acid at the end of a digestion

H_2SO_4 98 % [mL]	Kjeldahl Tablets [g]	Sample temperature [°C]	Approx. digestion time [h]	Remarks
20	–	338	8	Slow formation of NH_3
20	5	350	4	Slow formation of NH_3
20	10	370	1.5	Optimal digestion temperature
20	15	390	1.5	Nitrogen losses

[23] Römpps Chemie-Lexikon, Frankische Verlagshandlung Stuttgart, ISBN 3-440-04510-2 (1987)

2.2.9 Digestion by hydrogen peroxide

Digestion supported by the oxidative power of hydrogen peroxide often leads to a substantial reductions in digestion time and foaming. The digestion of milk using Kjeldahl Tablets for example needs 120 minutes digestion time compared to 30 minutes if hydrogen peroxide is added via the capillary funnels of the H_2O_2 suction module as oxidant. A typical digestion of milk with H_2O_2 would use 2 – 5 g sample, 15 – 20 mL of 98 % sulfuric acid and 30 mL of 30 % H_2O_2. The procedure is described in the BUCHI Application Note 'AN 054/2010' and available on request.

Addition of hydrogen peroxide into capillary funnels during digestion

2.2.10 Digestion parameters

The significant parameters for digestion of a given sample are the amounts of reagents and the temperature setting of the digestion unit.

The volume of sulfuric acid used is a function of the expected consumption of sulfuric acid in the redox reaction converting sulfuric acid to sulfur dioxide. The digestion time depends on the chemical structure of the sample, the temperature, the amounts of sulfate salt and the catalyst. A practical recommendation distinguishes a first period of time until the sample becomes translucent and a second period for the completion of the digestion. The second period of time is meant for the completion of the conversion of the nitrogen degradation products to ammonia[24]. As a rule of thumb, after the observation of a clear translucent solution, 30 minutes are sufficient to complete the reaction. By the end of the digestion a surplus of acid has to be present in a sufficient amount in order to keep the non-

See chapter 2.6
Calculation of the nitrogen content

Application Note No. 155/2016
Three digestion methods for protein determination in tofu
www.buchi.com/media-center

Application Finder
Find detailed method descriptions of nitrogen determinations using H_2O_2
www.buchi.com/applications

volatile ammonium ions in solution and prevent the loss of volatile ammonia. If the amount of sulfuric acid is too low the organic material may produce carbon smoke leaving the Kjeldahl sample tube. The excess sulfuric acid ideally is at a concentration which is high enough to keep the sulfate salt in solution such that after cooling no crystallization occurs.

The added sulfate salt increases the boiling temperature of the sulfuric acid solution. The ratio of sulfate salt to sulfuric acid should be high enough to allow a starting boiling temperature of 350 °C and must never lead to an increase of the sample temperature higher than 380 °C during the entire digestion. If the temperature is below 350 °C the reaction is slowed down and at temperatures above 390 °C nitrogen loss will be encountered. In this temperature range of 350 – 380 °C the Kjeldahl reaction takes place and the speed of reaction is further enhanced by the added catalysts.

See chapter 2.2.8
Ideal ratio of salt to sulfuric acid

The three periods of a Kjeldahl digestion

Digestion initial – clearing

Sample mixture is fuming and carbonizing. The black foam must not rise too high, as they must be rinsed during the 2. digestion step (completion) back into the sample tube to avoid losses of nitrogen.

Completion of digestion – total degradation

Maintaining the condenzation zone at 5 cm below the constriction of the sample tube.

Digestion finished

Digestate is clear and green, blue-green or colorless, depending on the catalyst used.

[24] H. Hadorn, R. Jungkunz und K.W. Biefer, Über die Stickstoffbestimmung in Lebensmitteln nach Kjeldahl und den Einfluss des Katalysators im Besondern, (1953) Mitt. Gebiete Lebensm. Hygiene 44:14

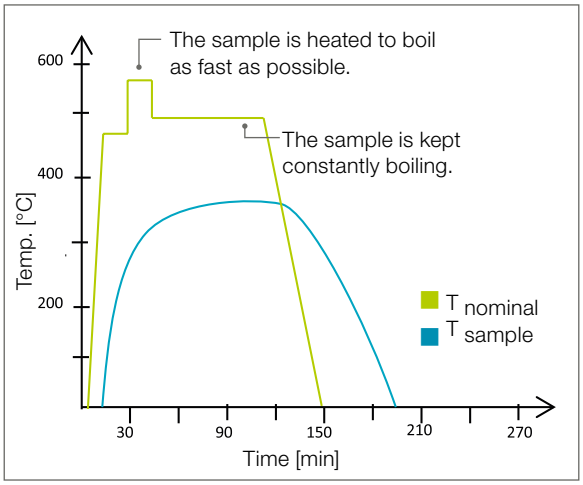
2.2.11 Digestion temperature

The digestion temperature is controlled by the boiling temperature of the mixture of sulfate salts and sulfuric acid. As discussed, foam formation is a function of the temperature. Monitoring the temperature along the digestion process is a way to control foam formation. In practice the sample temperature is increased stepwise parallel to the chemical degradation process under way. This can be done manually or automated by an instrumental feature with programmable time/temperature steps.

See chapter 2.2.8
Ideal ratio of salt to sulfuric acid

A typical temperature profile for the SpeedDigester (IR) could look as given below:

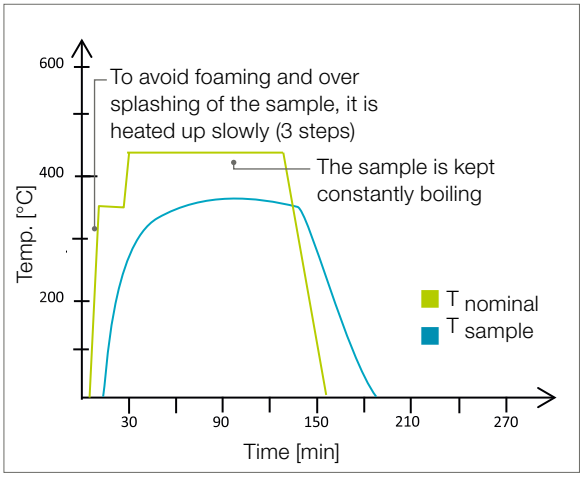
Standard Kjeldahl using 300 mL sample tube



Step	Temp. [°C]	Level	Time [min]
Preheating	480	8.5	
Digestion	480	8.5	10
	550	9.5	10
	490	8.5	65
Cooling			30

A typical temperature profile for the KjelDigester (block) could look as given below:

Standard Kjeldahl digestion using Kjeldahl Tablets



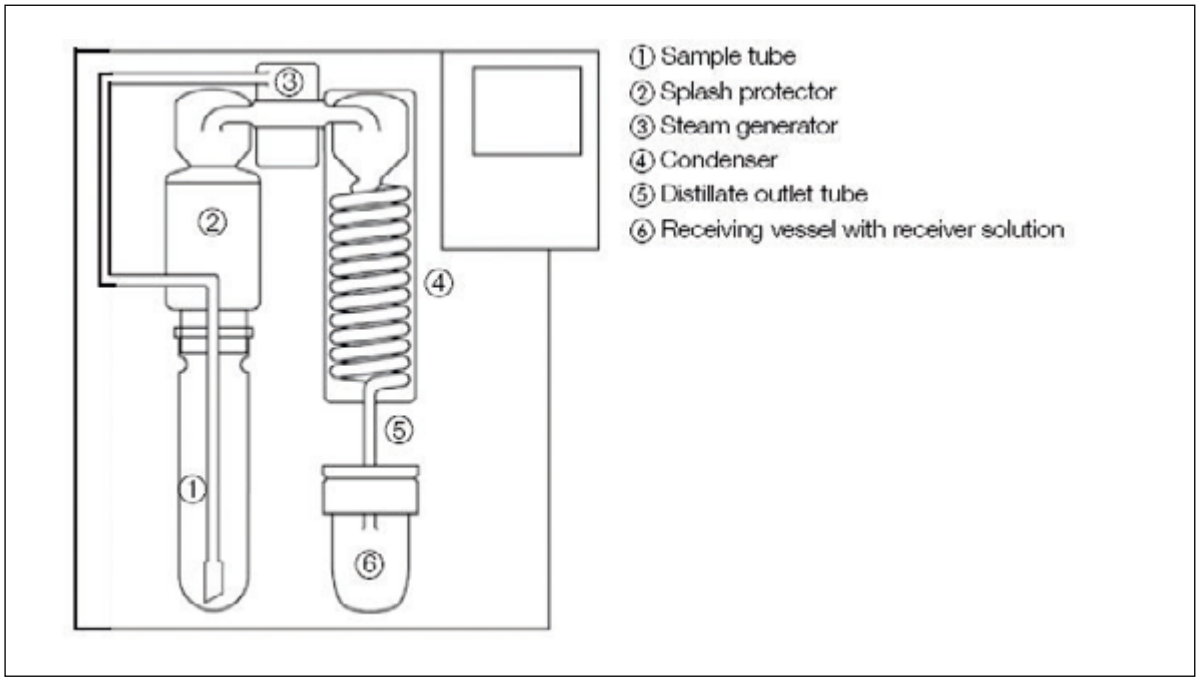
Step	Temp. [°C]	Time [min]
Preheating	300	0
Digestion	340	15
	420	105
Cooling		35

The two graphs above also show that the sample temperature is always lower than the instrument temperature. Which can be explained by losses of temperature by the heat transfer from the digester to the sample tube and from sample tube to the sample.

The ratio of sulfuric acid [mL] to sulfate salt [g] for a general sample is 2:1 and for samples containing fat 3:1. The digestion of fat consumes more sulfuric acid than a non-fat containing sample.

2.3 Distillation

After digestion the sample is allowed to cool to room temperature. Then the acidic digestion mixture is diluted with distilled water and the sample tube is transferred to a distillation unit.



Functional principle of steam distillation

Dilution

The acidic digestion mixture is diluted with distilled water before it is alkalized with naoh prior to distillation to free up the ammonia. The ammonia is steam distilled into an acidic receiver solution. The steam generator (3) of the distillation unit produces steam which is introduced into the sample tube (1) to drive out volatiles (here ammonia). After condensation in the condenser (4) the distillate is collected in a receiving solution (6).

Alkalization and distillation

In a chemical equilibrium the solvated ammonium ions (NH_4^+) produce ammonia gas (NH_3) by reacting with hydroxyl ions (OH^-) of excess sodium hydroxide.

Steam distillation

By steam distillation ammonia is separated from the sample and condensed together with water in the receiving vessel.

Condensate collection in receiving vessel

A common procedure to collect the ammonia (NH_3) in the receiver involves the presence of boric acid $B(OH)_3$ dissolved in water which forms ions with ammonia. The ammonia is quantitatively captured by the boric acid solution forming solvated ammonium ions.

2.3.1 Dilution of the digestion solution

Before the sulfuric acid is alkalized by adding concentrated sodium hydroxide solution the sample is 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. If digested samples cannot be processed directly after cooling, when standing for some hours, crystallization of the samples can be observed. In manual distillations this may lead to too low results for nitrogen if the solids are not dissolved. If the KjelSampler K-376 or K-377 is used solid samples would hamper sample transfer resulting in an error message and the determination to be skipped. Crystallization could be avoided by using 1 – 2 mL more sulfuric acid for the digestion process, or samples can be diluted with 10 – 20 mL of distilled water just after cooling.

2.3.2 Alkalization of the digestion solution

The strong acid keeps the ammonium ions dissolved in the sample tube. Neutralizing the acid by means of concentrated sodium hydroxide solution drives the chemical equilibrium between ammonium ions and ammonia towards the production of ammonia. In a basic environment ammonia can be driven out of the sample by means of steam distillation.

2.3.3 Preparation of the receiving vessel for distillate collection

The receiving vessel for the distillate collection is filled with an absorbing solution in order to capture the dissolved ammonia gas. Common solutions are aqueous boric acid of 2 or 4 % concentration if a boric acid titration is performed. In back titrations the absorbing solution is a precisely dosed volume of H_2SO_4 standard solution.

In the first case, the ammonia forms solvated ammonium cations and tetrahydroxyborate ($B(OH)_4^-$) anions (see equation above in chapter 2.3.1) and in the second case the ammonia reacts with sulfuric acid to ammonium ions. In the boric acid titration the tetrahydroxyborate is titrated with a standard solution of a strong acid (e.g. H_2SO_4 or HCl). In the case of back titration sodium hydroxide standard solution is used in order to determine the amount of sulfuric acid which reacted with ammonium ions. It is important that the condenser outlet tube and the electrode are completely immersed into the receiving solution.

See equation in chapter 2.0
Chemical reactions during alkalization

2.3.4 Steam distillation

After the alkalization of the acid a waiting period of some seconds, called «reaction time» can be set on the distillation unit in order to avoid splashes due to overheating when the hot steam enters the mixture already heated by the previous reaction. A distillation should last long enough such that more than 99.5 % of the ammonia is recovered in the receiving vessel. A typical distillation time is 150 to 300 seconds at a steam power of 100 %.

The starting volume for a distillation in 300 mL sample tubes should not be larger than 200 mL and in 500 mL tubes a volume of 300 mL should not be exceeded. If the starting volume is too large a risk of carryover of sample solution into the condenser and receiver is possible.

Distillation time

The actual distillation time needed for a quantitative determination can be evaluated and optimized by running verifications on the distillation unit under consideration. A reference material of choice is ammonium di-hydrogen phosphate. A recovery rate indicating a complete transfer of ammonia into the receiving vessel should be in the range of 98.5 – 101.5 %. If dry ammonium di-hydrogen phosphate is used and the determination is carried out with appropriate care, recoveries of > 99.5 % can be reached.

Steam power

Depending on the steam generator approximately 150 mL of condensate are distilled into the receiving vessel in five minutes time, if set to 100% steam power. The distillation units K-355, KjelFlex K-360 and the KjelMaster K-375 allow settings of 30 – 100 % steam power. This allows distillations at a lower pace which can be of help if the speed of distillation is prescribed by legal regulations or if optimizing recovered substance with a minimum of distilled volume is sought.

2.3.5 Distillation parameters

The diluted digestion mixture is alkalized with naoh prior to distillation to free up the ammonia. The ammonia is then steam distilled into an acidic receiver solution. Method parameters must be varied as required for boric acid titration or back titration.

Typical distillation parameters for a boric acid titration

Step		Why	How much	Rule-of-the-thumb
1. Dilution	H_2O dist.	Dilution of the strongly acidic solution, prevents violent reactions	25 – 90 mL	4 mL per mL used H_2SO_4, 2.5 mL when an autosampler is connected
2. Alkalization	Naoh 32 %	Conversion of NH_4^+ in NH_3 (gaseous)	15 – 90 mL	4.5 ml per mL used H_2SO_4
3. Preparation of the receiver	H_3BO_3 (pH 4.65)	To collect the distilled NH_3. NH_3 is bound as borate complex ($NH_4B(OH_4)$.	40 – 70 mL	2 % H_3BO_3 with KCl for low N contents 0.02 – 6.75 mg N/ sample tube 4 % H_3BO_3 for medium and high N content 6.75 – 125 mg N/sample tube
4. Distillation	Water steam (100 %)	Separation of NH_3 by boiling of the sample	150 – 300 s	Distillation time: · 150 s with KjelMaster · 180 s with KjelSampler · 240 s with others
5. Collection	NH_3	In boric acid receiver of pH 4.65		Condenser outlet tube and electrode must be completely immersed

Kjeldahl Knowledge Base
Procedure

KjelMaster System K-375 / K-377 for fully automated and high throughput Kjeldahl nitrogen determination

BUCHI Distillation Unit K-355

BUCHI KjelMaster K-375 with integrated titrator

BUCHI KjelFlex K-360 with optional external titrators

For the following analysis potentiometric as well as colorimetric titration can be performed.

Typical distillation parameters for a back titration are given below

Step		Why	How much	Rule-of-the-thumb
1. Dilution	H_2O dist.	Dilution of the strongly acidic solution, prevents violent reactions	25 – 90 mL	4 ml per mL used H_2SO_4, 2.5 mL when an autosampler is connected
2. Alkalization	NaOH 32 %	Conversion of NH_4^+ in NH_3 (gaseous)	15 – 90 mL	4.5 mL per mL used H_2SO_4
3. Preparation of the receiver	H_2SO_4 (0.25 mol/L)	To collect the distilled NH_3. Surplus of H_2SO_4 is titrated with naoh.	10 – 20 mL	Volume must be exactly dosed (usually 20 mL)
4. Distillation	Water steam (100 %)	Separation of NH_3 by boiling of the sample	150 – 300 s	Distillation time: · 150 s with KjelMaster · 180 s with KjelSampler · 240 s with others
5. Collection	NH_3	In sulfuric acid receiver		Condenser outlet tube and electrode must be completely immersed

2.4 Titration

After the completed steam distillation the determination is concluded by a quantification of ammonia. This is most commonly done by means of titration. The pH in the acidic receiver solution rises upon addition of ammonia and water (dilution). The nitrogen and protein content is then determined by titration of the borate complex.

Titrations may be carried out by means of a burette using an appropriate pH-indicator such as Sher mixed indicator[25] to indicate the endpoint of pH 4.65. A second option is to use a titrator to read the volume of consumed acid from the display of the titrator. The most sophisticated procedure is the use of a automated Kjeldahl distillation unit with a built-in titrator and have the result calculation done by the software of the instrument or an optional PC software (e.g. KjelLink PC software for KjelMaster K-375). Whatever the choice of the determination technique, the chemical reaction is the tetrahydroxyborate anion $B(OH)_4^-$ with a generalized strong acid HX (X = Cl^- etc.).

See chapter 2.4.3
Boric acid vs. Back titration

[25] Irving H. Sher, Two-Step Mixed Indicator for Kjledahl Nitrogen Titration, Analytical Chemistry, 831 (1955), BUCHI Sher mixed indicator (003512)

Potentiometric pH sensor with calibration buffers and KCl storage solution

Colorimetric sensor with Sher mixed indicator

2.4.1 Integrated vs. External titration

Manual titration

With the BUCHI Distillation Unit K-350 or K-355 titrations are carried out separately either manually using a burette or by means of an external titrator.

External but automated titration

With the BUCHI KjelFlex K-360 it is possible to connect and control a titrator and the titration can be done directly and automatically in the receiving vessel of the K 360.

Integrated tritration with result calculation

Finally the most sophisticated model KjelMaster K-375 is equipped with a built-in titrator for completely automated determinations and result calculation. The KjelMaster K-375 puts customers in the unique position of applying either potentiometric or colorimetric titration techniques. For a few official methods a colorimetric measurement is required. Changing between the two techniques is now easily achieved by “plug-and-measure” with no additional modifications on the KjelMaster and its integrated titrator.

2.4.2 Potentiometric vs. Colorimetric titration

The highly regulated environment of quality control requires authoritative qualifications, compliant titration techniques and traceable data management. For the detection of the pH two different techniques can be applied: potentiometric and colorimetric titration.

The potentiometric titration is based on the measurement of the electrical voltage using a pH electrode. For the colorimetric titration an indicator is used to measure the pH-dependent color changes with a colorimetric sensor that is measuring the absorbance of the light.

Using colorimetry with the KjelMaster it is necessary to daily determine the setpoint (patented), preliminary to blank and sample measurements. The setpoint defines the endpoint of the following titrations. The protection mesh has to be installed in order to shield the measuring beam from air bubbles. Two mixed indicators are supported by the BUCHI KjelMaster K-375: Sher indicator and bromocresol green / methyl red mixed indicator.

The pH of the boric acid solution has to be adjusted, dependent on the chosen indicator and boric acid concentration. Otherwise, the setpoint might not be detected. Due to the excellent properties of the Sher indicator, the titration speed is much higher compared to bromocresol green / methyl red.

Advantages and disadvantages of potentiometric and colorimetric titration

	Advantage	Disadvantage
Potentiometric direct pH measurement	· Lower detection limit[26] · „IntelliDist“ and back titration possible	· Daily calibration required · 6 – 12 months lifetime of electrode · Storage of pH electrode in saturated KCl
Colorimetric detection of the color change	· No calibration of the sensor necessary · Longer lifetime of the sensor (approx. 4 years) · Endpoint visible	· „IntelliDist[27]“ and back titration not possible · Indicator required (Sher or bromocresol green / methyl red indicator only!)[28] · protection mesh required to protect sensor from bubbles, to avoid titration errors · Daily determination of the Setpoint necessary

The method must be optimized for the use of the individual titration technique. The most important parameters are shown in the table below, for more detailed information please refer to the BUCHI Application Notes.

The most important method parameters using potentiometric or colorimetric titration

	Potentiometric	Colorimetric
Boric acid concentration	· 2 % + KCl 3 g/L · 4 %	· 2 % + indicator · 4 % + indicator
Boric acid volume in receiver	· Standard titration → 50 mL · Online titration → 60 mL	· Standard titration → 60 mL · Online titration → 60 mL
Stirrer speed during distillation	5	3
Stirrer speed during titration	7	10
Indicator	None	· Sher · Bromocresol green / methyl red (according to AOAC 2001.11)
Sensor	pH electrode with KCl storage solution and calibration buffers	Spectrosense with protection mesh
Titrant concentration	0.01 – 0.5 N	0.02 – 0.2 N
Titrant consumption (optimum)	3 – 17 mL	3 – 17 mL

[26] Due to lower concentrated titrants that can be used; see also best@buchi 58/2010 “Low detection and quantification limits”
[27] IntelliDist eliminates errors caused by a cooled down instrument. The countdown of the set distillation time only starts after the operating temperature is attained.
[28] Optimal point of inflection at 610 nm

2.4.3 Boric acid vs. Back titration

Two titration types are commonly used: boric acid and back titration

Boric acid titration

The boric acid titration is most commonly used as it is the direct detection and allows for automation without further equipment. The concentration of the captured ammonium ions in the boric acid are determined by means of an acid base titration commonly using standard solutions of sulfuric or hydrochloric acid. Depending on the amount of ammonium ions present, concentrations in the range of 0.01 N to 0.5 N are used. The receiving solution is boric acid adjusted to a pH of 4.65 to capture the nitrogen carried over as ammonia during the steam distillation. The subsequent endpoint titration is performed with an acid titration solution. This titration type does not require an accurate dosage of the boric acid.

Back titration

The alternative type of titration is called back titration. Here the receiving solution is a standardized acid of which an accurate volume is dispensed into the receiving vessel. After collecting the ammonia the excess acid is titrated with a basic titration solution at pH 7.00. Depending on the regulations to be followed, another endpoint pH might be chosen[29]. If the use of boric acid has to be avoided the back titration is the procedure of choice.

Advantages and disadvantages of boric acid titration and back titration

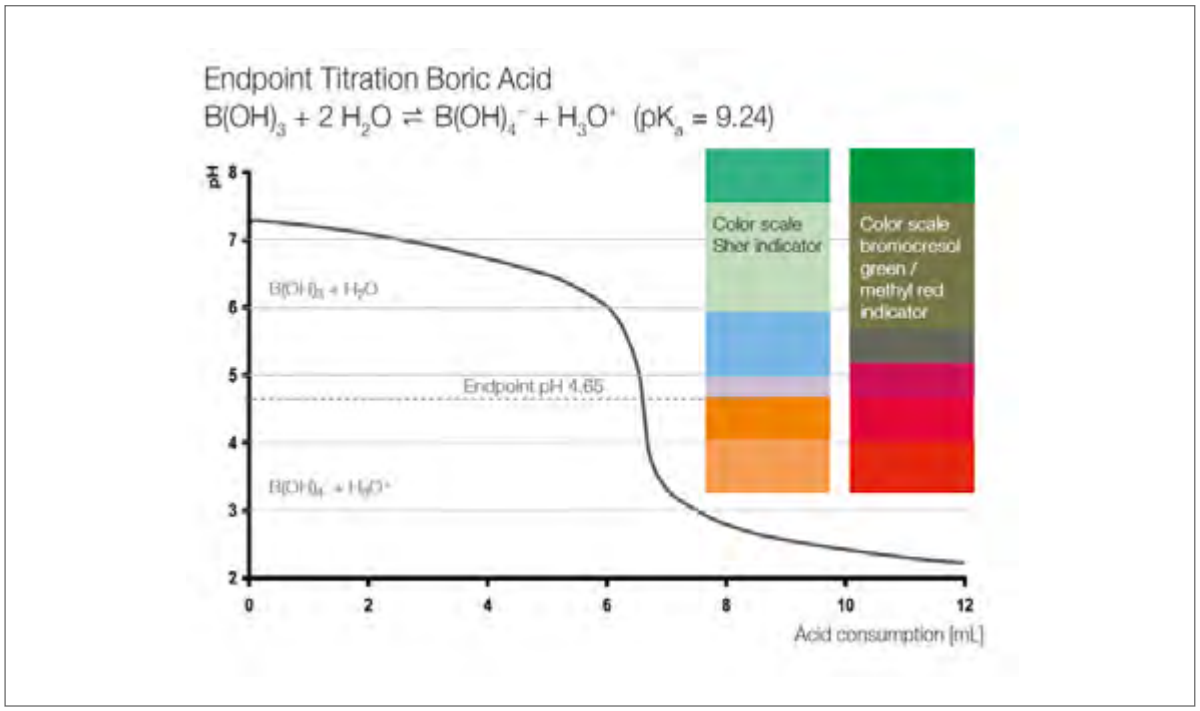
	Advantage	Disadvantage
Boric acid titration Receiver = H_3BO_3 Titrant = H_2SO_4 / HCl Endpoint = 4.65	Easy and approximate dosage of the receiver	Boric acid in use[30]
Back titration Receiver = H_2SO_4 Titrant = NaOH Endpoint = 7.00	No boric acid needed	Accurate dosage of the receiving solution by an additional dosing unit required. More expensive, as an additional dosing unit and more volumetric solutions are required

The two types of titrations are described in the following. It is necessary to verify that there are no air bubbles in the tubing of the titrant.

Air bubbles will cause a false titrant consumption reading, yielding high results. Incorrect results may also be caused by wrong titrant, air bubbles in the colorimetric sensor, false ratio of indicator, calculation errors or a defective or badly calibrated electrode.

[29] European Pharmacopoeia (Ph. Eur.) 2.5.9

[30] In June 2010 boric acid was added to the candidate list to the Substance of Very High Concern (SVHC). After the entry into force of the GHS regulation 1272/2008/EC and REACH change VO 790/2009/EC boric acid was listed as toxic to reproduction. The registration and review completed as part of REACH has meant the current classification of Boric Acid CAS 10043-35-3 / 11113-50-1 as of 1 December 2010 will be listed as H360FD (May damage fertility. May damage the unborn child.)



Typical titration curve with point of inflection at pH 4.65 and color scales

Boric Acid Titration

As shown in equations ammonia and boric acid form ammonium- and tetrahydroxyborate ions. The pH rises upon addition of NH_3 during the distillation when the NH_3 is captured by the transformation into NH_4^+-ions. In the titration with sulfuric acid standard solution the tetrahydroxyborate anions react to ammonium sulfate and boric acid.

The reaction scheme is given in the equation below.

$2\ NH_4^+\ B(OH)_4^- + H_2SO_4 \rightarrow (NH_4^+)_2SO_4^{2-} + 2\ B(OH)_3 + 2\ H_2O$

By the addition of acid titrant the ratio of tetrahydryoxborate and boric acid becomes smaller and the pH decreases. As can be seen from the figure below the point of inflection of the titration curve is at pH 4.65. This is the reason for preferably adjust the pH of the boric acid to 4.65 before distillation and use the endpoint pH 4.65 for the titration. The visible detection of the endpoint for a manual titration can be carried out by means of Sher mixed indicator. If a titrator is used the endpoint is set to pH = 4.65, the volume of acid titrant is determined and the nitrogen content calculated. Alternatively instead of the pH-electrode a colorimetric sensor can be used to detect the color change of the Sher indicator or the bromocresol green / methyl red mixed indicator.

Back Titration

Back titration is an indirect determination of the amount of distilled ammonia. As shown in the equation below an excess of sulfuric acid standard solution reacts with the NH_3 in the distillate. The residual sulfuric acid is titrated with sodium hydroxide standard solution and by difference the amount of ammonia is calculated.

Reaction scheme for back titration

- During distillation:
  H_2SO_4 (total) + 2 NH_3 → SO_4^{2-} + 2 NH_4^+
- During titration:
  H_2SO_4 (residual) + 2 OH^- → SO_4^{2-} + 2 H_2O

The chemical reaction during the distillation as well as the chemical reaction during the titration with sodium hydroxide standard solution is given in the equations above. The difference of the initial amount of sulfuric acid (H_2SO_4 (total)) and the amount of sulfuric acid present after the distillation (H_2SO_4 (residual)) corresponds to the distilled ammonia.

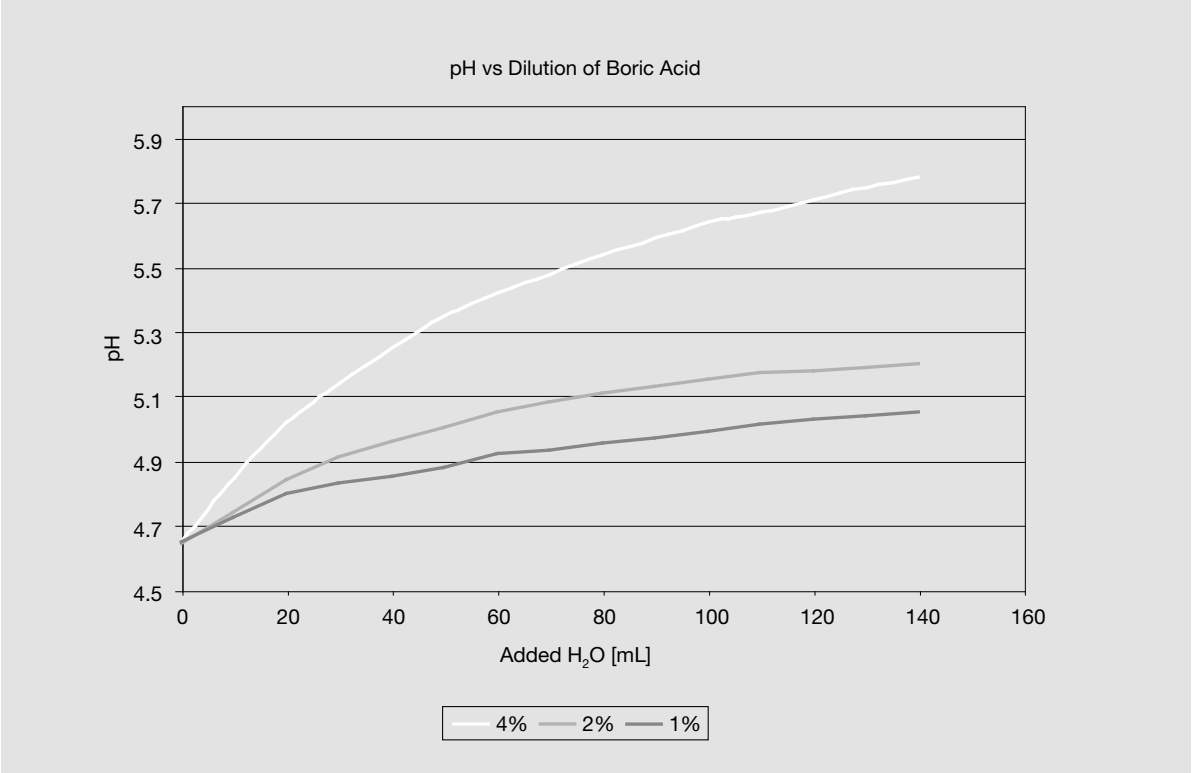
The titration of a strong acid with a strong base requires an indicator exhibiting a pKa close to 7 if a manual titration is performed. If a titrator is used an endpoint of pH 7 is appropriate. The amount of ammonia is calculated according to equations presented in chapter 2.6.2. The blank titration is carried out with an empty sample tube and basically corresponds to the initial amount of H_2SO_4. Small amounts of ammonia however, stemming from contaminations of the system, are accounted for by including blank determinations into the procedure.

2.5 Blanks

Blanks are necessary if a Kjeldahl distillation is carried out and contain all reagents apart from the test material (sample). In the following outline this is discussed for the case of boric-acid titrations. During distillation of a blank an increase of the pH-value in the receiving vessel is observed. This pH change is due to dilution of the boric acid by the addition of distillate and can be explained by equation for the pH of a weak acid. In the following table the effect is demonstrated by means of experimental pH readings as a function of the volume of added water to 60 mL of 4 %, 2 % and 1 % boric acid. In addition to the increase of pH due to dilution, also effects of traces of volatile bases, inevitably present in reagents and equipment, are taken into account by blank determinations.

Measured pH-values as a function of the volume of added water to 60 mL of 4 %, 2 % and 1 % boric acid

Dilution	pH expected in 60 mL boric acid		
Vol. H_2O mL	H_3BO_3 4 %	H_3BO_3 2 %	H_3BO_3 1 %
0	4.65	4.64	4.65
10	4.85	4.74	4.72
20	5.02	4.84	4.80
30	5.14	4.91	4.83
40	5.25	4.96	4.85
50	5.35	5.00	4.88
60	5.42	5.05	4.92
70	5.48	5.08	4.93
80	5.54	5.11	4.95
90	5.59	5.13	4.97
100	5.64	5.15	4.99
110	5.67	5.17	5.01
120	5.71	5.18	5.03
130	5.75	5.19	5.04
140	5.78	5.20	5.05



Representation of experimental pH-values as a function of different volumes of distillate added to 4 %, 2 % and 1 % boric acid

During distillation the extent of dilution depends on the distillation time and is identical for blank and sample determinations. The determination of the samples includes the pH increase due to dilution. This is taken into account in the calculations of the nitrogen contents in which the blank volumes are subtracted from the volumes found for the samples.

As can be seen above the increase of the pH-value depends on the concentration of the boric acid. At lower concentrations the increase in pH due to the distillation is less than for higher concentrations. This also leads to lower blank consumptions if less boric acid is used.

A typical blank volume in a boric-acid titration, using a distillation time of 4 minutes, is in the range of 0.1 to 0.2 mL if 0.25 mol/L H_2SO_4 is used as a titrant.

For a better understanding of the chemistry involved in the boric acid titration and the associated pH-increase effected by dilution during the distillation, a view at the chemical reaction and the chemical equilibria is given below:

Boric acid acts as a Lewis acid, an electron pair acceptor, in the chemical equilibrium described by equation:
$B(OH)_3 + 2\ H_2O \rightleftharpoons B(OH)_4^- + H_3O^+$

The chemical equilibrium is described by the law of mass action expressed by equation:

$$K = \frac{c(B(OH)_4^-) \cdot c(H_3O^+)}{c(B(OH)_3) \cdot c^2(H_2O)}$$

The derivation of the pH equation is based on equations K_a and pK_a. The pK_a-value for boric acid can be found in the literature[31].

$$K_a = \frac{c(B(OH)_4^-) \cdot c(H_3O^+)}{c(B(OH)_3)} = 5.8 \cdot 10^{-10} \text{ mol/L}$$

$$pK_a = 9.27$$

$$pH = pKa + \log \frac{c(B(OH)_4^-)}{c(B(OH)_3)}$$

2.6 Calculation of the nitrogen content

Calculations can aim at results expressed in absolute amounts of nitrogen mgN per sample or in terms of concentrations either %N, mgN / kg, mgN / L etc. The parameters needed for the calculations are:

Known parameters

c_{acid}	True concentration of standard acid solution[32]	0.005 – 0.5 mol/L
z	Valency of reaction	1 for HCl 2 for H_2SO_4
c_{NaOH}	Nominal concentration of standard base solution[33]	0.005 – 0.5 mol/L
M(N)	Atomic mass of nitrogen	14.00674 g/mol

Experimentally determined parameters

V_{blank}	Titrant consumption for blank	[mL]
m_{sample}	Sample weight	[g]
Vol_{sample}	Sample volume	[mL]
V_{sample}	Titrant consumption for sample	[mL]
f	Titer of the acid titrant	dimensionless
f_{NaOH}	Titer of NaOH titrant (for back titration)	dimensionless

Calculated results

W(N)	Weight of nitrogen in sample	[g]
w(N)	Weight fraction of nitrogen	[g N/g sample]
c(N)	Volume fraction of nitrogen	[g N/mL sample]
$\%(N)_{wt}$	Weight percent of nitrogen	[g/100 g sample]
$\%(N)_{vol}$	Volume percent of nitrogen	[g/100 mL sample]

[31] Handbook for Chemistry and Physics, D.R. Lide, Editor-in-chief, 88th Edition 2007–2008, p. 8–40.
[32] It is assumed that a commercially available standard acid solution is used
[33] Unless a fresh commercially available standard NaOH solution is used, the titer has to be determined

2.6.1 Calculation for boric acid titration

Nitrogen Content

Absolute amount of nitrogen [g]

$$W(N) = \frac{(V_{sample} - V_{blank}) \cdot M(N) \cdot c_{acid} \cdot f \cdot z}{1000} \quad (16)$$

Weight fraction of nitrogen [gN/g sample]

$$w(N) = \frac{(V_{sample} - V_{blank}) \cdot M(N) \cdot c_{acid} \cdot f \cdot z}{m_{sample} \cdot 1000} = \frac{W(N)}{m_{sample}} \quad (17)$$

Volume fraction of nitrogen [gN/mL sample]

$$c(N) = \frac{(V_{sample} - V_{blank}) \cdot M(N) \cdot c_{acid} \cdot f \cdot z}{Vol_{sample} \cdot 1000} = \frac{W(N)}{Vol_{sample}} \quad (18)$$

Weight percentage of nitrogen [%]

$$\%(N)_{wt} = \frac{(V_{sample} - V_{blank}) \cdot M(N) \cdot c_{acid} \cdot f \cdot z}{m_{sample} \cdot 10} = 100 \cdot w(N) \quad (19)$$

Volume percentage of nitrogen [%]

$$\%(N)_{Vol} = \frac{(V_{sample} - V_{blank}) \cdot M(N) \cdot c_{acid} \cdot f \cdot z}{Vol_{sample} \cdot 10} = 100 \cdot c(N) \quad (20)$$

2.6.2 Calculation for back titration

Nitrogen Content

Absolute amount of nitrogen [g]

$$W(N) = \frac{(V_{blank} - V_{sample}) \cdot M(N) \cdot c_{NaOH} \cdot f_{NaOH}}{1000} \quad (21)$$

Weight fraction of nitrogen [gN/g sample]

$$w(N) = \frac{(V_{blank} - V_{sample}) \cdot M(N) \cdot c_{NaOH} \cdot f_{NaOH}}{m_{sample} \cdot 1000} = \frac{W(N)}{m_{sample}} \quad (22)$$

Volume fraction of nitrogen [gN/mL sample]

$$c(N) = \frac{(V_{blank} - V_{sample}) \cdot M(N) \cdot c_{NaOH} \cdot f_{NaOH}}{Vol_{sample} \cdot 1000} = \frac{W(N)}{Vol_{sample}} \quad (23)$$

Weight percentage of nitrogen [%]

$$\%(N)_{wt} = \frac{(V_{blank} - V_{sample}) \cdot M(N) \cdot c_{NaOH} \cdot f_{NaOH}}{m_{sample} \cdot 10} = 100 \cdot w(N) \quad (24)$$

Volume percentage of nitrogen [%]

$$\%(N)_{Vol} = \frac{(V_{blank} - V_{sample}) \cdot M(N) \cdot c_{NaOH} \cdot f_{NaOH}}{Vol_{sample} \cdot 10} = 100 \cdot c(N) \quad (25)$$

2.6.3 Calculation of the protein content

Especially in food and feed applications the determination of Kjeldahl nitrogen is carried out with the intention to determine the protein content. Over the time from 1900 to 1982 the concept of multiplying the experimentally determined nitrogen content by an empirical protein factor (PF) was widely used. For a «general protein», the concept assumes an average nitrogen content of 16% which leads to a «general protein factor» of 6.25 as described by equation (26).

The parameter needed for the calculations is the empirical protein factor (PF)

$$\%P = \frac{\%(N) \cdot 100\%}{16\%} = \%(N) \cdot 6.25 = \%(N) \cdot f_{protein} \qquad (26)$$

Empirical protein factors for individual protein containing foodstuffs were determined based on their average nitrogen contents. In Table 16 specific protein factors are listed. All protein factors presented in the table are in accordance to regulations like e.g. AOAC and ISO. Already in 1982 the concept of protein factors was heavily disputed because with improved analytical tools available for the analysis of amino acids and proteins more precise protein determinations became feasible[34]. As a consequence adjusted protein factors were derived and are supported by official bodies[35 36 37 38].

[34] Die Stickstoffbestimmung nach Kjeldahl, Die Umrechnung von Stickstoff zu Protein, Literaturstudie und Erfahrungsbericht, M. Ugrinovits, Büchi Laboratoriumstechnik GmbH, Göppingen
[35] Schweizerisches Lebensmittelbuch, SLMB
[36] Association of Analytical Communities (AOAC)
[37] German industry norm and European norm DIN EN 32 645, Beuth Verlag GmbH, Berlin
[38] http://www.iso.org

2.6.4 Protein factors according to AOAC / ISO / DIN

Sample specific protein factors according to official regulations

General	6.25

Animal origin	
Eggs / egg products	6.25
Meat / meat products (general)	6.25
Fish	6.25
Milk / milk products	6.38
Casein / Caseinate	6.38
Baby and infant food on milk basis	6.38
Ice cream, frozen desserts	6.38

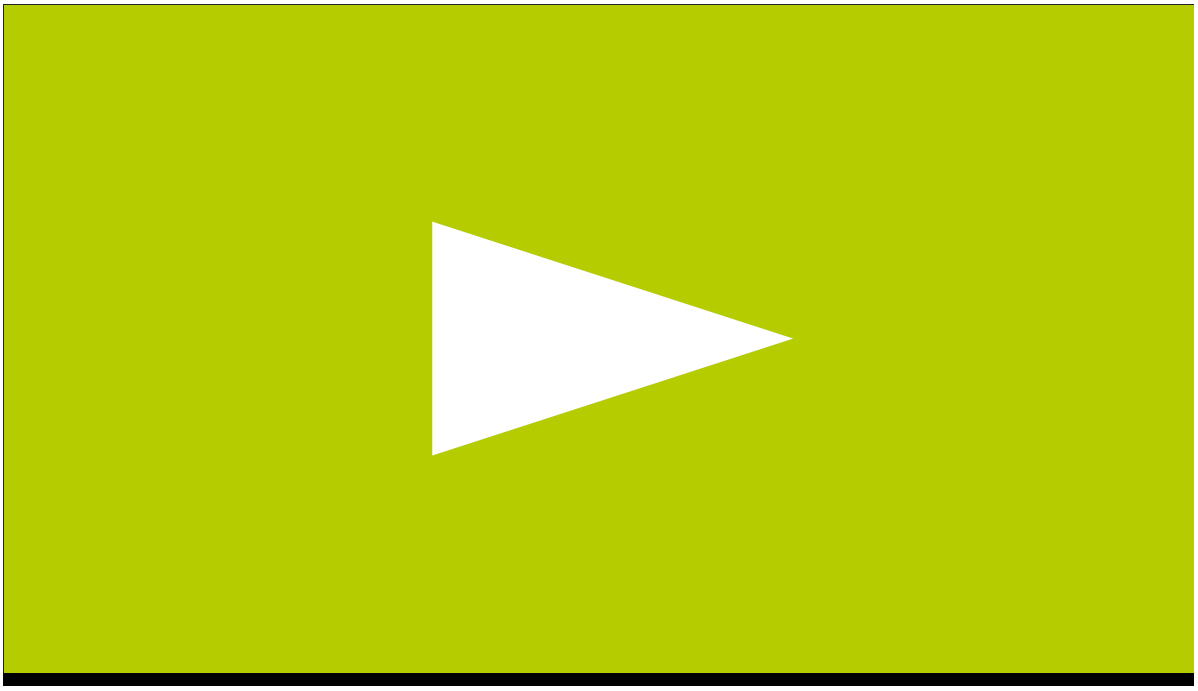
Plant origin	
Soy / soy products, tofu	5.70
Fruit products	6.25
Flour	5.70
Grain and pulses (general)	6.25
Wheat / hard wheat / wheat products	5.70
Barley	6.25
Oat	5.70
Rye	5.70
Maize	6.25
Pulses	6.25
Almonds	5.18
Peanuts, brasil nuts	5.46
Tree nut, coconut	5.30
Rice	5.26
Malt	6.38

Beverages	
Beer	6.25
Brewing sugars	6.25

Prepared food	
Baked products	5.70
Pasta (general)	6.25
Pasta (wheat)	5.70

Animal feed	
General	6.25
Wheat grains	5.70
Forage	6.25

Cosmetics	
Creme and ointment	2.144 (factor for urea)
Hair dye	1.216 (factor for ammonia)



Chemicals

Consumables from BUCHI for reliable digestions and distillations

BUCHI chemicals for Kjeldahl

BUCHI Kjeldahl Tablets

Consumables from BUCHI for reliable digestions and distillations

BUCHI offers not only high quality instrumentation but also approved consumables and chemicals for Kjeldahl that are tested for the most common reference applications. Kjeldahl Tablets, boric acid solutions, weighing boats, indicator, buffer solutions, potassium chloride, activated charcoal and reference substances for fast and reliable digestions, proper operation and reliable qualifications are available.

3.1 Kjeldahl Tablets

Today's requirements for a catalyst mixture lie not just in the shortening of digestion time but also in the possibility to deal with foamy samples and protection of the user as well as the environment.

See chapter 2.2.6 and 2.2.7
Catalyst used for digestion
Selecting the appropriate catalyst

Use the Kjeldahl Tablet Configurator to select the Kjeldahl Tablet for digestion that suits your needs best. Just select the most suitable answer to each question of the Kjeldahl Tablet Configurator, to get information about the most adequate Tablet(s) for your needs.

3.2 Boric acid

Two different concentrations of boric acid are recommended for Kjeldahl:

- 2 % (w/v) boric acid[39] for nitrogen contents ≤ 6.75 mg N absolute
- 4 % (w/v) boric acid[40 41] or nitrogen contents ≥ 6.75 mg N absolute

Kjeldahl Tablet Cofigurator
Use the Configurator to select the Kjeldahl Tablet for digestion that suits your needs best.
www.buchi.com/tablet-configurator

BUCHI boric acid solutions ready to be used with indicator and set pH

BUCHI Sher mixed indicator

3.3 Indicators for boric acid titration

To detect the endpoint during a colorimetric titration an indicator must be added to the boric acid. Two different indicators can be used for the colorimetric titration on the KjelMaster K-375:

1. Sher mixed indicator[42]
2. Bromocresol green / methyl red mixed indicator[43 44]

The point of inflection is depending on the indicator type as well as on the added indicator amount. For optimal performance the Sher mixed indicator is recommended, as it shows best performance in terms of endpoint detection speed and reliability. With the Sher indicator used in boric acid the color changes from green (pH > 7.5) to blue (7.4 to 4.8) and finally to the brown endpoint (pH 4.65). The optimal ratio of the Sher indicator to boric acid is 2.5 mL per 1 L boric acid (see BUCHI Application Notes for colorimetric titration). With the bromocresol green / methyl red indicator used in boric acid the color changes from green (pH > 6.5) to grey (pH 6) and finally to the red violet endpoint (pH 4.7) with 4 % boric acid or pH 5 with 2 % boric acid).

Note:

- Minor changes on the ratio could already cause a false detection of the endpoint.
- It is possible to use the bromocresol green / methyl red mixed indicator, but the color changes less sharply at pH 4.6 compared to the Sher indicator and only algorithm “Normal” can be used for the determination.
- The bromocresol green / methyl red mixed indicator causes red residues in hoses, tanks and other parts in the distillation unit!

[39] BUCHI boric acid 2 % with Sher indicator, pH 4.65 (11064972)
[40] BUCHI boric acid 4 % with Sher indicator, pH 4.65 (11064973)
[41] BUCHI boric acid 4 % with bromocresol green / methyl red indicator, pH 4.65 (11064976)
[42] BUCHI indicator according to Sher, 100 mL (003512)
[43] AOAC 2001.11
[44] ISO 5983-2

3.3.1 Sher mixed indicator for boric acid titration

The optimal ratio of Sher indicator[45] to boric acid is 2.5 mL Sher indicator per liter boric acid. Composition of the BUCHI mixed indicator according to Sher:

- Distilled water
- Ethanol 96 %
- p-Nitrophenol
- Sodium hydroxide 30 %
- Bromocresol green
- New Coccine

3.3.2 Bromocresol green / methyl red mixed indicator for boric acid titration

The pH of the boric acid must be adjusted between pH 4.6 and 4.8 for the 4 % (w/v) boric acid and between pH 4.95 – 5.1 for 2 % (w/v) boric acid in order to adjust the color of the boric acid to be dark red, not pink anymore. If the pH is too low the setpoint might not be detected correctly.

Preparation of the mixed indicator (according to ISO 5983-2, AOAC 2001.11 and other regulations):

1. Dissolve 100 mg bromocresol green in 100 mL methanol to get the bromocresol green indicator solution
2. Dissolve 100 mg methyl red in 100 mL methanol to get the methyl red indicator solution
3. Add 10 mL bromocresol green indicator solution per liter boric acid solution
4. Add 7 mL methyl red indicator solution per liter boric acid solution

[45] Irving H. Sher, Two-Step Mixed Indicator for Kjeldahl Nitrogen Titration, Analytical Chemistry, 831 (1955)

Regulations

Compliant without exception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Acide
Acid
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4.1 Official regulations

Regulations applied to BUCHI digestion and distillation units

Application	Sample	Regulation	BUCHI Application Note
Albumin (crude)	eggs liquide	AOAC 932.08	
Alcohol	wine beer	EEC 2676/90	FB06 K-355-001 Version A Alcohol FB07 K-360-003 V1.0 alcohol AN168/2014 AN172/2014
Alcohol	spirits liqueur	EEC 2870/2000	FB05 K-360-005 V1.0 wine and beer alcohol FB06 K-355-001 Version A Alcohol FB07 K-360-003 V1.0 alcohol FB08 K-360-004 spirits alcohol FB09 K-355_015 V1.0 sugar alcohol AN170/2014 AN171/2014
Ammonia	water waste water	AOAC, 973.49	
Ammonia	water waste water	DIN 38 406-E5-2	AN 033/2010
Ammonia	hair dye	EEC 3/514	
Ammonia	cosmetic products	EEC 83/514	AN 117/2013
Ammonia	water waste water	EPA, 350.2	
Ammonia	hair dye	LFGB §64 L84.11	AN 117/2013
Ammonium	fly ash	DIN 38406 - E5 - 2	AN 235/2016
Ammonium	water waste water	ISO 5664	
COD	waste water	DIN 38 409 Part 41	AN 081/2012
COD	waste water	ISO 6060	
Cyanide	waste water	EPA 9010C	
Formaldehyde	textiles	ISO 14184-2	
Heavy metal	sludge	DIN 13346	AN 095/2012
Heavy metal	sludge	DIN 38414-7	AN 095/2012
Heavy metal	waste soil	DIN EN 13657	AN 095/2012
Heavy metal	sample preperation	ISO 11464	AN 095/2012

Application	Sample	Regulation	BUCHI Application Note
Heavy metal	soil	ISO 11466	AN 095/2012
Hydroxyproline	meat	ISO 3496	AN 053/2010
Hydroxyproline	meat	LFBG §64 L06.00-8	AN 053/2010
NCN	milk	VDLUFA VI C30.4	AN 051/2010
Nicotine	tobacco tobacco products	ISO 2881	AN 223/2016
Nicotine	cigaretts	ISO 3400	AN 223/2016
Nitrate, Nitrite (Devarda)	fertilizer	DIN CEN/TS 15476	AN 116/2013 AN 235/2016
Nitrogen	mustard prepared	AOAC 920.173	
Nitrogen	sugars and sirups	AOAC 920.176	
Nitrogen	egg	AOAC 925.31	FB33 K-438-K-360-007 V1.0 nitrogen in egg AN 047/2010
Nitrogen	meat	AOAC 928.08	AN 023/2010 AN 077/2012 AN 076/2012 AN 114/2013 AN 242/2016
Nitrogen	food dressings spices	AOAC 935.58	
Nitrogen	laboratory wort	AOAC 950.10	
Nitrogen	ferilizer (nitrate free)	AOAC 955.04-C	AN 042/2010 AN 043/2010 AN 252/2016
Nitrogen	fertilizer (nitrate containing)	AOAC 955.04-D	AN 029/2010 AN 041/2010 AN 252/2016
Nitrogen	tobacco	AOAC 959.04	AN 223/2016
Nitrogen	ferilizer (nitrate free)	AOAC 960.52	AN 074/2011
Nitrogen	cacao products	AOAC 970.22	AN 022/2010
Nitrogen	plants	AOAC 978.04	
Nitrogen	resins plastics	ASTM D 1013-93	
Nitrogen	lubricating oil	ASTM D 3228 - 05	AN 030/2010
Nitrogen	Pharmaceuticals	European Pharmacopoeia (Ph. Eur.) 2.5.9	AN 045/2010 AN 264/2016 AN 279/2016

Kjeldahl Knowledge Base
Regulations

Application	Sample	Regulation	BUCHI Application Note
Total protein	Pharmaceuticals	European Pharmacopoeia (Ph. Eur.) 2.5.33 Method 7	AN 264/2016
Nitrogen (nitrates and nitrates absent)	Pharmaceuticals	US Pharmacopoeia (USP) 461 Method 1	AN 264/2016 AN 279/2016
Nitrogen (nitrates and nitrates present)	Pharmaceuticals	US Pharmacopoeia (USP) 461 Method 2	AN 264/2016
Nitrogen	soil	ISO 11261	AN 124/2013
Nitrogen	sludge	ISO 13342	AN 124/2013
Nitrogen	rubber	ISO 1656	
Nitrogen	agricultural food products	ISO 1871	
Nitrogen	starches derived products	ISO 3188	
Nitrogen	coal	ISO 333	
Nitrogen	meat	ISO 937	AN 023/2010 AN 077/2012 AN 076/2012 AN 114/2013
Nitrogen	meat	LFGB §64 L06.00-7	AN 023/2010 AN 077/2012 AN 076/2012
Nitrogen	tomato paste	LFGB §64 L26.11.03-11	
Nitrogen (bound)	water waste water	DIN 38 409 Part 28	
Nitrate, Nitrite (Devarda)	fertilizer	AOAC 892.01	AN 116/2013 AN 235/2016 (fly ash)
Nitrogen (Devarda)	fertilizer	DIN 38 409 H28	AN 116/2013
Nitrogen (total)	soil	DIN 19 684 Part 4	
NPN	milk	AOAC 991.21	
NPN	milk	ISO 8968-4	AN 050/2010
NPN	milk	LFGB §64 L01.00-10/4	
NPN	meat products	LFGB §64 L07.00-41	
Phenol	water	DIN 38 409 H16-3	
Phenol	water	EPA 9065	

Application	Sample	Regulation	BUCHI Application Note
Phenol	water	ISO 6439	
Protein	animal feed forage grain oilseed	AOAC 2001.11	AN186/2015
Protein	fruit products	AOAC 920.152	
Protein	beer	AOAC 920.53	FB01 K-438-K-360-004 V1.0 nitrogen colorimetric beer FB02 K-438-K-360-003 V1.0 nitrogen beer AN 024/2010 AN 108/2013
Protein	flour	AOAC 920.87	AN 027/2010
Protein	pasta	AOAC 930.25	AN 038/2010 AN 242/2016
Protein	milk milk products	ISO 8968-1	AN 020/2010 AN 031/2010 AN 028/2010 AN 021/2010 AN 078/2012 AN 075/2012 AN 180/2015
Protein	ice cream frozen desserts	AOAC 930.33	
Protein	baked products	AOAC 935.39	AN 039/2010 AN 242/2016
Protein	milk chocolate	AOAC 939.02	
Protein	brewing sugars	AOAC 945.23	
Protein	soy soy products tofu	AOAC 945.39	AN 035/2010 AN 036/2010 AN 155/2016
Protein	laboratory malt	AOAC 950.09	
Protein	grains, rice	AOAC 979.09	AN037/2010
Protein	meat	AOAC 981.10	AN 080/2012 AN 115/2013
Protein	fish fish products	AOAC 984.13	FB54 K-438-K-360-006 V1.0 colorimetric fish FB55 K-438-K-360-005 V1.0 fish

Kjeldahl Knowledge Base
Regulations

Application	Sample	Regulation	BUCHI Application Note
Protein	milk milk products milk products	AOAC 991.20	FB14 K-438-K-360-002 V1.0 milk Kjeldahl AN 020/2010 AN 031/2010 AN 028/2010 AN 078/2012 AN 075/2012 AN 180/2015 AN 197/2015
Protein	grain, pulses	ISO 20483	AN 110/2013
Protein	milk powder	AOAC 930.29	AN 180/2015
Protein	milk milk products	ISO 8968-2	FB14 K-438-K-360-002 V1.0 milk Kjeldahl AN 102/2013 AN 104/2013
Protein	milk milk products	ISO 8968-3	AN 103/2013 AN 105/2013 AN 197/2015
Protein	milk milk products	LFGB §64 L01.00-10/1	AN 020/2010 AN 031/2010 AN 028/2010 AN 021/2010 AN 078/2012 AN 075/2012
Protein	milk milk products	LFGB §64 L01.00-10/2	FB14 K-438-K-360-002 V1.0 milk Kjeldahl
Protein	Casein Caseinate	LFGB §64 L02.09-5	
Protein	sausages	LFGB §64 L08.00-7	
Protein	grain pulses	LFGB §64 L15.00-3	AN 025/2010 AN 110/2013
Protein	bread pastry from bread dough	LFGB §64 L17.00-15	
Protein	pastry cookies	LFGB §64 L18.00-13	
Protein	pulses	LFGB §64 L23.01-2	
Protein	baby and infant food on milk basis	LFGB §64 L48.01-26	
Protein	tomato ketchup	LFGB §64 L52.01.01-11	
Protein	juice lassie	SLMB 22/4.1 - 4.2	AN 046/2010

Application	Sample	Regulation	BUCHI Application Note
Protein	meat	SLMB 314.1	
Protein (crude)	nuts nut products	AOAC 950.48	AN 034/2010
Protein (crude)	animal feed	AOAC 954.01	AN 026/2010 AN 085/2012
Protein (crude)	animal feed	AOAC 976.05	AN 113/2013 AN 120/2013
Protein (crude)	animal feed	AOAC 984.13	
Protein (crude)	animal feed	AOAC 988.05	
Protein (crude)	animal feed	ISO 5983-1	AN 026/2010
Protein (crude)	animal feed	ISO 5983-2	AN 083/2012 AN 085/2012 AN 113/2013 AN 120/2013 AN 186/2015
Protein (raw)	egg egg products	LFGB §64 L05.00-15	AN 047/2010
Protein (raw)	meat products	LFGB §64 L07.00-7	
Protein N	milk	ISO 8968-5	
Protein N (NPN)	milk	AOAC 992.22	
Protein Nitrogen	milk	AOAC 991.22	
Protein Nitrogen	milk	AOAC 991.23	
SO2	food beverage	AOAC 990.28	AN 090/2012
SO2	food beverage	DTNB method	AN 066/2011
SO2	wine	OIV method	AN 065/2011 AN 072/2012
TKN	water waste water	AOAC 973.48	AN 040/2010 AN 049/2010 AN 091/2012 AN 118/2013 AN 191/2015
TKN	water waste water	DIN EN 25 663	AN 191/2015
TKN	water	EPA 351.2	
TKN	water waste water	EPA 351.3	AN 040/2010 AN 049/2010 AN 091/2012 AN 191/2015

Kjeldahl Knowledge Base
Regulations

Application	Sample	Regulation	BUCHI Application Note
TKN	water	EPA 351.4	
TKN	water waste water	ISO 5663	AN 091/2012 AN 118/2013 AN 191/2015
TVB-N	fish fish products	EC 95/149	FB52 K-355-006 A TVBN
TVB-N	fish fish products	LFGB § 64 L10.00-3	
TVB-N	fish products	LFGB § 64 L11.00-2	
TVB-N	crustaceans shellfish moluscs	LFGB § 64 L12.00-2	
Urea (micro Kjeldahl)	creme ointment	AOAC 960.52	AN 230/2016
Protein	royal jelly	ISO DIS 12824	
Protein soluble in potassium hydroxide solution	oilseeds soya meals rapeseed meals sunflower pellets	EN ISO 14244	

BÜCHI Labortechnik AG
Kjeldahl Product Overview

BUCHI provides Kjeldahl Solutions and support for the entire analytical workflow, from sample preparation to quantification. Depending on sample throughput, varying degrees of instrument integration and automation may be selected.

Sample preparation
Some applications require sample preparation prior to digestion and distillation. With a powerful mixer, non-homogeneous substances can be minced or crushed and homogenized to provide a representative sample.

Digestion
Digestion systems are used to convert nitrogen containing substances (e. g. proteins) in a sample (e. g. milk) into the appropriate form of nitrogen. Beyond classical Kjeldahl digestions, additional applications such as the determination of COD (Chemical Oxygen Demand) can be carried out with the SpeedDigester

Scrubbing
During the digestion process, acid fumes and reaction gases may emerge. A scrubber is connected to the digestion system for neutralizing acid fumes and adsorbing unpleasant odors.

Mixer B-400	SpeedDigester K-425 / K-436	Scrubber K-415
	SpeedDigester K-439	
	KjelDigester K-446	
	KjelDigester K-449	

Distillation

Digested samples can be processed directly in one of BUCHI's high-efficiency distillation units. The range of applications includes classical Kjeldahl tasks such as TKN (Total Kjeldahl Nitrogen) or Devarda as well as the direct distillation of other volatile components such as alcohol, sulfur dioxide, phenol, volatile acids, TVBN (Total Volatile Basic Nitrogen), formaldehyde and cyanide.

Titration

Reflecting different automation requirements, some distillation units actually combine the distillation step with the determination, for example by titration. The product range features an expandable distillation unit with the option of connecting it with a titrator of your choice as well as an automated unit with integrated titration.

Distillation Unit K-350

Distillation Unit K-355

KjelFlex K-360
with external titration

KjelMaster System K-375 / K-376 / K-377
with integrated titration and data management

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Close to customers

Our worldwide network of qualified distribution partners keeps us close to our customers. Since we are familiar with the requirements of our target markets, we can use our experience and knowledge to provide first-rate solutions.