A Guide To Kjeldahl Nitrogen Determination Methods and Apparatus

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This booklet has been developed to serve as a technical companion in the selection and use of Kjeldahl nitrogen determination methods and apparatus. This material has been prepared with the help of Kjeldahl chemists, consultants and Labconco engineers. It will not teach you to perform the Kjeldahl procedure but will help you understand steps in the process. This generic presentation is designed to help educate persons unfamiliar with Kjeldahl methodology and answer frequently asked questions raised by Kjeldahl users.

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History

Nitrogen determination has a long history in the area of analytical chemistry. Johan Kjeldahl first introduced the Kjeldahl nitrogen method in 1883 at a meeting of the Danish Chemical Society. As chairman of the chemistry department of the Carlsberg Laboratorium near Copenhagen, Kjeldahl was assigned to scientifically observe the processes involved in beer making. While studying proteins during malt production, he developed a method of determining nitrogen content that was faster and more accurate than any method available at the time. His method used simple equipment and could be performed by an inexperienced technician.

Applications

Since 1883, the Kjeldahl method has gained wide acceptance and is now used for a variety of applications. Kjeldahl nitrogen determinations are performed on food and beverages, meat, feed, grain, waste water, soil and many other samples. The method has been refined and tested for a wide variety of substances and approved by various scientific associations including:

AOAC International (formerly the Association of Official Analytical Chemists)

Association of American Cereal Chemists

American Oil Chemists Society

Environmental Protection Agency

International Standards Organization

United States Department of Agriculture

What is the Kjeldahl method?

The Kjeldahl method is a means of determining the nitrogen content of organic and inorganic substances. Although the technique and apparatus have been altered considerably over the past 100 years, the basic principles introduced by Johan Kjeldahl endure today.

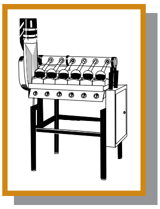
The Kjeldahl method may be broken down into three main steps:

Digestion - the decomposition of nitrogen in organic samples utilizing a concentrated acid solution. This is accomplished by boiling a homogeneous sample in concentrated sulfuric acid. The end result is an ammonium sulfate solution.

Distillation - adding excess base to the acid digestion mixture to convert NH_4^+ to NH_3, followed by boiling and condensation of the NH_3 gas in a receiving solution.

Titration - to quantify the amount of ammonia in the receiving solution.

The amount of nitrogen in a sample can be calculated from the quantified amount of ammonia ions in the receiving solution.



The Digestion Process

A general equation for the digestion of an organic sample is shown below as one basic example:

$$\text{Organic N} + H_2SO_4 \rightarrow$$

$$(NH_4)_2SO_4 + H_2O + CO_2 + \text{other sample matrix by-products}$$

A number of interrelated digestion conditions determine the rate of reaction and the completeness of the breakdown of nitrogen to ammonium sulfate. Among these are heat input to the acid digestion mixture, amount of inorganic salt added to elevate the acid boiling temperature, reflux rate of H_2SO_4 in the neck of the digestion flask, length of digestion, and catalyst addition. Adjusting any one of these factors has an influence on the others. Proper digestion conditions for a given sample matrix are achieved through establishing a balance of these factors in a controlled and repeatable fashion. In addition, if the sample contains nitrate or nitrite nitrogen, it is possible to chemically pretreat the digest to include or exclude this nitrogen source from the analysis as desired in a particular situation.

Acid Considerations

Sulfuric acid has been used alone for the digestion of organic samples. (As a convenience to reduce bumping of the digestion mixture, Alundum boiling chips or pumice are often added.) The amount of acid required is influenced by sample size and relative amount of carbon and hydrogen in the sample, as well as amount of nitrogen. A very fatty sample consumes more acid. Also, heat input and digestion length influences the amount of acid loss due to vaporization during the digestion process. Remember that a Kjeldahl flask is essentially a bulb with a condenser neck off to the side for refluxing of the acid.

Heat Input and Digestion Length

Typically the heating elements used for Kjeldahl digestions have variable settings. Heat input is frequently specified as “that setting which brings 250 ml of water at 25° C to a rolling boil in 5 minutes.”

Initially an organic sample usually chars and blackens. The reaction may at first be very vigorous depending on the matrix and the heat input. With organic decomposition the digestion mixture gradually clears as CO_2 evolves. Metallic ions might tint the clear digestion mixture. Note that solution clearing in itself is not an indication that all organic nitrogen has been broken down. Digestion length must be determined by recovery studies on known materials of similar matrix if a new method is being developed.

Salt Additions

The problem with using sulfuric acid alone for digestion is very long digestion times result with many samples due to the slow rate of organic decomposition. The addition of an inorganic salt to the digest elevates the boiling point of the H_2SO_4. The solution temperature of concentrated sulfuric acid alone is about 330° C. Addition of a salt such as K_2SO_4 can elevate the solution temperature of the digestion mixture to 390° C or more, depending on the ratio of salt to acid. This significantly increases the rate of organic decomposition in the digestion mixture, shortening the length of time required for digestion.

There are several precautions to keep in mind concerning salt addition. First, it is possible to raise the solution temperature of the digestion mixture too much. If the temperature goes much above 400° C during any phase of the digestion, volatile nitrogen compounds may be lost to the atmosphere.

Remember that as acid is gradually consumed during the digestion process, for the various reasons mentioned above, the salt acid ratio of the digest gradually rises. This means that the hottest solution temperatures are attained at the end of the digestion. Heat input, consumption of acid by organic material and vaporization, salt/acid ratio, digestion length, and physical design of the Kjeldahl flask, are all interrelated. Each has an effect on the final solution temperature.

A second precaution is that if the salt/acid ratio is too high, a considerable amount of material will “salt out” upon cooling of the digest. Concentrated acid pockets can be contained within the cake. These can react violently when concentrated base is added in the distillation process. A certain amount of salting out can be managed by diluting the digest with water while it is still somewhat warm, but not too hot.

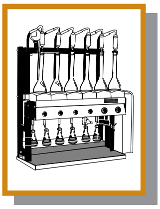
Catalyst Additions

Several catalysts have been employed by Kjeldahl chemists over the years to increase the rate of organic breakdown during the acid digestion. Mercuric oxide has been the most effective and widely used. However, mercury forms a complex with ammonium ions during digestion. The addition of sodium thiosulfate or sodium sulfide after digestion and before distillation will break the complex and precipitate mercuric sulfide. This is also important from a safety point of view, as mercury vapor might escape to the atmosphere during the distillation process.

Because of environmental concerns over the handling and disposal of mercury, other catalysts are coming more into favor. Many methods employ copper sulfate. Titanium oxide and copper sulfate in combination have been found to be more effective than copper sulfate alone. Selenium is frequently used. Commercially prepared mixtures of potassium sulfate and a catalyst are available from laboratory chemical suppliers. Bulk custom mixtures are also available.

Nitrate and Nitrite Reduction

Kjeldahl digestions do not always recover all forms of nitrogen in a sample. Nitrate and nitrite ions in a sample must first be reduced prior to acid digestion for quantitative recovery. Salicylic acid followed by sodium thiosulfate has been used to pretreat the mixture to ensure complete reduction. Other reduction schemes have been devised. Or other pretreatments have been used to prevent nitrates from being reduced at all during the charring process, leaving a clear digest with no contribution from nitrate ions.



The Distillation Process

The acid digestion mixture is diluted and made strongly alkaline with NaOH, liberating NH_3 as follows:

$$\overset{\text{ammonium sulfate}}{(NH_4)_2SO_4} + 2NaOH \xrightarrow{\text{heat}} \overset{\text{ammonia gas}}{2NH_3\uparrow} + Na_2SO_4 + 2H_2O$$

The Kjeldahl flask is attached to a water condenser and is heated to boil off the NH_3 gas from the digest. The tip of the condenser is submerged in a flask of acidic receiving solution, either standard acid or boric acid solution, to again trap the distilled NH_3 in receiving solution.

Digestion Mixture Dilution

The acid digestion mixture is usually cooled and diluted with ammonia-free water. As mentioned above, with digestion mixtures containing high salt/acid ratios, dilution prevents or minimizes caking. Sometimes this is done while the digestion mixture is still quite warm (with caution!), and the K_2SO_4 has not yet salted out. With some digestion mixtures, a cake forms, but dissolves or breaks up if time is allowed following dilution. Alternatively, warming or sonicating after dilution may break up the cake.

Cake material may cause low nitrogen recoveries in the distillation step. Also, entrapped acid in a cake may react violently with base during the distillation process.

Dilution of the digestion mixture before making it alkaline and distilling also reduces the likelihood of bumping. Additional boiling chips added just before distillation also reduces bumping, especially towards the end of the distillation as the solution becomes more concentrated. Two or three drops of tributyl citrate may be added as an aid to reduce foaming.

NaOH Addition

Concentrated NaOH (usually 50% solution) is added slowly down the neck of the flask. Being heavier, it forms a layer underneath the diluted acid digestion mixture. Generally, for each 5 ml of concentrated sulfuric acid used in the digestion, 20 ml of 50% sodium hydroxide is required to make the digest strongly alkaline (pH of >11). The flask is connected to the condenser and mixed before heating and distillation begins.

For samples not requiring a digestion step, such as direct ammonia determinations in water, the sample is buffered to a pH of 9.5 with a solution of sodium tetraborate and sodium hydroxide, to decrease hydrolysis of any complex organic nitrogen compounds present.

Distillation

The majority of the NH_3 is distilled and trapped in the receiving acid solution within the first 5 or 10 minutes of boiling. But depending on the volume of the digestion mixture and the method being followed, 15 to 150 ml of condensate should be collected in the receiving flask to ensure complete recovery of nitrogen. Further extension of the distillation times and volumes collected simply results in more water being carried over to the receiving solution. Excess water does not change the titration results.

Distillation times and distillate volumes collected should be standardized for all samples of a given methodology. The rate of distillation is affected by condenser cooling capacity and cooling water temperature, but primarily by heat input. Typically the heating elements used for distillation have variable temperature controllers. A distillation rate of about 7.5 ml/minute is most commonly cited in accepted methods.

Connecting bulbs or expansion chambers between the digestion flask and the condenser is an important consideration to prevent carryover of the alkaline digestion mixture into the receiving flask. The slightest bit of contamination of the receiving solution can cause significant error in the titration step.

When very low levels of nitrogen are being determined, it is advisable to “precondition” the distillation apparatus prior to distillation. This can be done by distilling a 1:1 mixture of ammonia-free water and 50% NaOH for 5 minutes just before sample distillation to reduce contamination from atmospheric ammonia.

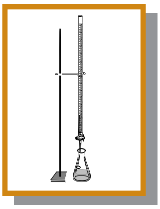
Receiving Solutions

If the receiving solution is standardized HCl or H_2SO_4, it is desirable to have only a slight excess left after the NH_3 is distilled and trapped in the receiving solution to minimize the back titration. Based on the anticipated amount of nitrogen in the sample, a target amount of standard acid can be calculated from the following formula:

$$\text{mls of standard acid to add to receiving flask} = \frac{[(\%\ \text{nitrogen expected in sample}) \times (\text{sample aliquot used}) \times \text{gram sample wt.}] + 2}{(\text{normality of standard acid}) \times 1.4007 \times \text{digestion dilution volume}}$$

If boric acid is used, the exact concentration is not needed because the titration directly measures the amount of ammonia in the distillate by neutralizing the 1:1 complex formed by ammonia and boric acid. Large quantities of boric acid may be added to the receiving solution so complete absorption of the ammonia is assured.

Receiving solution volumes may be increased by the addition of ammonia-free water so that the tip of the delivery tube is immersed. Delivery tubes should always be allowed to drain momentarily into the receiving flask before removal from the distillation apparatus. The receiving solution should remain below 45° C during distillation to prevent loss of ammonia.



The Titration Process

There are two types of titration: back titration, commonly used in Macro Kjeldahl; and direct titration. Both methods indicate the ammonia present in the distillate with a color change and allow for calculation of unknown concentrations.

Nitrogen Determination by BACK TITRATION

The ammonia is captured by a carefully measured excess of a standardized acid solution in the receiving flask. The excess of acid in the receiving solution keeps the pH low, and the indicator does not change.

ammonia		standard sulfuric acid		ammonium sulfate		excess sulfuric acid
$2NH_3$	+	$2H_2SO_4$	→	$(NH_4)_2SO_4$	+	H_2SO_4

(no color change)

The excess acid solution is exactly neutralized by a carefully measured standardized alkaline base solution such as sodium hydroxide. A color change is produced at the end point of the titration.

ammonium sulfate		measured excess acid		measured sodium hydroxide				ammonium sulfate		
$(NH_4)_2SO_4$	+	H_2SO_4	+	$2NaOH$	→	$(Na)_2SO_4$	+	$(NH_4)_2SO_4$	+	$2H_2O$

(color change)

Nitrogen Determination by DIRECT TITRATION

If boric acid is used as the receiving solution instead of a standardized mineral acid, the chemical reaction is:

ammonia gas		boric acid		ammonium-borate complex		excess boric acid
NH_3	+	H_3BO_3	→	$NH_4^+:H_2BO_3^-$	+	H_3BO_3

(color change)

The boric acid captures the ammonia gas, forming an ammonium-borate complex. As the ammonia collects, the color of the receiving solution changes.

ammonium borate complex		sulfuric acid		ammonium sulfate		boric acid
$2NH_4H_2BO_3^-$	+	H_2SO_4	→	$(NH_4)_2SO_4$	+	$2H_3BO_3$

(color change in reverse)

The addition of sulfuric acid exactly neutralizes the ammonium borate complex, and a reverse color change is produced.

The boric acid method has two advantages: only one standard solution is necessary for the determination and the solution has a long shelf life.

Indicator Solutions

Many different indicators have been used to provide a "sharp end point" color change. The analyst's use of specific types of indicators can be a personal choice. The combination of methyl red and methylene blue indicators is frequently used in many methods. The color change of the indicator must fall within the equivalent point of the reaction. For standard acid/base titrations, methyl orange is usually the preferred indicator. If color change end points are difficult to detect, reference solutions made from a blank with an indicator can be very helpful.

Calculations

The calculations for % nitrogen or % protein must take into account which type of receiving solution was used and any dilution factors used during the distillation process. The equations given here are in long form. They are often simplified in the published standard methods. In the equations below, "N" represents normality. "ml blank" refers to the milliliters of base needed to back titrate a reagent blank if standard acid is the receiving solution, or refers to milliliters of standard acid needed to titrate a reagent blank if boric acid is the receiving solution. When standard acid is used as the receiving solution, the equation is:

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

If the sample weight is in milligrams, the molecular weight of nitrogen should be changed to 1400.67.

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

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

If it is desired to determine % protein instead of % nitrogen, the calculated % N is multiplied by a factor, the magnitude of the factor depending on the sample matrix. Many protein factors have been developed for use with various types of samples. The list below represents just a few of the factors described in the standard methods of analysis published by the American Association of Cereal Chemists (AACC) and AOAC International.

PROTEIN FACTORS	PROTEIN SOURCES
6.38	milk and dairy
6.25	other grains
5.95	rice
5.70	wheat flour

Equipment and Apparatus

Very early Kjeldahl digestions and distillations were performed using stone fume hoods and gas mantles as a heat source. In the 1920's these were replaced by what is now known as classical macro-Kjeldahl digestion and distillation apparatus. Macro setups use Kjeldahl flasks from 500 to 800 ml volume and handle sample sizes from 0.5 to about 5.0 g.

A smaller version of this apparatus is referred to as micro-Kjeldahl equipment. The equipment consists of smaller benchtop heater units, and Kjeldahl flasks of 30 to 100 ml volume.

A third more recent variation in equipment makes use of ceramic or aluminum heating blocks designed to accept a number of straight digestion tubes at once. "Block digestors" are often used in conjunction with benchtop distillation units with steam generators to shorten the distillation time.

Each of these three types of apparatus are described in more detail below. In all cases, since the Kjeldahl process involves significant corrosive fumes, appropriate attention must be given to fume removal. Equipment must be suitably constructed of corrosion-resistant materials.

Classical Macro-Kjeldahl Apparatus

Many governmental and regulating agencies have developed methodologies that specify the classical macro-Kjeldahl apparatus. For example, standard methods for low level nitrogen determinations in water (0-10 mg/1) require a sample size of 250 to 500 ml, and therefore large Kjeldahl flasks. Methodologies in the agricultural industry often require larger sample sizes and involve matrices which can significantly foam.

Equipment engineered to accommodate macro methods can be substantial. Apparatus designed to process 2 to 12 samples simultaneously is typical, and involves a bank of heaters and a fume manifold for digestion, and a second bank of heaters with condensers for distillation. The apparatus is available freestanding or contained within an integral hood. Freestanding installations require additional laboratory room modifications to handle fumes and heat. In either case the equipment is large, expensive, involves significant installation considerations, and ongoing utility and reagent costs.

The digestion apparatus has separate heating mantles to retain each flask. Typically each mantle has an individual temperature controller. The long necks of the Kjeldahl flasks are positioned to release digestion fumes into a common manifold. Manifold aspiration can be provided either by a mechanical blower with exhaust through ductwork to the outside, or by a large water aspirator that provides a water spray to dilute the fumes for disposal down a drain.

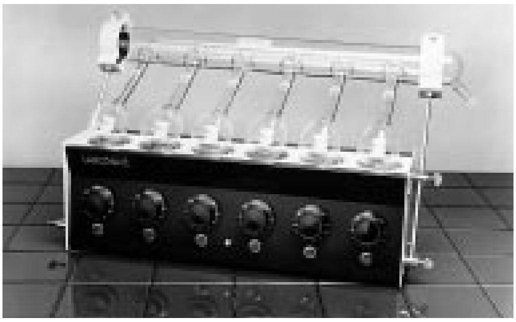
Like the digestion apparatus, the distillation apparatus has separate heating mantles to retain each flask. With the flask in place on the mantle, the neck of each flask is attached to a "connecting bulb" or an expansion chamber that acts as a trap to keep any of the concentrated liquid digest from mechanically carrying over through the condensers and into the receiving solution.

Macro-Kjeldahl Combination Digestion/Distillation Apparatus.

Downstream from the connecting bulb is the condenser, which is a tube of stainless steel surrounded by a second water jacket tube. Typically multiple condenser/jacket units are ganged together in a single assembly. A glass delivery tube is attached to the end of the condenser. The delivery tube has a ball-shaped tip with small holes to help disperse large bubbles and eliminate pressure fluctuations during distillation. The tip is submerged in the flask of receiving solution to ensure that the distilling ammonia is completely captured.

Micro-Kjeldahl Apparatus

These are miniature versions of the macro-digestion apparatus which are movable and intended for use in a laboratory hood. They are designed to digest small samples in 30 or 100 ml digestion flasks. Individually controlled heaters allow multiple flasks to be handled simultaneously. Some micro-digestors include a one-piece glass manifold for fume removal. The glass manifold is connected to a standard water aspirator to dilute and remove fumes that do not reflux in the flask necks. Whether or not a glass manifold is used, the digestion apparatus should be operated inside a laboratory hood.



The Micro Digestor uses a glass manifold to remove fumes.

Digested samples can be diluted and an aliquot taken for distillation on a micro steam distillation unit. Micro steam distillation units are available to accept digested samples of up to 4 ml concentrated acid and volumes of about 55 ml. An electric immersion heater produces steam heat that causes the sample to boil and release ammonia gas. The vapors pass into a condenser where contact with water-cooled glass condenses the vapors, which drip into the receiving solution via a delivery tube. Distillation time is approximately 5 minutes.

The RapidStill I is a microdistillation unit.

Methodologies have been specifically developed for micro-Kjeldahl apparatus. These most often are methods that involve homogeneous samples, relatively high nitrogen levels in the sample, and small sample sizes normally less than 0.25 g.

The installation considerations, initial expense, space requirements, and ongoing utility and reagent cost are much less with micro equipment.

Block Digestors

Block digestors are high temperature ceramic or aluminum blocks with wells to accept straight walled digestion tubes. They can accommodate from 2 to 25 tubes at once. Block digestors are heavy but movable self-contained units designed to be used in a laboratory hood. Digestion tubes range up to 300 ml capacity, suitable for sample sizes up to about 2 g or 75 ml.

Since narrow, straight-walled digestion tubes are used instead of traditional Kjeldahl flasks with a bulb and neck, and since a number of tubes are grouped closely together in a vertical configuration in the block, acid reflux parameters are different. This means salt/acid ratios and heat inputs appropriate for macro-Kjeldahl methods do not exactly transfer to digestion in a block. Methods have been developed specifically for block digestors similar, but not exactly identical, to macro-Kjeldahl methods.

Rapid Digestor-4 with fume removal system

Typically block digestors have one controller that adjusts the temperature of the entire block. The controller can be automated, allowing for timed ramping and multiple temperature settings during the course of a digestion.

Most often block digestors are used together with fume removal devices, even though they are operated in a hood. One such system consists of a glass manifold with individual bulb eductors in a rack designed to rest on top of a set of digestion tubes in the block. Another system uses individual exhaust caps with flexible tubing. In either case the manifold is connected to a water aspirator. Or, instead of a water aspirator, there are alternative units that employ mechanical aspiration and include bubbling the acid fumes through a neutralizing base solution.

Block digestion followed by steam distillation is sometimes referred to as “rapid Kjeldahl” partly because a number of block digestion methodologies are shorter than classical macro-Kjeldahl digestions and benchtop steam distillation units in use are faster than classical distillations.

Rapid Steam Distillation Apparatus

The development of block digestors as an alternative to macro-Kjeldahl digestion has been paralleled by the development of benchtop steam distillation units. Basically, this equipment consists of a steam generator to inject steam into the alkaline digestion mixture and an evaporator-type condenser, where contact with water-cooled glass coils causes the vapors to condense and drip into the receiving solution via a delivery tube. Usually the unit is designed to accept straight digestion tubes from block digestors, though there is no reason in theory why any digestion techniques could not be combined with any distillation technique. Steam distillation is much more rapid than classical macro-distillation, typically taking from 3 to 8 minutes. On the other hand, only one distillation at a time can be done.

Rapid steam distillation units are available with different features, ranging from relatively manual models to highly automated models. Basic models dispense dilution water and base under control of a push button. Some models include a timer to control the length of distillation, shutting down automatically. Some units automate the entire distillation process once the digestion flask is in place, and others automatically titrate to end point after distillation, and calculate and display the results in a printed report.

Titrators

Of course, receiving solutions may be individually hand titrated using an indicator solution and buret, but several models of benchtop automatic titrator instruments are also available. Some units will titrate one receiving solution at a time to a set end point. Others will automatically titrate a number of receiving flasks sequentially. Still other models provide a printout of results along with digital readout.

The RapidStill I is a steam distillation apparatus.

Other Equipment and Procedures

Following Kjeldahl digestion, there are other procedures available to determine nitrogen (or protein) in the digestion mixture. The most popular involves the spectrophotometric method for ammonium determinations of digestion mixtures. The Nessler and Phenate color development methods are the most widely used. The Biuret method, a dye binding procedure, is another type. Automated analyzers which use color development chemistry are available and can perform large numbers of assays. However, the apparatus and maintenance costs are high.

Manual Nessler colorimetric methods are often used in ammonia determinations. Digested samples are prepared for a color development and their absorbency is read with a spectrophotometer at a range of 400 to 500 nm. Standards are also run and a standard curve is prepared which plots absorbency versus milligrams of ammonia. The standard curve is then compared to the sample results.

The conditions required for optimal color development and stability can be rigorous for all of these procedures. However, spectrophotometric analysis has the ability to determine very small quantities of ammonia — as low as 20 micro-grams per liter under optimal conditions.

Ammonia specific ion electrodes give the analyst another means for the determination of ammonia. The electrode uses a hydrophobic gas permeable membrane to separate the sample solution from an internal ammonium chloride solution. Dissolved ammonia diffuses through the membrane and changes the internal pH, which is sensed by a pH meter that has an expanded millivolt scale. Ammonia concentrations of standards versus potential in millivolts are plotted on a semi-logarithmic graph, and sample concentrations are read from the standard curve. The ammonia specific ion electrode can be precise and has few interferences.

In addition, there is alternative nitrogen determination equipment which involves techniques other than Kjeldahl digestion. There are instruments which incorporate techniques of Dumas combustion, neutron activation, near infrared reflectance spectrometry or direct distillation. The first two instrument/techniques determine total nitrogen regardless of its form within the sample. Their acceptance in most laboratories has been limited due to the necessary changes involved in reporting these types of nitrogen values. These changes would involve broad multi-industrial re-evaluations of comparative nitrogen-protein analysis.

Near infrared reflectance spectrometry and direct distillation use methods based on approximate relationships between nitrogen content and protein. Both methods depend on calibrations with standards which have been determined by traditional Kjeldahl analysis.

Most Common Problems

Like any other method, the Kjeldahl procedure is subject to problems with methodology, technique and equipment. Understanding the principles behind the Kjeldahl process and using appropriate quality control procedures should prevent most problems from occurring. When developing a new or modifying an existing method, one should proceed with caution and experimental verification.

Should nitrogen results differ from those expected, knowledge of common problems makes finding and correcting the error easier. Common problems include the following: sample size and type incompatibility with amounts and types of acid, salt or catalyst used; contaminated samples, standards, reagents or equipment; inadequate or excessive digestion time; inadequate or excessive distillation dilution volume or aliquot volume; foaming or bumping during digestion; uneven digestion; inadequate or excessive sample temperature; precipitation of salt or caking or digested sample; leaks in apparatus connections; inadequate sodium hydroxide addition or sodium hydroxide carryover; and calculation errors.

Glossary

Acid Normality The measurement of an acid strength, ie: the lower the normality the weaker the acid.

Aliquot A part which is a definite fraction of a whole, ie: as taking a portion of a sample.

Alundum (Al_2O_3) A granular material that may be added to the digestion mixture to decrease bumping during the initial digestion phase.

Back Titration Method of titration to determine the amount of excess acid that was consumed in the receiving solution during Kjeldahl distillation.

Base Normality The measurement of base strength, i.e.: the lower the normality, the weaker the base.

Biuret Method A dye-binding procedure using an analytical reagent especially designed for proteins.

Blank The amount of nitrogen contained in the reagents used. In Kjeldahl calculations, the blank is subtracted so that it does not falsely increase the % nitrogen or % protein of the sample.

Catalyst Any substance of which a fractional percentage notably affects the rate of a chemical reaction without itself being consumed or undergoing a chemical change. Most catalysts accelerate reactions, but a few retard them.

Classical Macro-Kjeldahl A method designed to digest or distill multiple samples that contain high levels of nitrogen.

Connecting Bulb A glass component of a classical Kjeldahl distillation system. Separates liquid from vapors prior to the vapors entering the condenser and effectively traps caustic mist that may carry over to the receiving solution.

Delivery Tube A glass component of a Kjeldahl system that delivers distillate to a receiving flask. The long neck, round bottom and thick walls help eliminate pressure fluctuations in the distillation phase.

Digestion In Kjeldahl methods, the destruction of organic matter with an acid. Also used as a noun meaning digestion mixture.

Direct Distillation A method based on approximate relationships between nitrogen content and protein without performing a digestion step.

Direct Titration Method of titration to determine the amount of ammonia that has been absorbed into the receiving solution during Kjeldahl distillation.

Distillation A separation process in which a liquid is converted to vapor and the vapor then condensed to a liquid.

Dumas Combustion A combustion method measuring nitrogen gas involving in the total destruction of the sample.

End Point The point during a titration at which a marked color change is observed.

Homogeneous Adjective describing a mixture comprised of two or more compounds or elements uniformly dispersed in each other.

Hydrolysis A chemical reaction in which water reacts with another substance to form two or more new substances.

Hydrophobic Incapable of dissolving in water.

Indicator Solution A solution added to a sample during titration that produces a color change indicating end point.

Kjeldahl Method An analytical method for determining nitrogen content in certain organic and inorganic compounds. The method uses digestion, distillation and titration to arrive at the nitrogen content of the sample.

Macro-Kjeldahl A Kjeldahl method designed to handle high levels of nitrogen; classical Kjeldahl.

Micro-Kjeldahl A Kjeldahl method that requires smaller volumes of chemical reagents and smaller sample sizes than macro or classic Kjeldahl.

Molecular Weight The sum of the atomic weights of the atoms in a molecule.

Near Infrared Reflectance Spectrometry Protein identification method involving the measurement of the radiant energy reflected by the sample in infrared wavelengths.

Nessler Method Manual colorimetric method where digested samples are prepared for color development and their absorbency read with a spectrophotometer.

Neutron Activation A sensitive technique for identifying and measuring very small amounts of various elements. A sample is exposed to neutron bombardment in a nuclear reactor for the purpose of producing radioisotopes from the stable elements.

Nitrate $[NO_3^-]$ Form of nitrogen usually found in fertilizers. It must be reduced before digestion in the Kjeldahl method.

Nitrite $[NO_2^-]$ Form of nitrogen usually found in fertilizers. It must be reduced before digestion in the Kjeldahl method.

pH A measurement that represents the acidity or alkalinity of an aqueous solution.

Phenate Method Manual colorimetric method where digested samples are prepared for color development and their absorbency read with a spectrophotometer.

Precipitate Small particles that have settled out of a liquid or gaseous suspension by gravity, or that result from a chemical reaction.

Rapid Kjeldahl Same method as classical Kjeldahl, except, through efficient heating and compactness, requires less time to complete the digestion step. Digestion times range from 6-60 minutes compared to up to two hours for classical digestion.

Receiving Solution Solution used to capture ammonia separated through distillation.

Reflux The liquid condensed from the rising vapor.

Spectrophotometry Identification method involving an optical device that measures radiant energy within selected wavelengths in the visual, ultraviolet or infrared regions of the spectrum.

Steam Distillation Distillation using steam to drive off NH_3 gas in Kjeldahl determinations.

Titration A quantitative analytical process that is basically volumetric. Generally, a standard solution, that is, one containing a known concentration of substance X (titrant), is progressively added to a measured volume of a solution of a substance Y (titrand) that reacts with the titrant. The addition is continued until the end point is reached.

Water Aspirator A device that provides suction through the use of flowing water past a small orifice.

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