

Experiment No. _____

Date _____

Kjeldahl Method for Determination of Nitrogen

INTRODUCTION

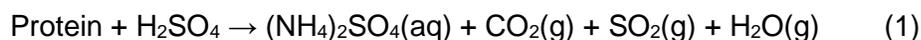
Nitrogen is one of the five major elements found in organic materials such as protein. The Kjeldahl method of nitrogen analysis is the worldwide standard for calculating the protein content in a wide variety of materials ranging from human and animal food, fertilizer, waste water and fossil fuels.

The Kjeldahl method consists of three steps:

- A. Digestion
- B. Distillation
- C. Titration

A. Digestion of the sample

Digestion is 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. The general equation for the digestion of an organic sample is shown below:



Sulfuric acid has been used alone for the digestion of organic samples. 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. 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.

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.

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.

B. Distillation

Distillation is 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. This is accomplished by;

- 1) raising the pH of the mixture using sodium hydroxide (NaOH solution). This has the effect of changing the ammonium (NH_4^+) ions (which are dissolved in the liquid) to ammonia (NH_3), which is a gas.



2) separating the nitrogen away from the digestion mixture by distilling the ammonia (converting it to a volatile gas, by raising the temperature to boiling point) and then trapping the distilled vapors in a special trapping solution of boric acid (H_3BO_3). The ammonia is bound to the boric acid in the form of ammonium borate complex.



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.

C. Titration:

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

REAGENTS AND APPARATUS

- Potassium sulfate, K_2SO_4 +Se tablets (2 for each digestion tube)
- Concentrated H_2SO_4 (in the hood)
- 35 % (w/v) NaOH for each digestion tube (already prepared).
- 4.0 % (w/v) Boric acid, H_3BO_3 (already prepared)
- 0.1 M HCl (already prepared, exact concentration will be given)
- Methyl orange (in droppers)
- Erlenmeyer flasks
- Burette
- Digestion tubes
- **Milk: Do not forget to bring any brand of milk.**

PROCEDURE

- 1) **Sample:** 5.0 mL fresh cow's milk in a digestion tube.
- 2) **Reagents for digestion:** to each milk sample and also to an empty digestion tube (blank) add the followings:
 - 2 tablets of K_2SO_4 + Se catalyst
 - 10.0 mL of concentrated H_2SO_4 (98%)
- 3) **Digestion:** heat for ca. 30 minutes at 420°C.
ATTENTION: Do not inhale the gases evolve in reaction 1.
- 4) **Cooling and diluting:** let the digestion tubes to cool to 50-60 °C and add to each add 50 mL of distilled water.
ATTENTION: Let the tubes stand in air to cool, cold water may break the tubes.
- 5) **Distillation:**
 - i. Place the digested samples in digestion tubes to the distilling unit and add 50.0 mL of 35% (w/v) NaOH.
 - ii. The sample is distilled until 100 mL of distillate are collected in 25.0 mL of 4.0 % (w/v) boric acid.
- 6) **Titration:** add 2-3 drops indicator to the Erlenmeyer flask and titrate it with 0.1 M HCl.
- 7) Calculate the amount of protein (% protein) and compare the result with the value give on the milk.

PRE-LAB STUDIES

Read the introduction from the manual.

- 1) List name of three steps in Kjeldahl Method.
- 2) What is the purpose of digestion?
- 3) Describe the digestion procedure.
- 4) Write three catalysts that can be used in Kjeldahl Method. Which one will be used in this experiment?
- 5) Write down the purpose of distillation and explain each step in the distillation by writing chemical reaction(s).

POST-LAB STUDIES

- 1) What is the aim of using K_2SO_4 in this experiment?
- 2) What is back titration? How is it used in Kjeldahl Method?
- 3) Which reagent will be used to change ammonium ions to ammonia? Write down the reaction?
- 4) Explain the role of H_3BO_3 in this experiment.

Name surname:

Section:

Date:

DATA SHEET FOR KJELDAHL METHOD

Volume of HCl for replicate 1:

Volume of HCl for replicate 2:

% protein in milk sample:

% protein written on the label:

Concentration of HCl, M:

TA's Name and Signature: