Application Guide Kjeldahl Determination

Using KjelROC Digestion Systems and the KjelROC Analyzer

As the Kjeldahl method involves use of highly corrosive and hazardous chemicals at high temperature it is strongly recommended to use protective glasses and gloves. The safety section in the KjelROC Operation Manual should be read before starting any analysis work.

Introduction

Since the seventies Block Digestion followed by Steam Distillation in the same test tube has more or less become a standard in the Kjeldahl analyses. Many different application hints are available and often a combination can be the best. Dependent on sample type and reason for doing the analysis must decide which application to follow. This Guide is describing the method in a general way and will help to use the KjelROC Digestion systems in combination with the KjelROC Analyzer. To benefit from all features it is recommended to read the different KjelROC User Manuals. Here details e.g. wireless and the possibilities to integrate with LIMS are described. Also more sample specific applications are available as “KjelROC Quick Guides”.

A ground and well homogenised sample is weighed and put into the Test Tube. Catalyst Tablet containing salt and a catalyst as well as concentrated sulphuric acid is added making sure the acid is wetting the same before putting the tube into the Digestor. Amount of sample and the needed time for digestion can vary a lot dependent on sample type. However, all protein in the sample is during the digestion converted to ammonium sulphate.

After cooling the Test Tube with the digested sample is put into the KjelROC Analyzer. Dilution water and alkali is dispensed into the tube and thereafter steam distillation starts. The liberated ammonia is collected in a receiver solution (often 1% boric acid) and titrated with an acid during the distillation. The added titrant volume in combination with the concentration and earlier recorded sample weight allows the Analyzer to calculate the result and present it on the touch screen. After completed analysis the test tube is automatically drained and the KjelROC Analyzer is ready for next sample.

Reagents

For digestion

- Kjeldahl Tablets, different types are available see LA 1001"The importance of the catalyst"
- Sulphuric acid, concentrated p.a. quality
- Alkali, ≈20% to be used in the Scrubber bottle no.2

For proper work liquids should be added to the Scrubber Flasks, 800 ml of tap water in the left flask (no 1) and a mixture of 400 ml water plus 400 ml alkali (30-40%) in the second. As long as the liquid in flask no 2 is alkaline the air coming out from the Scrubber will be odourless. The pH of the liquid can easily be checked by just adding a small amount of the mixed indicator used for the receiver solution. The number of batches that can be analysed before changing the liquids depends on the suction effect applied as well as the volume added acid. During "normal" conditions the contents in the flasks should be replaced after digestion of 60-80 tubes.

The Scrubber motor is not affected by eventual acidic gases. However, the stink from evaporated gases if the outlet tube from the Scrubber is not placed inside a fume cupboard is neither pleasant nor healthy.

For distillation

- Distilled or deionised Water for dilution and steam production. If the tap water is free from Nitrogen it can be used. However, cleaning of the Steam Generator has to be done on a frequent basis if the water contains calcium or other minerals building up deposits.
- Alkali, 30 – 40% Sodium Hydroxide
- Titrant acid, HCl (or H_2SO_4) with a well-defined concentration e.g. 0.1000 or 0.2000 Mol/L The concentration used in the KjelROC Analyzer is the Normality which if HCl is used is the same as the Mol/L but two times this value if H_2SO_4 is used.

Often the titrant can be prepared from ampoules. If the instructions for dilution are followed the accuracy normally is enough for routine work.

Sometimes when a large volume at low price is desired the titrant is prepared out from concentrated, constant boiling, hydrochloric acid. This method requires careful standardisation of the acid, often by using sodium carbonate. The procedure can be found in chemical hand books or on the Webb.

Ready-made titrant with certificated concentration is often a good choice for accurate and convenient work.

- Receiver solution, 1% boric acid with mixed indicators.
- Ready-made receiver solutions are sometimes available but often it is more economical to prepare it in the laboratory, if so please follow the below instruction.

Preparation of Receiver Solution with mixed Indicators

10L is prepared as follows;

1. Dissolve 100 g boric acid in approximately 1 L boiling water. Add about 7 L more water and let cool.
2. Solve 100 mg bromocresol green and 70 mg methyl red in 170 ml methanol (or ethanol)
3. Pour the indicator solution into the boric acid solution and make up to 10 L with water.
4. Take 25 ml of the receiver solution in a beaker or E-flask and add 100 ml water. If the colour turns slightly green or just neutral grey the solution is OK. If still red add weak alkali (≈0.1M) until it turns grey. Calculate the volume needed for all 10 L. After eventual alkali addition the colour should be checked again. The colour must never stay red after addition of water as it is impossible to know how far from the equilibrium it is and this will influence the result.A blank value between 0.02 to 0.10 ml is optimal.

Dependent of workload it might be better to prepare a larger volume as the time to check and the eventual adjustment of colour with alkali will be the same.

The shelf time for the prepared receiver solution depends on the temperature in the laboratory. If repeated blank distillations start to vary it is often a signal to discharge the old liquid, clean the storage tank and prepare new.

Sample preparation and weighing

Sampling and preparation of the sample prior to analysis is important for the result. Dry samples should be ground, the finer the better. Semi solid samples, like meat, can be minced using a kitchen mixer or a proper homogenizer.

Weigh the sample using an analytical balance, if liquid samples use a pipette or measuring cylinder. Note the sample weight/volume in a form or directly key it into a computer for later transfer to the KjelROC Analyzer. During weighing the result presentation is selected. To make it simple it is recommended to only have the actual activated. If LIMS is used the easiest might be to just select the titrant volume in ml as result, the calculations probably already are installed in the LIMS.

A high sample weight is often better as it is giving a more representative result. However, the reagents used must match the nitrogen level. If the titrant concentration is 0.2000 Mol/L it covers the nitrogen range 10 – 200 mg with a good precision. This is routine levels in e.g. a laboratory analysing raw materials as grain, soya and fish meal. For samples with lower nitrogen levels a weaker titrant is recommended. Please see the table at the end of this Application Guide.

The KjelROC burette volume is 50ml and if necessary it makes refill during the analysis.

As high fat content samples consume more sulphuric acid during the digestion, either a lower sample weight or a higher dosing of the acid is needed.

One or several blanks, all chemicals used for digestion but no sample, often is put on a random place in the Rack. Dependent on GLP used this is done for every Rack, once a day or only when a new batch chemicals is begun.

Digestion

Put the weighed sample into the Test Tube. Add the required amount salt/catalyst mixture. For convenient and reproducible conditions often Kjeldahl Tablets are used. The salt, most common potassium sulphate K_2SO_4, is added to rise the boiling temperature and thereby shorten the digestion time. Different catalysts like cupper, selenium or titanium are used to speed up the reaction. The most efficient Mercury is nowadays banned in most countries due to environmental rules. In Opsis LA 1001, the efficiency of different Kjeldahl Tablets was compared. No significant difference could be seen and when environmental influence is of importance the Missouri tablets with 0.3% cupper (order no.KT-211A) are recommended.

Add the sulphuric acid H_2SO_4 and make sure the sample is wet by the acid by gently swirling the tube before putting it back into the Tube Rack. For dry samples like grain, feed mixes and raw materials as fish meal 10 to 12 ml acid is often enough. If the sample has a high fat content more acid is necessary as fat will consume some during the digestion. If the digest mixture directly after completed digestion, when still hot, is a liquid it indicates that the added acid has been enough. However, if the digest when raised from the Digestor looks like a solid cake, more acid is needed for this type of sample. Another reason can be that too much suction has been used. The Scrubber / water aspirator should always be adjusted to a minimum after the initial ten minutes.

Some recommend the use of hydrogen peroxide, 30% H_2O_2, to speed up the reaction and also reduce the foaming some sample types might create. However, the time saved is often not more than what the extra addition takes. Also the handling of this chemical can be troublesome as the reaction with many samples is quite violent. Foaming problems can often be overcome by using slightly more acid or start at a lower temperature.

Dependent on the Digestor alternative available (KjelROC Digestor Advanced with Motor Lift or Auto with or without Manual Stand) the tubes are connected to the Exhaust and loaded into the Digestor and the operation started /stopped as described in the actual Instruction Manual.

Before starting a digestion the Scrubber or water aspirator always should be adjusted to full capacity. After the first ten minutes the exhaust capacity should be adjusted to a minimum but still high enough to prevent gas to escape. This is to minimise the acid consumption. If too much acid is evaporated the salt concentration will be too high and there is a great risk for Nitrogen losses. Only if the sample contains a lot of liquid the exhaust should be fully on for a longer time. Please refer to the Quick Guide for the specific sample.

Many samples can be digested starting directly with a pre-heated Digestor e.g. with a temperature of 420°C. Some needs more attention. All Opsis Digestors come with pre-set programmes for different sample types. Water is an example where the first step is at a lower temperature to avoid violent bumping during the reduction of the start volume, approximately 100 ml. Thereafter the temperature is raised to the final level for the true digestion. To collect evaporated water an optional flask, available from Opsis, should be connected between Exhaust and Scrubber.

The time needed to complete the digestion can vary a lot dependent on sample type, salt/acid ratio and the catalyst used. Some claim that the digest should be completely clear others that an internal standard, often an amino acid e.g. glycine or lysine, must reach the theoretical result. As many samples can be completed earlier than a pure amino acid routine work can be done more efficient by testing the Nitrogen / Protein level of the actual samples after different times e.g. 45, 60, 75 and 90 minutes. The time where all the samples of interest have reached a stable level is the optional time. To give a margin of extra ten minutes can be wise. Sometimes it is impossible to obtain a “clean digest”. E.g. for samples like soil or when tablets with titanium oxide are used as they contain insoluble matters.

On all Opsis LiquidLINE Digestors temperature and time can easily be set by the user to fit any individual need.

Distillation including simultaneous titration and calculation of the result

Note: After digestion the test tubes with hot digest has to cool for at least ten minutes to avoid violent reaction during water dilution and alkali addition.

The distillation step is compared with the digestion very simple as all samples can be handled the same. Also the distillation, including the titration and calculation, is easy to confirm by performing a recovery test using an ammonium salt as standard. Please refer to KjelROC Analyzer Operation Manual chapter 7.2.1 “Recovery test”.

When switching on the KjelROC Analyzer it automatically prepares for analysis. Dependent on settings the operator has to log-in before selecting the function. The pre-set programmes are displayed.

Note: If new titrant is used the concentration has to be entered as described in KjelROC Analyzer Operation Manual chapter 5-9-3 Set-Up Instrument settings

If any “Refill Alarms” are displayed, fill up the corresponding storage tank. If an alarm is activated during distillation normally the Rack can be completed before action is taken.

If the instrument has not been used for a while it is recommended to run a couple of blanks. The easiest way is to select “Auto blank”. Three, or as many as pre-set, blanks will be processed. It is quite normal that the first blank will differ but when the Analyzer is warm and all tubes are filled with fresh reagents the blank values will stabilise. The last blank value will be stored and used in the coming calculations. If preferred the average of all or selected blanks can be entered.

The real blank, all digestion chemicals but no sample, is normally analysed together with the other samples. If the chemicals used do not affect the blank many laboratories save labour by just using the “auto blank”, only dilution water and alkali in the test tube during distillation.

When good quality chemicals are used no difference between the two blank alternatives can be seen.

If not already transferred via the PC, to save time it is recommended to key in the sample weights as the “Auto blanks” are running. New weights can also be entered when previous sample(s) are distilled.

Select “Kjeldahl” and connect the first test tube to be distilled. Close the safety door and press start. (Optionally just close the door)

1. 30 ml receiver solution is added into the titration vessel
2. 70 ml dilution water is added into the test tube
3. 50 ml alkali is dispensed into the test tube
4. Steam distillation starts and continues until end volume and end-point colour is reached. Dependent on how much titrant was added, distillation will continue to compensate for this.
5. When the analysis is ready the test tube will automatically drain.
6. (Optionally no drain can be selected. This is recommended if the digested sample contains solids like boiling beads in water samples or sand from soil samples)
7. The automatic cycle stops and next tube can be processed.

During distillation the titrant volume as well as the result is displayed. During distillation it is possible to enter more weights.

All volumes above are the default factory-set volumes and should be set differently if other concentrations and conditions are selected. Here are some hints.

Receiver solution: The condenser outlet should be below the liquid surface. The receiver volume is controlled by the second level pin in the titration vessel. It is quite normal that the liquid is sucked into the lower part of the condenser during dilution/alkali addition due to the heat. However, the volume is easily held in the condenser, working as a trap for any ammonia liberated.

Dilution water: If a larger volume of digestion acid is used it might be necessary to increase the water volume. However, it might be better to investigate if the digestion procedure is optimised. Too much acid will longer the time and does cost money.

Alkali: The sulphuric acid remaining after digestion has to be neutralised and an access of alkali is needed to liberate the ammonia. Theoretically 1ml concentrated H_2SO_4 needs 3.64 ml of 40% (10M) NaOH to be neutralised. If the alkali concentration is lower e.g. 32% (8 M) instead 4.28 ml is required. Please consider that not all acid added at the digestion remains, some is consumed and some lost through evaporation/ exhaust.

Distillation volume: 100 ml gives very good results for all levels of nitrogen, allowing enough steam to rinse splash-head/condenser between samples. If samples with almost the same and relatively low nitrogen level e.g. grain samples, a lower distillation volume will save time and still the results will be satisfying.

The table below will give some indications on what titrant concentrations to use. As the burette dispenses 1.9 µl per step also relatively low titrant volumes, around 1ml, still give accurate results. Also the automatic refill function of the burette during analysis allows a wide nitrogen range
without changing titrant.

Titrant concentration N	Titrant Volume ml	 mg N	%N if 1g sample	%Protein (6.25) if 1g sample
0.2000	5 - 45	14 - 125	1.4 - 12	9 - 79
0.1000	5 - 45	7 - 63	0.7 - 6	4.5 - 40
0.0050	5 - 45	3.5 - 30	0.35 - 3	2 - 20
0.0010	5 - 45	0.7 - 6	0.07 - 0.6	0.4 - 4

Opsis AB, Box 244
SE-244 02 Furulund Sweden
Telephone +46 46 72 25 00
Telefax +46 46 72 25 01
E-mail info@opsis.se
www.liquidline.se

Made in Sweden

LA 1000
2015 02