METHOD #: 351.3	Approved for NPDES (Editorial Revision 1974, 1978)
TITLE:	Nitrogen, Kjeldahl, Total (Colorimetric; Titrimetric; Potentiometric)
ANALYTE:	CAS # N Nitrogen 7727-37-9
INSTRUMENTATION:	Spectrophotometer
STORET No.	00625

1.0 Scope and Application

1.1 This method covers the determination of total Kjeldahl nitrogen in drinking, surface and saline waters, domestic and industrial wastes. The procedure converts nitrogen components of biological origin such as amino acids, proteins and peptides to ammonia, but may not convert the nitrogenous compounds of some industrial wastes such as amines, nitro compounds, hydrazones, oximes, semicarbazones and some refractory tertiary amines.

1.2 Three alternatives are listed for the determination of ammonia after distillation: the titrimetric method which is applicable to concentrations above 1 mg N/liter; the Nesslerization method which is applicable to concentrations below 1 mg N/liter; and the potentiometric method applicable to the range 0.05 to 1400 mg/L.

1.3 This method is described for macro and micro glassware systems.

2.0 Definitions

2.1 Total Kjeldahl nitrogen is defined as the sum of free-ammonia and organic nitrogen compounds which are converted to ammonium sulfate $(NH_4)_2SO_4$, under the conditions of digestion described below.

2.2 Organic Kjeldahl nitrogen is defined as the difference obtained by subtracting the free- ammonia value (Method 350.2, Nitrogen, Ammonia, this manual) from the total Kjeldahl nitrogen value. This may be determined directly by removal of ammonia before digestion.

3.0 Summary of Method

3.1 The sample is heated in the presence of conc. sulfuric acid, K_2SO_4 and $HgSO_4$ and evaporated until SO_3 fumes are obtained and the solution becomes colorless or pale yellow. The residue is cooled, diluted, and is treated and made alkaline with a hydroxide-thiosulfate solution. The ammonia is distilled and determined after distillation by Nesslerization, titration or potentiometry.

4.0 Sample Handling and Preservation

4.1 Samples may be preserved by addition of 2 mL of conc. H_2SO_4 per liter and stored at 4°C. Even when preserved in this manner, conversion of organic

nitrogen to ammonia may occur. Preserved samples should be analyzed as soon as possible.

5.0 Interference

5.1 High nitrate concentrations (1OX or more than the TKN level) result in low TKN values. The reaction between nitrate and ammonia can be prevented by the use of an anion exchange resin (chloride form) to remove the nitrate prior to the TKN analysis.

6.0 Apparatus

6.1 Digestion apparatus: A Kjeldahl digestion apparatus with 800 or 100 mL flasks and suction takeoff to remove SO_3 fumes and water.

6.2 Distillation apparatus: The macro Kjeldahl flask is connected to a condenser and an adaptor so that the distillate can be collected. Micro Kjeldahl steam distillation apparatus is commercially available.

6.3 Spectrophotometer for use at 400 to 425 nm with a light path of 1 cm or longer.

7.0 Reagents

7.1 Distilled water should be free of ammonia. Such water is best prepared by the passage of distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Regeneration of the column should be carried out according to the manufacturer's instructions.
NOTE 1: All solutions must be made with ammonia-free water.

7.2 Mercuric sulfate solution: Dissolve 8 g red mercuric oxide (HgO) in 50 mL of 1:4 sulfuric acid (10.0 mL conc. H_2SO_4: 40 mL distilled water) and dilute to 100 mL with distilled water.

7.3 Sulfuric acid-mercuric sulfate-potassium sulfate solution: Dissolve 267 g K_2SO_4 in 1300 mL distilled water and 400 mL conc. H_2SO_4. Add 50 mL mercuric sulfate solution (7.2) and dilute to 2 liters with distilled water.

7.4 Sodium hydroxide-sodium thiosulfate solution: Dissolve 500 g NaOH and 25 g $Na_2S_20_3 \bullet 5H_2O$ in distilled water and dilute to 1 liter.

7.5 Mixed indicator: Mix 2 volumes of 0.2% methyl red in 95% ethanol with 1 volume of 0.2% methylene blue in ethanol. Prepare fresh every 30 days.

7.6 Boric acid solution: Dissolve 20 g boric acid, H_3BO_3, in water and dilute to 1 liter with distilled water.

7.7 Sulfuric acid, standard solution: (0.02 N) 1 mL = 0.28 mg NH_3-N. Prepare a stock solution of approximately 0.1 N acid by diluting 3 mL of conc. H_2SO_4 (sp. gr. 1.84) to 1 liter with CO_2-free distilled water. Dilute 200 mL of this solution to 1 liter with CO_2-free distilled water. Standardize the approximately 0.02 N acid so prepared against 0.0200 N Na_2CO_3 solution. This last solution is prepared by dissolving 1.060 g anhydrous Na_2CO_3, oven-dried at 140°C, and diluting to 1 liter with CO_2-free distilled water.

NOTE 2: An alternate and perhaps preferable method is to standardize the approximately 0.1 N H_2SO_4 solution against a 0.100 N Na_2CO_3 solution. By proper dilution the 0.02 N acid can the be prepared.

7.8 Ammonium chloride, stock solution: 1.0 mL = 1.0 mg NH_3-N. Dissolve 3.819 g NH_4Cl in water and make up to 1 liter in a volumetric flask with distilled water.

7.9 Ammonium chloride, standard solution: 1.0 mL = 0.01 mg NH_3-N. Dilute 10.0 mL of the stock solution (7. 8) with distilled water to 1 liter in a volumetric flask.

7.10 Nessler reagent: Dissolve 100 g of mercuric iodide and 70 g potassium iodide in a small volume of distilled water. Add this mixture slowly, with stirring, to a cooled solution of 160 g of NaOH in 500 mL of distilled water. Dilute the mixture to 1 liter. The solution is stable for at least one year if stored in a pyrex bottle out of direct sunlight.

NOTE 3: Reagents 7.7, 7.8, 7.9, and 7.10 are identical to reagents 6.8, 6.2, 6.3, and 6.6 described under Nitrogen, Ammonia (Colorimetric; Titrimetric; Potentiometric-Distillation Procedure, Method 350.2).

8.0 Procedure

8.1 The distillation apparatus should be pre steamed before use by distilling a 1:1 mixture of distilled water and sodium hydroxide-sodium thiosulfate solution (7.4) until the distillate is ammonia-free. This operation should be repeated each time the apparatus is out of service long enough to accumulate ammonia (usually 4 hours or more).

8.2 Macro Kjeldahl system

8.2.1 Place a measured sample or the residue from the distillation in the ammonia determination (for Organic Kjeldahl only) into an 800 mL Kjeldahl flask. The sample size can be determined from the following table:

Kjeldahl Nitrogen in Sample, mg/L	Sample Size mL
0-5	500
5-10	250
10-20	100
20-50	50.0
50-500	25.0

Dilute the sample, if required, to 500 mL with distilled water, and add 100 mL sulfuric acid-mercuric sulfate-potassium sulfate solution (7.3). Evaporate the mixture in the Kjeldahl apparatus until SO_3 fumes are given off and the solution turns colorless or pale yellow. Continue heating for 30 additional minutes. Cool the residue and add 300 mL distilled water.

8.2.2 Make the digestate alkaline by careful addition of 100 mL of sodium hydroxide - thiosulfate solution (7.4) without mixing.

NOTE 5: Slow addition of the heavy caustic solution down the tilted neck of the digestion flask will cause heavier solution to underlay the

aqueous sulfuric acid solution without loss of free-ammonia. Do not mix until the digestion flask has been connected to the distillation apparatus.

8.2.3 Connect the Kjeldahl flask to the condenser with the tip of condenser or an extension of the condenser tip below the level of the boric acid solution (7.6) in the receiving flask.

8.2.4 Distill 300 mL at the rate of 6-10 ml/min., into 50 mL of 2% boric acid (7.6) contained in a 500 mL Erlenmeyer flask.

8.2.5 Dilute the distillate to 500 mL in the flask. These flasks should be marked at the 350 and the 500 mL volumes. With such marking, it is not necessary to transfer the distillate to volumetric flasks. For concentrations above 1 mg/L, the ammonia can be determined titrimetrically. For concentrations below this value, it is determined colorimetrically. The potentiometric method is applicable to the range 0.05 to 1400 mg/L.

8.3 Micro Kjeldahl system

8.3.1 Place 50.0 mL of sample or an aliquot diluted to 50 mL in a 100 mL Kjeldahl flask and add 10 mL sulfuric acid-mercuric sulfate- potassium sulfate solution (7.3). Evaporate the mixture in the Kjeldahl apparatus until SO_3 fumes are given off and the solution turns colorless or pale yellow. Then digest for an additional 30 minutes. Cool the residue and add 30 mL distilled water.

8.3.2 Make the digestate alkaline by careful addition of 10 mL of sodium hydroxide thiosulfate solution (7.4) without mixing. Do not mix until the digestion flask has been connected to the distillation apparatus.

8.3.3 Connect the Kjeldahl flask to the condenser with the tip of condenser or an extension of the condenser tip below the level of the boric acid solution (7.6) in the receiving flask or 50 mL short-form Nessler tube.

8.3.4 Steam distill 30 mL at the rate of 6-10 ml/min., into 5 mL of 2% boric acid (7.6).

8.3.5 Dilute the distillate to 50 mL. For concentrations above 1 mg/L the ammonia can be determined titrimetrically. For concentrations below this value, it is determined colorimetrically. The potentiometric method is applicable to the range 0.05 to 1400 mg/L.

8.4 Determination of ammonia in distillate: Determine the ammonia content of the distillate titrimetrically, colorimetrically, or potentiometrically, as described below.

8.4.1 Titrimetric determination: Add 3 drops of the mixed indicator (7.5) to the distillate and titrate the ammonia with the 0.02 N H_2SO_4 (7.7), matching the endpoint against a blank containing the same volume of distilled water and H_3BO_3 (7.6) solution.

8.4.2 Colorimetric determination: Prepare a series of Nessler tube standards as follows:

mL of Standard 1.0 mL = 0.01 mg NH_3-N	mg NH_3-N / 50.0 mL
0.0	0.0
0.5	0.005
1.0	0.010
2.0	0.020
4.0	0.040
5.0	0.050
8.0	0.080
10.0	0.10

Dilute each tube to 50 mL with ammonia free water, add 1 mL of Nessler Reagent (7.10) and mix. After 20 minutes read the absorbance at 425 nm against the blank. From the values obtained for the standards plot absorbance vs. mg NH_3-N for the standard curve. Develop color in the 50 mL diluted distillate in exactly the same manner and read mg NH_3-N from the standard curve.

8.4.3 Potentiometric determination: Consult the method entitled Nitrogen, Ammonia: Potentiometric, Ion Selective Electrode Method,(350.3) in this manual.

8.4.4 It is not imperative that all standards be treated in the same manner as the samples. It is recommended that at least 2 standards (a high and low) be digested, distilled, and compared to similar values on the curve to insure that the digestion-distillation technique is reliable. If treated standards do not agree with untreated standards the operator should find the cause of the apparent error before proceeding.

9.0 Calculation

9.1 If the titrimetric procedure is used, calculate Total Kjeldahl Nitrogen, in mg/L, in the original sample as follows:

$$\text{TKN, } mg/L = \frac{(A - B)N \times F \times 1{,}000}{S}$$

where:

A = milliliters of standard 0.020 N H_2SO_4 solution used in titrating sample.
B = milliliters of standard 0.020 N H_2SO_4 solution used in titrating blank.
N = normality of sulfuric acid solution.
F = milliequivalent weigh to nitrogen (14mg).
S = milliliters of sample digested.

If the sulfuric acid is exactly 0.02 N the formula is shortened to:

$$\text{TKN, } mg/L = \frac{(A - B) \times 280}{S}$$

9.2 If the Nessler procedure is used, calculate the Total Kjeldahl Nitrogen, in mg/L, in the original sample as follows:

$$\text{TKN, } mg/L = \frac{A \times 1{,}000}{D} \times \frac{B}{C}$$

where:

A = mg NH_3-N read from curve.
B = mL total distillate collected including the H_3BO_3.
C = mL distillate taken for Nesslerization.
D = mL of original sample taken.

9.3 Calculate Organic Kjeldahl Nitrogen in mg/L, as follows: Organic Kjeldahl Nitrogen = TKN--(NH_3-N.)

9.4 Potentiometric determination: Calculate Total Kjeldahl Nitrogen, in mg/L, in the original sample as follows:

$$\text{TKN, } mg/L = \frac{B}{D} \times A$$

where:

A = mg NH_3-N/L from electrode method standard curve.
B = volume of diluted distillate in mL.
D = mL of original sample taken.

10.0 Precision

10.1 Thirty-one analysts in twenty laboratories analyzed natural water samples containing exact increments of organic nitrogen, with the following results:

Increment as Nitrogen, Kjeldahl mg N/liter	Precision as Standard Deviation mg N/liter	Accuracy as Bias, %	Accuracy as Bias mg N/liter
0.20	0.197	+15.54	+0.03
0.31	0.247	+ 5.45	+0.02
4.10	1.056	+ 1.03	+0.04
4.61	1.191	- 1.67	-0.08

(FWPCA Method Study 2, Nutrient Analyses)

Bibliography

1. Standard Methods for the Examination of Water and Wastewater, 14th Edition, p 437, Method 421 (1975).
2. Schlueter, Albert, "Nitrate Interference In Total Kjeldahl Nitrogen Determinations and Its Removal by Anion Exchange Resins", EPA Report 600/7-77-017.