

Optimized Kjeldahl Titration

The impact of different titration technologies in Kjeldahl analysis



Summary

Several factors will affect the performance of a Kjeldahl Analyzer, i.e. an instrument that automatically distill and titrate a sample to determine Protein. The OPSIS LiquidLINE KjelROC Analyzer is designed to achieve the highest performance for this analysis with a colorimetric titration, a unique titration algorithm (predictive titration) and several design choices to minimize carry-over effects and creation of air bubbles.

This document will describe these options and how they impact the final result of the analysis.

Note that Kjeldahl digestion is not explained in this document. More information about digestion can be found in the OPSIS LiquidLINE Application Guide for Kjeldahl^[1].

The Kjeldahl Method

The Kjeldahl method is the reference standard to determine Protein in different samples, often referred as TKN analysis (Total Kjeldahl Nitrogen). The Kjeldahl method specifies that you should digest, distill and titrate your samples.

Initially, a century ago, titration was done manually using a burette. It was time consuming, the risk for errors were high and it required good analytical skills. In the 1980's several instruments were therefore developed to simplify and automate the titration. A Kjeldahl Analyzer can now perform the distillation and titration automatically.

Kjeldahl Analyzer Function

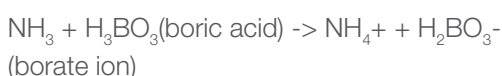
The Kjeldahl method describes that water and alkali shall be dispensed into a sample tube and thereafter distillation should start. Normally distillation is achieved with steam from a steam generator. The liberated ammonia is collected in a receiver solution (often 1% boric acid) and thereafter titrated. Based on the end-point a result can be calculated by the instrument.

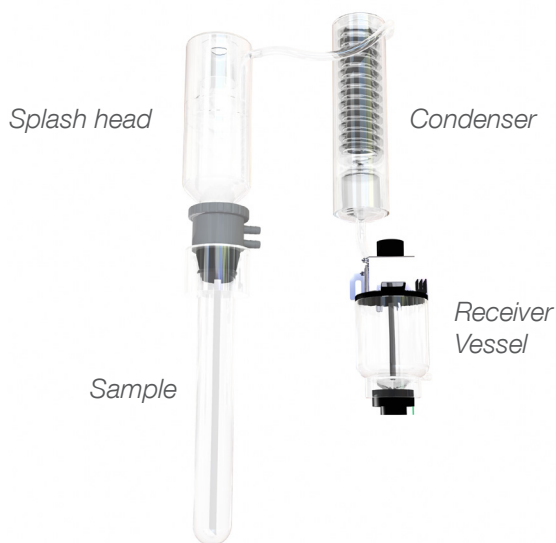
The chemical process for Kjeldahl can be described in the following chemical reactions:

The solution in a sample tube is made alkaline by addition of sodium hydroxide, which converts the ammonium sulfate into ammonia gas:



The ammonia gas that is formed is liberated from the solution and moves out of the digestion tube and into the receiving vessel - which contains an excess of boric acid (receiver solution). The low pH of the solution in the receiving flask converts the ammonia gas into the ammonium ion, and simultaneously converts the boric acid to the borate ion:





Kjeldahl Distillation and Titration; Distill the sample with steam from a steam generator, cool the ammonia gas and collect in a receiver vessel.

The nitrogen content is then estimated by titration of the ammonium borate formed with standard sulfuric or hydrochloric acid, using a suitable indicator to determine the end-point of the reaction.



This step-by-step process is identical for all instruments that perform a Kjeldahl analysis.

The precision of the result will depend on the analytical chemist when handling and moving samples, weighing procedure, preparation of titrant, analytical variations and finally variations introduced by the instrument.

Titration Technologies

An instrument capable of automatic titration typically uses a pH electrode for direct measurement of the pH level or a color sensor to detect the change in an indicator solution. Both methods have certain advantages and disadvantages.

Titration with pH electrode

A pH electrode can accurately detect a pH level in a solution as long as it is correctly calibrated.

The technique is based on differences in electric current and therefore requires regular calibration. Without repeated calibration against known solutions it is not possible to know that the measurement is correct.

A pH electrode might also be slow to recognize changes in the pH level. It is therefore more suitable to complete the Kjeldahl distillation before starting to measure the pH level with an electrode, i.e. separate distillation and titration. A pH electrode has a limited lifespan, typically a couple of years.

Colorimetric Titration

Colorimetric titration can also accurately detect a specific pH level in a solution.

The colorimetric titration is based on color change in an indicator solution and is therefore dependent on choice of indicator. Bromocresolgreen and Methylred are often used as the color change matches the theoretical pH for NH_3 titration. Colorimetric titration does not require any calibration, since the indicator will always change color at the same pH value.

Continuous titration works well with Colorimetric titration.

Summary

Both pH electrode as well as Colorimetric titration achieve similar acceptable results.

OPSIS LiquidLINE KjelROC Analyzer uses Colorimetric titration.

Distillation and Titration speed

Separate Distillation and Titration

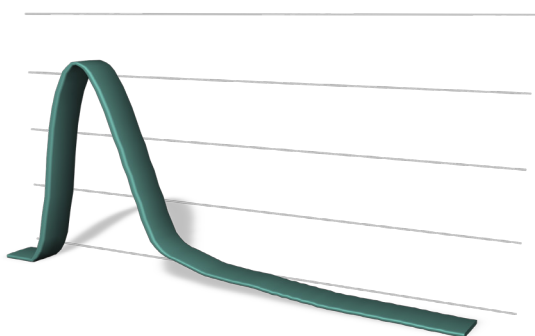
Many laboratories still use two separate instruments, distillation unit and a titrator for their Kjeldahl Analysis. This means an increased risk for handling errors and larger variations in results. It is also relatively slow since it requires a completed distillation before the titration can start.

A Kjeldahl Analyzer that separates distillation and titration will remove the handling errors but will still be a slow instrument.

Continuous Titration

Continuous titration is significantly faster and, if correctly designed, will achieve similar results as when separating distillation and titration.

The major challenge is achieving rapid response when distillate arrives and still avoid over-titration when there is less ammonia at the end. A common method is to measure if the end-point has been reached, if not then add more titrant until a change can be detected. However, today's digital sensors allows development of even more accurate titration algorithms.



Typical nitrogen/ammonia distillation; no ammonia gas during the first half minute, rapid increase of ammonia and thereafter a slow decrease.

Summary

Continuous titration is faster and can achieve as good results as separate distillation and titration.

OPSIS LiquidLINE KjelROC Analyzer uses an advanced color sensor that is equipped with a new, specifically developed algorithm to optimize the continuous titration. The OPSIS LiquidLINE Predictive titration* algorithm allows the Kjeldahl Analyzer to accurately compare the actual color with the end-point at any time. Adding of titrant is therefore very precise while significantly faster than if the distillation and titration were separate.

Titration Resolution

Dosing Device

It is important with a high resolution of the dosing device, in addition of good control of the titrant molarity. A burette is typically used as dosing device for a Kjeldahl Analyzer. The resolution of each dosing step will affect the precision of the final result.

The OPSIS LiquidLINE KjelROC Analyzer uses a small dosing step, 1.95 µl/step, which gives high precision.

Instrument Introduced Errors

There are also other errors that can affect the titration and the result.

Air bubbles

A common issue with Kjeldahl instruments is air bubbles created inside tubes. Air bubbles appear when liquid is not moved for a period, typically when the instrument is not used over the night. If air bubbles are dosed from the burette it will result in incorrect results. The OPSIS LiquidLINE KjelROC Analyzer is designed to minimize the creation of air bubbles, by optimizing the location of

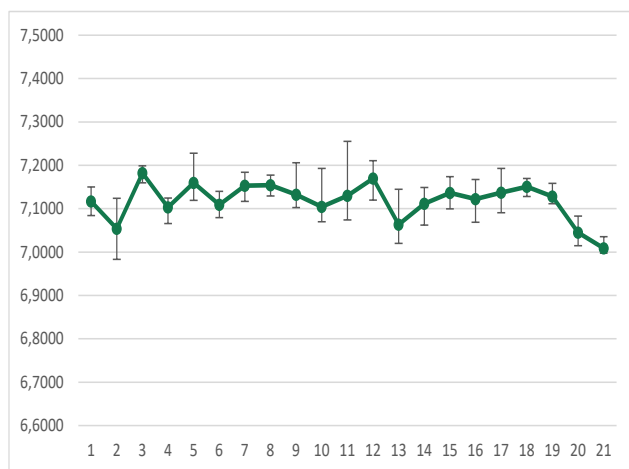
* patent pending

components such as the titration tank and burette. PTFE (Teflon) tubes are also used to further minimize the risk of dosing air bubbles.

Carry-over effects

Carry-over effects can be a problem, i.e. that previous analysis affects later results. The most important when avoiding this is to have a well designed splash head, a component that ensures that only gas and no sample or alkali liquid is forwarded to the condenser and receiver flask. The splash head need to be completely cleaned by the instrument between analyses.

The OPSIS LiquidLINE KjelROC Analyzer is delivered with a glass splash head which keeps carry-over effects to a minimum.



A summary of recovery tests, using $(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$ on 20 different KjelROC Analyzers. Average and max/min values ($n=10$). The relative standard deviation were, on average, 0.36% for these instruments^[3].

Result

Reference samples should be analyzed to verify the performance of a Kjeldahl Analyzer. Most common is to analyze a substance with a known amount of Nitrogen, such as Ammonium Iron Sulfate ($(\text{NH}_4)_2\text{Fe}(\text{SO}_4)_2$).

The analysis should give a complete recovery, no carry-over effects and a low standard deviation (rsd). The OPSIS LiquidLINE KjelROC Analyzer has a relative standard deviation (rsd) of less than 0.75%^{[2][3]}. A specific program testing the recovery, called Performance Tracking, is available in the software and is used in the production control of all instruments.

Good design leads to an instrument with very low detection point and a low quantification limit for samples. This is due to low variation of the blank, and therefore limited noise when analyzing low levels of Nitrogen. The Lowest Quantification Limit (LOQ) of the OPSIS LiquidLINE KjelROC Analyzer can be as low as 0.013 mgN^{[4][5]}.

OP SIS LiquidLINE KjelROC Analyzer:

- Used Technology: Colorimetric Titration
- Titration Algorithm: Predictive Titration*
- Design: PTFE tubes, Glass splash head, High precision burette at 1.95 $\mu\text{l}/\text{step}$
- Detection Range: 0.1-225 mgN
- Precision: < 0.75% (rsd)
- Lowest Detection Limit (LOD): 0.004 mgN
- Lowest Quantification Limit (LOQ): 0.013 mgN

References:

- [1] LA1000 Kjeldahl Application Guide
- [2] LA1001 The Importance of Catalyst in Kjeldahl
- [3] LTE1032 Verification of KjelROC Analyzer rsd
- [4] LTE1036 Report on determination of LOD/LOQ on KjelROC
- [5] DIN 32645 Chemical Analysis - Decision limit, detection limit and determination limit

* patent pending

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