**NH3-N and TOTAL KJELDAHL NITROGEN (TKN) MEASUREMENTS**

**1. OBJECTIVE AND IMPORTANCE OF EXPERIMENT**

Nitrogen exists in four forms that are of interest in water resources. These are ammonia (NH4+), nitrite (NO2–), nitrate (NO3–), and organic nitrogen.

Chemists working with wastes and freshly polluted waters learned that most of the nitrogen is originally present in the form of organic (protein) nitrogen and ammonia. As time progresses, the organic nitrogen is gradually converted to ammonia nitrogen, and later on, if aerobic conditions are present, oxidation of ammonia to nitrite and nitrate occurs.



Waters that contained mostly organic and ammonia nitrogen were considered to have been recently polluted and therefore of great potential danger. That’s why NH3 – TKN experiments are most commonly preferred experiments to express pollution levels.

**1.1 NH4-N**

Nitrite nitrogen seldom appears in concentrations greater than 1 mg/L, even in waste-treatment-plant effluents. Its concentration in surface waters and groundwaters is normally much below 0.1 mg/L. For this reason sensitive methods are needed for its measurement. Even though ion chromatography provides this sensitivity, the colorimetric procedure is preferred because it is cheaper method.

NH4+ ↔ NH3 + H+

is considered to be ammonia nitrogen. Essentially four different methods for determining ammonia nitrogen are included in “Standard Methods”: two colorimetric procedures, a volumetric procedure, and an instrumental method using an ammonia-selective membrane probe.

When samples contain more than 2 mg/L of ammonia nitrogen, the concentration can be determined by titration with a standard solution of sulfuric acid after distillation and absorption of ammonia in boric acid.

Ammonium ion exists in equilibrium with ammonia and hydrogen ion. At pH levels above 8, the equilibrium is displaced far enough to the right so that ammonia is liberated as a gas along with the steam produced when a sample is boiled, as follows:

 NH4+ → NH3 ↑ + H+

The distillate is collected by passage of the vapor through a condenser and then into an acid solution contained in a flask.

The chemistry involved is as follows:

* First, boric acid is an excellent buffer. It combines with ammonia in the distillate to form ammonium and borate ions, as shown in the equation:
1. NH3 + H3BO3 → NH4+ + H2BO3–
* The ammonia may then be measured by back titration with a strong acid such as sulfuric acid. When the pH of the boric acid solution has been decreased to its original value, an amount of strong acid equivalent to the ammonia has been added.

(2) H2BO3– + H+ → H3BO3

**Note:** Boric acid is normally colorless. Mixed indicator solution gives a purple color. When this solution meets with NH3 (ammonium) during distillation in 1st equation, the color turns green. When we titrate it by acid such H2SO4, the color get backs its original color, which is purple.

**1.1.1. EXPERIMENTAL PROCEDURE**

***1.1.1.1. Materials and Equipment***

* Erlenmeyer Flask
* Glass Test Tubes (For the VELP Distillation apparatus)
* Pipette
* Graduated Cylinder
* Stirring rod (magnet)
* Stirrer
* Distillation apparatus by VELP
* Distilled water
* NaOH solution (35%)
* H2SO4 (0,02 N) titrant.
* Boric Acid Solution (with Mixed Indicator Solution, purple color)

***1.1.1.2. Steps of the Experiment***

* Select the sample volume as described in Standard Methods;

|  |  |
| --- | --- |
| Ammonia Nitrogen in Sample (mg/L) | Sample Volume (mL) |
| 5 – 10 | 250 |
| 10 – 20 | 100 |
| 20 – 50 | 50 |
| 50 – 100 | 25 |

 \* If NH3 is more than 100 mg/L, either you dilute it or take lesser

 amount than 25 mL. But do not have lesser than 5 mL sample.

* Turn on the VELP distillation apparatus and cooling water.
* Add 25 mL boric acid to an Erlenmeyer flask.
* Add selected volume of sample into the glass test tubes.
* Place one hose in 35% NaOH solution and the other hose in distilled water.
* Have 3 minutes distillation (Color of boric acid turns purple to green in the presence of NH3-N).
* Take the Erlenmeyer flask, put a magnet in it and place it on a stirrer.
* Titrate by 0,02N H2SO4 until the color reach purple, the original color of boric acid at the beginning.

***1.1.1.3. Calculations***

mg NH3 – N / L = [A x 280] / mL sample

A = volume of H2SO4 titrated for sample, mL

* 1. **Total Kjeldahl Nitrogen (TKN):**

The Danish chemist Johan Kjeldahl, 1849 *–* 1900, developed what today is well known as the Kjeldahl method for analyzing nitrogen in organic substances. The aim of the digestion procedure is to break all nitrogen bonds in the sample and convert all of the nitrogen into ammonium ions. So TKN is equal the sum of organic nitrogen and ammonium.

The original method as it was presented by Kjeldahl has been continuously improved. These developments have improved environmental and personal safety aspects, increased the speed and versatility of the method, and simplified the entire analytical procedure.

The Kjeldahl method has three different steps, digestion, distillation and titration. Due to its many areas of application, it is not practical to look at a single Kjeldahl digestion as a procedure to handle every sample type.

Sample weight for solids;

Using a titrant concentration of 0.1 – 0.2 N acid the rough guide below can be used for selecting the sample size. The analytical sample should ideally contain 30 *–* 140 mg N.

Protein content mg sample

< 5 % 1000 – 5000

5 – 30 % 500 – 1500

> 30 % 200 – 1000

Sample volume for liquids;

When analyzing Total Kjeldahl Nitrogen (TKN) in water/wastewater where nitrogen content can be quite low, larger sample amounts need to be used.

Nitrogen conc. mg N/L mL Volume of sample

< 20 100

20 – 50 50

50 – 100 25

To obtain low detection limits a titrant concentration of 0.01 N should be used. Analyzing the digest using a flow analysis technique smaller sample amounts can be used.

Most of the organic nitrogen that occurs in domestic wastes is in the form of proteins or their degradation products: polypeptides and amino acids. The Kjeldahl method employing sulfuric acid as the oxidizing agent is standard procedure. A concentrated salt-copper mixture is used to hasten the oxidation of some of the more resistant organic materials. An example for organic nitrogen:

CH3CHNH2COOH + 7 H2SO4 + Heat + Catalyses 🡪 3 CO2 + 6 SO2 + 8 H2O + NH4HSO4

(Alanine)

The oxidation proceeds rapidly at temperatures slightly above the boiling point of sulfuric acid (340oC). The boiling point of acid is increased to about 360 to 370oC to enhance the rate of oxidation by addition of sodium or potassium sulfate. The complete digestion of organic matter is essential if all organic nitrogen is to be released as ammonia.

**1.2.1. EXPERIMENTAL PROCEDURE**

***1.2.1.1. Materials and Equipment***

* Erlenmeyer Flask
* Glass Test Tubes (For the VELP Distillation apparatus)
* Glass beads
* Pipette
* Graduated Cylinder
* Stirring rod (magnet)
* Stirrer
* Distillation apparatus by VELP
* Digester by VELP
* Distilled water
* Digestion reagent
* NaOH solution (35%)
* H2SO4 (0,02 N) titrant.
* Boric Acid Solution (with Mixed Indicator Solution, purple color)

***1.1.2.2. Steps of the Experiment***

* Select the sample volume as described in Standard Methods.
* Turn on the VELP digester and cooling water.
* Add your sample into the glass test tubes.
* Add 50 mL digestion reagent.
* Add 5-6 glass beads to prevent bumping (foaming) during digestion.
* Heat the digester up above 380oC.
* Copious fumes are observed when sulfuric acid reaches its boiling point. Digestion is just beginning at this stage.
* Continue to boil briskly until solution becomes transparent and pale green. Complete destruction of organic matter is indicated by transparency of the sample.
* Then turn each heating unit up to its maximum setting (400 or 420oC) and digest for an additional 30 min to ensure complete destruction of all organic matter.
* Take the glass tubes when the operation cycle ends and wait until it gets cooler.
* Once the organic nitrogen has been released as ammonia nitrogen, it may be measured as described in ammonia nitrogen procedure.
* Calculation of organic nitrogen is made in the same manner as for ammonia nitrogen.