भारतीय मानक IS 3025 (Part 34/Sec 1) : 2023

Indian Standard

जल एवं अपशिष्ट जल के नमूने लेने तथा परीक्षण (भौतिक एवं रसायन) की पद्धतियां

भाग 34 नाइट्रोजन

अनभाग 1 विभिन्न प्रकार के नाइट्रोजन जैसे अमोनियाकल, नाइट्रेट, नाइट्राइट और कार्बनिक नाइट्रोजन का निर्धारण

(दूसरा पुनरीक्षण)

Methods of Sampling and Test (Physical and Chemical) for Water and Wastewater

Part 34 Nitrogen

Section 1 Determination of Various Types of Nitrogen like Ammoniacal, Nitrate, Nitrite and Organic Nitrogen

(Second Revision)

ICS 13.060.50

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December 2023 **Price Group 8**

Water Quality Sectional Committee, CHD 36

FOREWORD

This Indian Standard (Part 34/Sec 1) (Second Revision) was adopted by the Bureau of Indian Standards, after the draft finalized by the Water Quality Sectional Committee had been approved by the Chemical Division Council.

In waters and wastewaters, the forms of nitrogen of greatest interest are, in order of decreasing oxidation state, namely, nitrate, nitrite, ammonia and organic nitrogen. All these forms of nitrogen, as well as nitrogen gas are biochemically inter-convertible and are components of nitrogen cycle. All these forms are of interest to water chemist.

Ammonia is present in surface and wastewaters. Its concentration is generally low in ground waters because it is absorbed in soil particles and clays and is not leached readily from soils. Nitrates generally occur in trace quantities in surface water but may attain high levels in some ground waters. In excessive limits, it contributes to the illness known as methenoglobinemia in infants. Nitrite is an intermediate oxidation state of nitrogen, both in oxidation of ammonia to nitrate or in the reduction of nitrate. Nitrous acid which is formed from nitrite in acidic solution can react with secondary amines to form nitroso amines, many of which are known to be carcinogens. Organic nitrogen is defined functionally as organic bound nitrogen in tri-negative state. Analytically organic and ammoniacal nitrogen can be determined together and called as Kjeldahl nitrogen.

The Committee responsible for formulation of IS 3025 : 1964 'Methods of sampling and test (physical and chemical) for water used in industry' and IS 2488 (Part 4) : 1974 'Methods of sampling and test for industrial effluents' decided to revise the standard and publish it in separate parts. This standard superseded **5** of IS 2488 (Part 4) : 1974 and **47**, **48**, **49** of IS 3025 : 1964 and was one among the different parts published under IS 3025 series of standards. The first revision of this standard was published in 1988, in which ultraviolet spectrophotometric screening method for determination of nitrate estimation in water and wastewater which is based on ASTM Standard, 1985, Section II was incorporated.

In this revision the following modifications have been incorporated:

a) Nesslerization method for determination of ammoniacal nitrogen has been deleted;

b) Phenate method has been updated;

c) Chromotropic acid and devarda's alloy reduction method for determination of nitrate nitrogen have been deleted; and

d) Ultraviolet spectrophotometric screening method and nitrate electrode method for determination of nitrate nitrogen have been incorporated.

This Section of IS 3025 (Part 34) covers phenate, titrimetric, and ammonia selective electrode method for determination of ammoniacal nitrogen, cadmium reduction method, and its advanced methods, ultraviolet spectrophotometric screening, nitrate electrode methods for determination of nitrate nitrogen in waters and wastewaters. It also covers determination of nitrite nitrogen and organic nitrogen by Macro Kjeldahl Method, and Semi-Micro-Kjeldahl Method.

The standard IS 3025 (Part 34) has been published in 4 sections. The other sections of this standard are:

Sec 2	Determination of ammonium nitrogen — Method by flow analysis (CFA and FIA) and spectrometric detection
Sec 3	Determination of nitrite nitrogen and nitrate nitrogen and the sum of both by flow analysis CFA and FIA and spectrometric detection
Sec 4	Determination of total nitrogen after UV digestion method using flow analysis CFA and FIA and spectrometric detection

In the preparation of this standard, considerable assistance has been derived from 'Standard methods for the examination of water and wastewater', 23rd edition 2017 published by the American Public Health Association, Washington, USA.

The composition of the Committee responsible for the formulation of this standard is given in Annex A.

In reporting the result of a test or analysis made in accordance with this standard, is to be rounded off, it shall be done in accordance with IS 2 : 2022 'Rules for rounding off numerical values (*second revision*)'.

Indian Standard

METHODS OF SAMPLING AND TEST (PHYSICAL AND CHEMICAL) FOR WATER AND WASTEWATER

PART 34 NITROGEN

SECTION 1 DETERMINATION OF VARIOUS TYPES OF NITROGEN LIKE AMMONIACAL, NITRATE, NITRITE AND ORGANICNITROGEN

(Second Revision)

1 SCOPE

This standard (Part 34/Sec 1) prescribes methods for determination of various types of nitrogen like ammoniacal, nitrate, nitrite andorganic nitrogen in water and waste water and sludge samples.

2 REFERENCES

The standards given below contain provisions which through reference in this text constitute provisions of this standard. At the time of publications, the editions indicated were valid. All standards are subject to revision and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent edition of these standards:

IS No.	*Title*
IS 7022 (Part 1) : 1973	Glossary of terms relating to water, sewage and industrial effluents: Part 1
IS 7022 (Part 2) : 1979	Glossary of terms relating to water, sewage and industrial effluents: Part 2
IS 17614 (Part 1) : 2021/ISO 5667-1 : 2020	Water quality — Sampling: Part 1 Guidance on the design of sampling programmes and sampling techniques
IS 17614 (Part 3) : 2021/ISO 5667-3 : 2018	Water quality — Sampling: Part 3 Preservation and handling of water samples

3 TERMINOLOGY

For the purpose of this standard definitions given in IS 7022 (Part 1) and IS 7022 (Part 2) shall apply.

4 SAMPLING

Sampling and sample preservation shall be done as prescribed in IS 17614 (Part 1) and IS 17614 (Part 3).

5 AMMONIACAL NITROGEN

5.1 General

The following three methods are used for determination of ammoniacal nitrogen in water and wastewater:

a) Titrimetric method;

b) Phenate method; and

c) Ammonia selective electrode method.

5.2 Preliminary Distillation Step

5.2.1 The two major factors that influence selection of the method for determination of ammonia are concentration and presence of interferences. Where interferences are present and greater precision is necessary, a preliminary distillation step is necessary. This is compulsory for titrimetric method.

5.2.2 *Preparation of Distilling Apparatus*

Add 500 ml water and 20 ml of borate buffer solution (*see* **8.3.2.3**) to a distillation flask and adjust *p*H to 9.5 with 6 N sodium hydroxide solution. Add a few glass beads and use this mixture to steam out the distillation apparatus, until distillate shows no trace of ammonia.

5.2.3 *Sample Preparation*

Use 500 ml of dechlorinated sample or a portion diluted to 500 ml with water so as to maintain ammonia > 100 µg/l. Remove residual chlorine by adding hypo at the time of collection/dechlorinating agent equivalent to chlorine residual. If necessary, neutralize to *p*H 7 with dilute acid or alkali. Add 25 ml of borate buffer and adjust *p*H to 9.5 with 6 N sodium hydroxide solution using a *p*H meter.

5.2.4 To minimize contamination, leave distillation apparatus assembled after steaming out and until just before starting the sample distillation. Disconnect steaming out flask and immediately transfer sample flask to distillation apparatus. Distil at the rate of 6 ml/min to 10 ml/min with the tip of the delivery tube below the surface of acid receiving solution. Use 50 ml indicating boric acid (*see* **5.3.3.2**) solution for titrimetric method.

Distil ammonia into 50 ml of 0.04 N sulphuric acid for the phenate method and for the ammonia

selective electrode method. Collect at least 200 ml of distillate. Lower the collected distillate free of contact with the delivery tube and continue distillation during the last minute or two to cleanse condenser and delivery tube. Dilute to 500 ml with water.When phenate method is used, neutralize the distillate with 1 N sodium hydroxide solution.

5.3 Titrimetric Method

5.3.1 *Principle*

The method is used only on samples that have been carried through preliminary distillation (*see* **5.2**). Use the values for selecting sample volume for the distillation and titration method as specified in Table 1.

Table 1 Sample Volume for the Distillation and Titration Method

(*Clause* 5.3.1)

Sl No.	**NH_3-N in Sample**, mg/l	**Sample Volume**, ml
(1)	(2)	(3)
i)	5 to 10	250
ii)	10 to 20	100
iii)	20 to 50	50.0
iv)	50 to 100	25.0

5.3.1.1 The ammonia in distillate is titrated with standard sulphuric acid.

5.3.2 *Apparatus*

5.3.2.1 *Distillation assembly*

Borosilicate glass flask of 800 ml to 2 000 ml capacity attached to a vertical condenser so that the outlet tip may be submerged below the surface of the receiving acid solution.

5.3.3 *Reagents*

5.3.3.1 *Mixed indicator solution*

Dissolve 200 mg of methyl red indicator in 100 ml of 95 percent ethyl or isopropyl alcohol.

Dissolve 100 mg of methylene blue in 50 ml of 95 percent ethyl or isopropyl alcohol. Combine these two. Prepare monthly.

5.3.3.2 *Indicating boric acid solution*

Dissolve 20 g of hydroboric acid in ammonia free water, add 10 ml of mixed indicator solution and dilute to 1 litre.

5.3.3.3 *Standard sulphuric acid titrant* — 0.02 N

5.3.4 *Procedure*

5.3.4.1 Proceed as prescribed in **5.2** using indicating boric acid solution as absorbent for distillate.

5.3.4.2 Titrate ammonia in distillate against standard sulphuric acid until indicator turns a palelavender. Carry a blank through all steps of the procedure and apply the necessary correction to the results.

5.3.5 *Calculations*

$$\text{Ammonical nitrogen, mg/l} = \frac{(A-B) \times 280}{V} \quad \ldots(1)$$

where

A = volume, in ml, of sulphuric acid used for sample;

B = volume in ml, of sulphuric acid used for blank; and

V = volume, in ml, of sample taken for test.

5.4 Phenate Method

5.4.1 *Principle*

An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite and phenol which is catalyzed by a sodium nitroprusside.

5.4.2 *Interference*

Alkalinity over 500 mg/l as calcium carbonate or acidity over 100 mg/l as calcium carbonate or turbidity interferes. Remove these by preliminary distillation.

5.4.3 *Apparatus*

5.4.3.1 *Spectrophotometer or filter photometer*

For use at 640 nm. The photometer is equipped with a red-orange filter. The light path of these photometers should be 1 cm, approximately.

5.4.3.2 *Magnetic stirrer*

5.4.4 *Reagents*

5.4.4.1 *Ammonia-free water*

Prepare ammonia free water by passing distilled water through anion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Alternatively, re-distil distilled water by adding 0.1 ml of concentrated sulphuric acid to1 litre of water. It may also be made by treating distilled water with sufficient bromine or chlorine water to produce a free halogen residual of 2 mg/l to 5 mg/l. Re-distil after standing at least for 1 h. Discard the first 100 ml distillate.

5.4.4.2 *Sodium hypochlorite* — 5 percent commercial grade.

Use commercial grade 5 percent sodium hypochlorite. This will slowly decompose once the seal on the bottle cap is broken. Hence replace every two month.

5.4.4.3 *Sodium nitro prusside* — 0.5 percent *w/v.*

Dissolve 0.5 g sodium nitro prusside in 100 ml deionized water. Store in amber colour bottle for up to 1 month.

5.4.4.4 *Phenol solution*

Mix 11.1 ml liquefied phenol (> 89 percent) with 95 percent ethyl alcohol and make upto 100 ml final volume. Prepare weekly.

5.4.4.5 *Stock ammonium solution*

Dissolve 381.9 mg of anhydrous ammonium chloride in water and dilute to 1 000 ml (1.00 ml = 0.122 mg as NH_3 or 0.1 mg as N).

5.4.4.6 *Standard ammonium solution*

Dilute 5.00 ml of stock solution to 1 000 ml with water (1.00 ml = 0.500 µg of nitrogen or 0.607 µg of ammonia).

5.4.4.7 *Alkaline citrate solution*

Dissolve 200 g trisodium citrate and 10 g sodium hydroxide (NaOH) in deionized water. Dilute to 1 000 ml.

5.4.4.8 *Oxidizing agent*

Mix 100 ml of alkaline citrate solution with 25 ml sodium hypochlorite solution.

5.4.5 *Procedure*

5.4.5.1 *Preparation of standards*

Prepare a series of standard solutions covering the concentrations of 1 000 mg, 100 mg, 10 mg, 1 mg, and 0.1 mg of nitrogen (ammonical) by making decimal dilutions of stock ammonical chloride solution with water.

5.4.5.2 *Treatment of sample*

Take 25 ml sample in a 50 ml flask and add 1 ml phenol solution, 1 ml sodium nitroprusside solution, and 2.5 ml oxidizing solution. Cover the samples with plastic wrap or paraffin wrapper film. Let colour develop at room temperature in subdued light for at least 1 h.

Measure absorbance at 640 nm. Prepare a blank and at least two other standards by diluting the stock ammonium solution into the sample concentration range. Treat the standard the same as samples.

5.4.6 *Calculation*

5.4.6.1 Calculate ammonia concentration as follows:

$$\text{Ammoniacal nitrogen, mg/l} = \frac{A \times B}{C \times S} \times \frac{D}{E} \quad \ldots (2)$$

where

A = absorbance of sample;

B = ammoniacal nitrogen in the standard, in µg;

C = absorbance of standard;

S = volume of sample, in ml;

D = volume of total distillate collected, including the acid absorbents, neutralizing agents and ammonia free water added, in ml; and

E = volume of distillate used for colour development, in ml.

NOTE — The ratio D/E applies only to distilled samples.

5.5 Ammonia Selective Electrode Method

5.5.1 *Principle*

It uses a hydrophobic gas permeable membrane to separate the sample solution from an electrode internal solution of ammonium chloride. Dissolved ammonia is converted into NH_3 (aqueous) by raising *p*H to above 1 litre with a strong base. NH_3 (aqueous) diffuses through the membrane and changesthe internal solution *p*H that is, sensed by a *p*H electrode. The fixed level of chloride in the internal solution is sensed by a chloride ion selective electrode that serves as the reference electrode. Potentiometric measurements are made with a *p*H meter having an expanded millivolt scale or with a specific ion-meter.

5.5.2 *Interference*

Amines are a positive interference. Mercury and silver interfere by complexing with ammonia.

5.5.3 *Apparatus*

5.5.3.1 *Electrometer*

A *p*H meter with expanded millivolt scale capable of 0.1 mV resolution between − 700 mV and + 700 mV or a specific ion meter.

5.5.3.2 *Ammonia-selective electrode*

5.5.3.3 *Magnetic stirrer*

5.5.4 *Reagents*

5.5.4.1 *Ammonia free water — see* **5.4.4.1**

5.5.4.2 *Sodium hydroxide solution/ ethylenediaminetetraacetic acid* (*EDTA*) — 10 N

Dissolve 400 mg NaOH in 800 ml water. Add 45.2 g EDTA and stir to dissolve. Cool and dilute to 1 000 ml.

5.5.4.3 *Stock ammonium chloride solution — see* **5.4.4.5**

5.5.4.4 *Standard ammonium chloride solution — see* **5.4.4.6**

5.5.5 *Procedure*

5.5.5.1 *Preparation of standards*

Prepare a series of standard solutions covering the concentrations of 1 000 mg, 100 mg, 10 mg, 1 mg,

and 0.1 mg of nitrogen (ammonical) by making decimal dilutions of stock ammonical chloride solution with water.

5.5.5.2 *Electrometer calibration*

Place 100 ml of each standard solution in a 150 ml beaker, immerse electrode in standard of lowest concentration and mix with a magnetic stirrer. Do not stir so rapidly that air bubbles are sucked into the solution because they will get trapped on the electrode membrane. Maintain the same stirring rate and temperature of about 25 °C throughout calibration and testing procedures. Add sufficient volume of 10 N sodium hydroxide solution to raise *p*H above 1 1itre. Keep electrode in solution until a stable millivolt reading is obtained. Do not add sodium hydroxide solution before immersing electrode because ammonia may be lost from a basic solution. Repeat procedures with remaining standards, proceeding from lowest to highest concentration. Wait for at least 5 min before recording millivolts for standards and samples containing ≤ 1 mg of nitrogen (ammoniacal) per 1.

5.5.5.3 *Preservation of standard curve*

Using semi-logarithmic graph paper, plot ammonia concentration in milligrams of nitrogen (ammoniacal) per litre on the log axis vs. potential in millivolts on the linear axis starting with the lowest concentration at the bottom of the scale. If the electrode is functioning properly, a ten-fold change of ammoniacal nitrogen concentration produces a potential change of 59 mV.

5.5.5.4 *Calibration of specific ion meter*

Following manufacturer's instructions, follow steps given in **5.5.5.1** and **5.5.5.2**.

5.5.5.5 *Measurement of samples*

Dilute, if necessary, to bring ammoniacal nitrogen to within calibration curve range. Place 100 ml sample in 150 ml beaker and follow procedure given in **5.5.5.2**. Record the volume of 10 N of sodium hydroxide added in excess of 1 ml. Read ammoniacal nitrogen concentration from standard curve.

5.5.6 *Calculation*

Ammoniacal nitrogen, in mg/l

$$= \left[\frac{101+D}{100+C}\right] \times A \times B \qquad \ldots (3)$$

where

A = dilution factor;

B = concentration of ammoniacal nitrogen per litre, mg/l from calibration curve;

D = volume of 10 N NaOH added to sample, in ml; and

C = volume of added 10 N sodium hydroxide to calibration standards, in ml.

6 NITRATE NITROGEN

6.1 The three methods for determination of nitrate, nitrogen in waters and wastewaters are as follows:

a) Cadmium reduction method, and its advanced methods;

b) Ultraviolet Spectrophotometric screening method; and

c) Nitrate electrode method.

6.2 General

For concentration below 1.0 mg/l of nitrate nitrogen, cadmium reduction method is suitable. There are various advance automated methods and flow injection methods were available to measure nitrates for continuous measurement. For concentration from 0.2 mg/l to 11 mg/l, and for fresh waters UV screening method may be made applicable. For 1 mg/l to 50 mg/l of nitrates and effluents, ion electrode method is suitable ion chromatography method also can be used for nitrate determination.

6.3 Cadmium Reduction Method

6.3.1 *Principle*

Nitrate is reduced to nitrite in presence of cadmium. The nitrite produced is determined by diazotizingwith sulphanilamide and coupling with N-(1-naphthyl) ethylene diamine to form a highly coloured azodye which is measured calorimetrically.

6.3.2 *Interference*

Higher concentrations of copper, iron, etc, lower the reduction efficiency. Add EDTA to remove this interference. Oil and grease can also interfere, similarly as well as residual chlorine. Remove oil and grease by extraction with organic solvents and residual chlorine by adding sodium thiosulphate. Suspended solids also interfere; hence filtration is needed before reduction process.

6.3.3 *Apparatus*

6.3.3.1 *Reduction column*

Commercially available one or construct the column from a 100 ml. volumetric pipette by removing the top portion. The column can also be constructed by two pieces of tubing joined end to end [join a 10 cm length of 3 cm internal diameter (ID) tubing to a 25 cm length of 3.5 cm ID tubing]. A liquid levelling device is useful as given in Fig. 1.

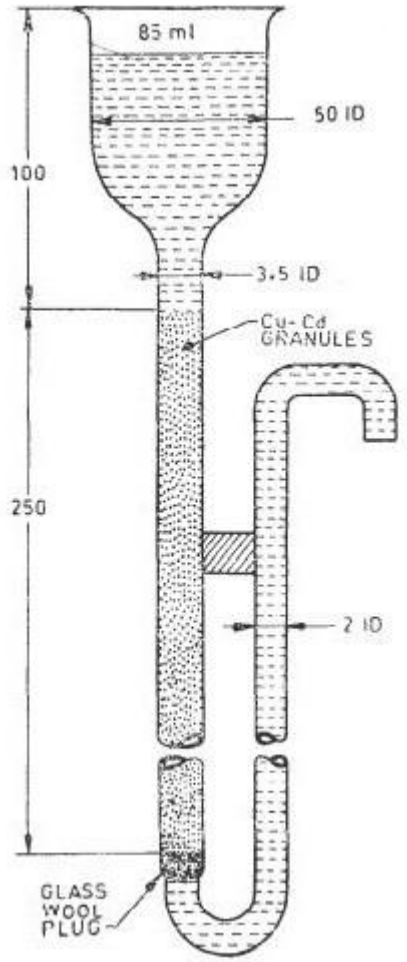

FIG. 1 REDUCTION COLUMN

All dimensions in millimetres.

6.3.3.2 *Colorimeter* — One of the following:

a) *Spectrophotometer* — for use near 543 nm with a light path of 1 cm or longer; and

b) *Filter photometer* — provided with a yellow green filter having maximum transmittance near 540 nm and a light path of 1 cm or longer.

6.3.4 *Reagents*

6.3.4.1 *Nitrate free water*

The absorbance of a reagent blank prepared with this water should not exceed 0.01. Use for all solutions and dilution.

6.3.4.2 *Copper-cadmium granules*

Wash 25 g of 40 to 60 mesh cadmium granules with 6 N hydrochloric acid and rinse with water. Swirl cadmium with 100 ml of 2 percent copper sulphate solution for 5 min or until blue colour partially fades. Decant and repeat with fresh copper sulphate until a brown colloidal precipitate develops. Wash copper-cadmium copiously with water (at least 10 times) to remove all precipitated copper.

6.3.4.3 *Sulphanilamide reagent*

Dissolve 5 g of sulphanilamide in a mixture of 50 ml concentrated phosphoric acid (85 percent) and 300 ml of water. Dilute to 400 ml with water. The reagent is stable for many months.

6.3.4.4 *N-(1-naphthyl)-ethylenediaminedihydrochloride* (*NED dihydrochloride*) *solution*

Dissolve 500 mg of NED di-hydrochloride in 500 ml of water. Store in dark coloured bottle. Replace as soon asa brown colour develops.

6.3.4.5 *Ammonium chloride – EDTA solution*

Dissolve 13 g of ammonium chloride and 1.7 g of disodium ethylene diamine tetra acetate in 900 ml of water. Adjust *p*H to 8.5 with liquor ammonia and dilute to 1 litre.

6.3.4.6 *Dilute EDTA*

Dilute 300 ml of the above solution (*see* **6.3.4.5**) to 500 ml with water to get a dilute solution.

6.3.4.7 *Hydrochloric acid* — 6 M

Add 500 ml conc. hydrochloric acid to 400 ml

reagent water and dilute to 1 litre and this solution is stable for 1 year.

6.3.4.8 *Copper sulphate solution* — 2 percent (*w/v*).

Dissolve 20 g of copper sulphate ($CuSO_4.5H_2O$) in 500 ml waterand make up to 1 litre.

6.3.4.9 *Stock nitrate solution*

Dissolve 0.721 8 g of dry potassium nitrate in water and dilute to 1 000 ml. Preserve with 2 ml of chloroform/l (1 ml = 100 µg of nitrate nitrogen).

6.3.4.10 Dilute 50 ml of stock nitrate solution to 500 ml with water to get standard solution (1.00 ml = 10.0 µg nitrate nitrogen).

6.3.4.11 *Stock nitrite solution*

Dissolve 0.607 2 g of dried potassium nitrite in nitrite free water and make up to 1 000 ml (1.00 ml = 100 µg nitrite nitrogen). Preserve with 2 ml of chloroform and keep in a refrigerator. The solution is stable for 3 months.

6.3.4.12 Dilute 50.0 ml of above stock nitrite solution to 500 ml with nitrite free water (1.00 ml = 10.0 µg of nitrite nitrogen).

6.3.5 *Procedure*

6.3.5.1 *Preparation of reduction column*

Insert a glass wool plug into the bottom of reduction column and fill with water. Add sufficient copper-cadmium granules to produce a column 18.5 cm long. Maintain water level above Cu-Cd granules to prevent entrapment of air. Wash column with 200 ml dilute ammonium chloride EDTA solution. Activate column by passing through it, at 7 ml/min to 10 ml/min, 100 ml of a solution comprising 25 ml of a 1.0 mg nitrate-nitrogen)/l standard and 75 ml of ammonium chloride- EDTA solution.

6.3.5.2 *Treatment of sample*

If turbidity or suspended solids are present, remove by filtering through a 0.45 pm pore diameter membrane or glass fibre filter. Adjust *p*H to between 7 and 9 as necessary. To 25.0 ml sample or a portion diluted to 25.0 ml, add 75 ml of ammonium chloride — EDTA solution and mix. Pour mixed sample into column and collect at the rate of 7 ml/min to 10 ml/min. Discard first 25 ml. Collect the rest in original sample flask. There is no need to wash the column between samples butif columns are not to be reused for several hours or longer, pour 50 ml dilute ammonium chloride — EDTA solution on to the top and let it pass through the system. Store Cu-Cd column in this solution and never allow it to dry.

6.3.5.3 As soon as possible and not more than 15 minutes after reduction, add 2.0 ml sulphanilamide reagent to 50 ml of sample. Let the reagent react for 2 min to 8 min. Add 2 ml of NED di-hydrochloric acid solution and mix immediately. Between 10 min and 2 h afterwards, measure absorbance at 543 nm against a distilled water reagent blank. Using the standard nitrate nitrogen solution, prepare standards in the range of 0.05 mg to 1.0 mg of nitrate nitrogen/l by diluting the following volumes of standards to 100 ml in volumetric flasks: 0.5 ml, 1.0 ml, 2.0 ml, 5.0 ml and 10.0 ml. Carry out reduction of standards exactly as described for samples. Compare at least one nitrite standard to a reduced nitrate standard at the same concentration to verify reduction column efficiency. Reactivate copper-cadmium granules when reduction efficiency falls below 75 percent.

6.3.6 *Calculation*

Obtain a standard curve by plotting absorbance of standards against nitrate- nitrogen concentration. Compute sample concentration directly from standard curve. Report as milligrams of oxidized nitrogen per litre (sum of nitrate nitrogen plus nitrite nitrogen) unless the concentration of nitrite nitrogen is separately determined and corrected for.

Concentration of nitrate-nitrogen, in mg/l

$$= \frac{\text{Absorbance-intercept of the regression line}}{\text{Slope of the regression line}} \quad \ldots (4)$$

Concentration of nitrate, in mg/l = Concentration of nitrate-nitrogen, in mg/l × 4.43 …..(5)

6.4 Ultraviolet Spectrophotometric Screening Method

6.4.1 *Principle*

Use this technique only for screening samples that have low organic matter contents that is uncontaminated natural waters and potable water supplies. The NO_3^- calibration curve follows beer's law up to 11 mg N/l. Measurement of UV absorption at 220 nm enables rapid determination of NO_3^- because dissolved organic matter may also absorb at 220 nm while NO_3^- does not absorb at 275 nm, a second measurement made at 275 nm may be used to correct the NO_3^- value.

Consequently, this method is not recommended if a significant correction for organic matter absorbance is required, although it may be useful in monitoring NO_3^- levels within a water body with a constant type of organic matter. Sample filtration is intended to remove interference from suspended particles. Acidification with 1 N HCl is designed to prevent interference from hydroxide or carbonate concentration up to 1 000 mg $CaCO_3$/l. Chloride has no effect on the determination.

6.4.2 *Interference*

Dissolved organic matter, surfactants, NO_3^- and Cr^{6+} interfere. Various inorganicions not normally found

in natural water, such as chlorite and chlorate may interfere. Inorganic substances can be compensated for by independent analysis of their concentrations and preparation ofindividual correction curves.

6.4.3 *Apparatus*

6.4.3.1 *Spectrophotometer*

For use at 220 nm and 275 nm with matched silica cells of 1 cm or longer light path.

6.4.4 *Reagents*

6.4.4.1 *Nitrate-free water*

Use re-distilled or distilled, de-ionized water of highest purity toprepare all solutions and dilutions.

6.4.4.2 *Stock nitrate solution*

Dry potassium nitrate (KNO_3) in an oven at 105 °C for 24 h. Dissolve 0.721 8 g in water and dilute to 1 000 ml (1 ml = 100 µg NO_3^-–N). Preserve it with 2 ml chloroform. This solution is stable for 6 months.

6.4.4.3 *Intermediate nitrate solution*

Dilute 100 ml stock nitrate solution 1 000 ml with water (1 ml = 10 µg NO_3^-–N). Preserve with 2 ml chloroform. This solution is stable for 6 months.

6.4.4.4 *Hydrochloric acid, HCl* — 1 N

Dilute 8.33 ml concentrated HCl in 100 ml of distilled water.

6.4.5 *Procedure*

6.4.5.1 *Treatment of sample*

To 50 ml clear sample add 1 ml hydrochloric acid (HCl) solution and mix thoroughly.

6.4.5.2 *Preparation of standard curve*

Prepare NO_3^-–N calibration standards in the range 0 mg to 7 mg NO_3^-–N/l by diluting to 50 ml the following volumes of intermediate nitrate solution: 0 ml, 1 ml, 2 ml, 4 ml, 7 ml,…35 ml. Treat NO_3–N standards in same manner as samples.

6.4.5.3 *Spectrophotometric measurement*

Read absorbance against distilled water set at zero absorbance. Use a wavelength of 220 nm to obtain NO_3–N reading and a wavelength of 275 nm to determine interference due to dissolved organic matter.

6.4.6 *Calculation*

For samples and standards, subtract two times the absorbance reading at 275 nm from the reading at 220 nm to obtain absorbance due to NO_3^-–N. Construct a standard curve by plotting absorbance due to NO_3^- against NO_3^-–N concentration of standard. Using corrected sample absorbances, obtain sample concentrations directly from standard curve. If correction value is more than 10 percent of the reading at 220 nm, do not use this method.

6.5 Nitrate Electrode Method

6.5.1 *Principle*

The specific nitrate electrode to be used in the ion meter is having a selective sensor and measures the nitrate concentration from 0.14 mg/l to 1 400 mg/l. The electrode response is linearly related to logarithmic activity of nitrate activity.

6.5.2 *Interferences*

Chlorides, bicarbonates, cyanides, sulphides in higher concentration may interfere. Hence buffer solution with silver sulphate is needed if interferences are observed. If nitrites are also present sulphamic acid is to be used.

6.5.3 *Apparatus*

6.5.3.1 *ISE meter*

Use a *p*H/mv/ISE meter which is capable of measuring 0.1 mv resolution.

6.5.3.2 Nitrate electrode with a double junction electrode or a combined electrode with suitable reference solution (ammonium sulphate) as per manufactures instructions for storage and usage.

6.5.3.3 *Magnetic stirrer with teflon bars for constant stirring*

6.5.3.4 *Pipettes*

6.5.4 *Reagents*

6.5.4.1 *Stock nitrate solution*

Dissolve 0.721 8 dry potassium nitrate in 1 000 ml water (1 ml = 100 µg/nitrate). Otherwise use standard CRM grade reagents.

6.5.4.2 *Standard nitrate solution*

Dilute 1.0 ml, 10.0 ml and 50 ml stock nitrate solution to 100 ml with reagent water to obtain the standard solutions of 1 mg, 10 mg, 50 mg NO_3^-–N/l and measure with the instrument.

6.5.4.3 *Calibration verification standard*

Dissolve 10 ml of stock nitrate solution standard and dilute to 100 ml (1 ml = 10 mg NO_3^-–N/l).

6.5.4.4 *Buffer solution* [*total ionic strength adjustment buffer* (*TISAB*)]

Dissolve 17.32 g aluminium sulphate ($Al_2(SO_4)_3.18H_2O$) and 3.43 g silver sulphates and 1.28 g boric acid and 2.52 g sulphamic acid in about 800 ml reagent water. Adjust to *p*H 3.0 by slowly adding 0.10 N NaOH. Dilute to 1 000 ml with water and add to amber colour bottle. Alternatively use commercial grade buffers containing CDTA reagent.

6.5.4.5 *Sodium hydroxide* — 0.1 M

6.5.4.6 *Reference electrode filling solution*

Dissolve 0.53 g ammonium sulphate in a reagent water and dilute to 100 ml.

6.5.5 *Procedure*

6.5.5.1 *Treatment of sample*

Take equal amount of sample or standard and buffer in a 50 ml beaker soas to immerse electrode properly and measure the values.

6.5.5.2 *Calibration of ISE meter*

Follow the manufactures instruction and operate the instrument in concentration mode. Set units in mg/l and enable non-linear calibration features. Perform three point calibration using 1 mg, 10 mg, 50 mg NO_3^-–N/l standards. Remove electrodes and blot dry with tissue paper. The slope must be negative, with an absolute value > 50 mv/decade. If the instrument is used in millivolts mode then the slope must be 57 mv/decade ± 3 mv/decade and follow the manufactures instruction.

6.5.6 *Calculation*

The ISE meter once calibrated will read the value directly. For mv measurement ona *p*H meter, find the slope, intercept, and correlation coefficient of the calibration line via least square linear regression using an electronic spread sheet or calculator.

$$E = I + S \times \text{Log}_{10}(C) \quad \text{....(6)}$$

where

E = electrode potential;

I = interception of calibration line;

S = slope of calibration line; and

C = NO_3^-–N concentration in standard.

Use I and S to calculate NO_3^-–N in the sample

$$C = 10^{(E-I)/S} \quad \text{... (7)}$$

7 NITRITE NITROGEN

7.1 General

Nitriteis an intermediate oxidation state of nitrogen. It is an etiological agent of methemoglobinemia. The NO_2 dissolves in water and produces nitrous acid which reacts with secondary amines to form nitrosamines which are carcinogens. It is an indicator of recent pollution in the nearby area in rivers and lakes.

7.2 Principle

Nitrite is determined through formation of a reddish purple azo dye produced at *p*H 2.0 to 2.5 by coupling diazotized sulphanilamide with N-(1 naphthyl)-ethylene diaminedihydrochloride (NED dihydrochloride). The colour obeys Beers' law up to 180 µg/l with 1 cm path length at 543 nm.

7.3 Interference

Nitrogen trichloride (NCl_3) imparts a false red colour when normal order of reagents addition is followed. It can be minimized by adding NED di-hydrochloride first and then sulphanalic acid. Ions like Sb^{3+}, Au^{3+}, Fe^{3+}, Bi^{3+}, Pb^{2+}, Hg^{2+}, Ag^+, $PtCl_6^{2-}$ interfere. Cupric ions cause low results.

7.4 Apparatus

7.4.1 *Spectrophotometer or Photometer*

For use at 543 nm in case of spectrophotometer having a green filter and having maximum absorbance near 540 nm.

7.4.2 *Nessler Tubes* — matched, 50 ml capacity.

7.5 Reagents

7.5.1 *Nitrite-Free Water*

If the distilled water is not nitrite free, prepare as follows:

a) Add to 1 litre of distilled water, a small crystal each of potassium permanganate and barium hydroxide or calcium hydroxide. Re-distil in an all-borosilicate glass bottle; and

b) Add 1 ml of concentrated sulphuric acid and 0.2 ml of manganese sulphate (36.48 g $MnSO_4.H_2O$/100 ml) solution to each 1 litre of distilled water and make pink with 1 ml to 3 ml of potassium permanganate solution (400 mg/l). Re-distil as in (a) above. Use this water in making all reagents and dilutions.

7.5.2 *Color Reagent*

To 800 ml water**,** add 100 ml of 85 percent phosphoric acid and 10 g of sulphanilamide. After dissolving sulphanilamide completely, add 1 g NED reagent [N-(1-naphthyl)-ethylenediamine dihydrochloride. Mix to dissolve, then dilute to 1 litre with water. Solution is stable for one month if stored in a dark amber colour bottle in refrigerator.

7.5.3 *Hydrochloric Acid* — 1 : 3

7.5.4 *Sodium Oxalate* — 0.05 N

Dissolve 3.350 g of sodium oxalate (primary standard grade) in 1 000 ml of water.

7.5.5 *Ferrous Ammonium Sulphate* — 0.05 N

Dissolve 19.607 g of ferrous ammonium sulphate in 20 ml of concentrated sulphuric acid and water and dilute to 1 litre. Standardize the solution by using standard dichromate.

7.5.6 *Potassium Permanganate Solution* ($KMNO_4$) — 0.05 N

Dissolve 1.6 g $KMNO_4$ in distilled water and keep in a brown bottle and age it for one week. Decant the supernatant and standardize as follows. Weigh 100 mg to 200 mg of anhydrous sodium carbonate into 400 ml beaker. Add 100 ml distilled water and stir to dissolve. Then add 10 ml of 1 + 1 H_2SO_4 and heat rapidly to 90 °C to 95 °C. Titrate rapidly with potassium permanganate to be standardized. Take care that the slight pink end point should persist for one minute. Do not let the temperature decrease to less than 85 °C. 100 mg of sodium carbonate may consume 6 ml of $KMN0_4$ solution. Run a blank on distilled water as per the above procedure.

$$\text{Normality of } KMNO_4 = \frac{W}{(A-B) \times 0.067} \quad \ldots. (8)$$

where

W = mass, in mg, of sodium oxalate;

A = volume, in ml, of titrant for sample; and

B = volume, in ml, of titrant for blank.

7.5.7 *Stock Nitrite Solution*

Dissolve 1.232 g of sodium nitrite in water and dilute to 1 000 ml (1 ml = 250 μg of N). Preserve it by using 1 ml of chloroform. Standardize the nitrite solution by using sodium oxalate and standard potassium permanganate solution.

7.5.7.1 *Standardization*

Pipette out 50 ml of standard potassium permanganate and add 5 ml concentrated sulphuric acid and 50 ml of stock nitrite solution into a glass stoppered conical flask. Warm gently to 70 °C to 80 °C and shake it. On hot plate, discharge permanganate colour by adding sufficient 10 ml portions of 0.025 M sodium oxalate. Titrate the excess of sodium oxalate with 0.05 $KMNO_4$ to the faint pink end point. Carry a blank with water. Alternatively 0.05 M ferrous ammonium sulphate can also be used instead of sodium oxalate and heating step can be avoided.

Calculate the NO_2^-–N content of stock nitrite solution as follows:

$$NO_2^-\text{–N, mg/ml (A)} = \frac{(B \times C) - (D \times E) \times 7}{F} \quad \ldots. (9)$$

where

A = concentration, in mg/ml, of NO_2^-–N in stock $NaNO_2$;

B = total volume, in ml, of $KMNO_4$ used;

C = normality, in ml, of standard $KMNO_4$;

D = total volume, in ml, of standard reductant used;

E = normality of standard reductant; and

F = volume, in ml, of stock nitrite solution taken for titration.

Generally 1 ml of 0.05 $KMNO_4$ consumed by the $NaNO_2$ corresponds to 1 725 µg of $NaNO_2$ or 375 µg of NO_2–N

Concentration of nitrite, mg/l = concentration of nitrite-nitrogen, mg/l * 3.29 …(10)

7.5.8 *Intermediate Nitrite Solution*

Calculate the volume of stock nitrite solution G which is required for the intermediate nitrite solution such that

$G = 12.5/A$,

where

A is the stock solution in mg/l. Dilute the volume G up to 250 ml by using distilled water (1.00 ml = 50.0 µg of N).

7.5.9 *Standard Nitrite Solution*

Dilute 10.00 ml volume of the intermediate nitrite solution to 1 litre with distilled water (1.00 ml = 0.500 µg of N).

7.6 Procedure

7.6.1 If the sample is turbid, filter through a 0.45 µg membrane filter. To 50.0 ml of clear sample neutralized to *p*H 7 or to a portion diluted to 50 ml, add 2 ml of colour regent. Let the reagent react for 2 min to 8 min. Let stand for at least 10 min but not more than 2 h. Measure absorbance at 543 nm. As a guide, use the following light paths for the indicated nitrite nitrogen concentrations as specified in Table 2.

Table 2 Light Paths for the Indicated Nitrite Nitrogen Concentrations

(*Clause* 6.6.6)

Sl No.	Light Path Length	Nitrite Nitrogen
	cm	µg/l
(1)	(2)	(3)
i)	1	2–25
ii)	5	2 – 6
iii)	10	< 2

Run parallel checks frequently against nitrite standards.

7.6.1.1 *Colour standards for visual comparison*

Prepare a suitable series of visual colour standards in the nessler tubes by adding the following volumes of standard nitrite solutions and diluting to 50 ml with water: 0 ml, 0.1 ml, 0.2 ml, 0.4 ml, 0.7 ml, 1.0 ml, 1.4 ml, 1.7 ml, 2.0 ml and 2.5 ml, corresponding, respectively to 0 µg, 1.0 µg, 2.0 µg, 4.0 µg, 7.0 µg, 10 µg, 14 µg, 17 µg, 20 µg and 25 µg of nitrite/l. Develop colour as described in **7.6.1**.

7.6.1.2 Compare samples to visual standards in matched Nessler tubes between 10 and 120 min after adding NED di-hydrochloride reagent. Select the concentration where the sample tube colour matches the standard tube colour.

7.7 Calculation

Calculate nitrite nitrogen from the following: prepare a Standard curve by plotting absorbanceof standards against NO_2^-–N Concentration and compute the sample concentration from curve.

8 ORGANIC NITROGEN

8.1 General

The two methods for determination of organic nitrogen are as follows:

a) Macro Kjeldahl method; and
b) Semi-Micro-Kjeldahl method.

The major factor that influences the selection of Macro or Semi-Micro Kjeldahl method is the concentration of organic nitrogen. The Macro-Kjeldahl method is applicable to sample containing either low or high concentration of organic nitrogen whereas the semi-micro method is applicable to samples containing high concentration of organic nitrogen. Macro-Kjeldahl method shall be the referee method.

8.2 Macro-Kjeldahl Method

8.2.1 *Principle*

In the presence of sulphuric acid, potassium sulphate, and mercuric sulphate catalyst, amino-nitrogen of many organic materials is converted to ammonium sulphate. Free ammonia and ammonium nitrogen are also converted into ammonium sulphate. During sample digestion, a mercury ammonium complex is formed and then decomposed by sodium thiosulphate. After decomposition, the ammonia is distilled from an alkaline medium and absorbed in boric or sulphuric acid. The ammonia is determined colorimetrically or by titration with a standard mineral acid or electrode method also.

8.2.2 A*pparatus*

8.2.2.1 *Digestion apparatus*

Kjeldahl flasks with a total capacity of 800 ml yield the best results. The heating device meeting this specification should provide the temperature range of 365 °C to 370 °C for effective digestion. Alternatively ready to use digestive assemblies can also be used.

8.2.2.2 *Distillation apparatus*

8.2.2.3 *Apparatus for ammonia determination*

8.2.2.4 *Colorimetric equipment*

Spectrophotometer or photometer suitable for use at 400 nm to 500 nm. The photometer should be equipped with a violet filter.

8.2.3 *Reagents*

8.2.3.1 *Digestion reagent*

Dissolve 134 g of potassium sulphate and 7.3 g $CuSO_4$ in about 800 ml water. Carefully add 134 ml concentrated H_2SO_4. when it has cooled to room temperature, dilute the solution to 1 litre with water. Mix well and does not allow to crystalize by maintaining a temperature close to 20 °C.

8.2.3.2 *Sodium hydroxide–sodium thiosulphate*

Dissolve 500 g of sodium hydroxide and 25 g of sodium thiosulphate ($Na_2S_2O_35H_2O$) in water and dilute to 1 litre.

8.2.3.3 *Borate buffer solution*

Add 88 ml of 0.1 N sodium hydroxide solution to 500 ml of approximately 0.025 M sodium tetraborate solution and dilute to 1 litre.

8.2.3.4 *Sodium hydroxide* — 6 N

8.2.3.5 All other reagents needed for estimation of ammonical nitrogen as per method no 2.0 titration method.

8.2.4 *Procedure*

8.2.4.1 *Selection of Sample Volume and Sample Preparation*

Place a measured volume of sample in an 800 ml Kjeldahl flask. Select sample size from the following table:

Sl No.	*Organic Nitrogen in Sample, mg/l*	*Sample Size, ml*
(1)	(2)	(3)
i)	0 to 1	500
ii)	1 to 10	250
iii)	10 to 20	100
iv)	20 to 50	50.0
v)	50 to 100	25.0

If necessary, dilute sample to 300 ml, neutralize to *p*H 7, and dechlorinate as given in **5.2.3**.

8.2.4.2 *Ammonia removal*

Add 25 ml borate buffer and then 6 N sodium hydroxide until *p*H 9.5 is reached. Add a few glass beads or boiling chips and boil off 300 ml. If desired, distil this fraction and determine ammoniacal nitrogen. Alternatively, if ammonia has been determined by the distillation method, use residue in distilling flask for organic nitrogen determination.

8.2.4.3 *Digestion*

Cool and add carefully 50 ml of digestion reagent to distillation flask. Add a few glass beads and after mixing, heat under a hood or with suitable ejection equipment to remove acid fumes. Boil briskly until the volume is greatly reduced and copious white fumes are observed. Then continue digestion for additional 30 minutes. As digestion continues, coloured or turbid samples will turn clear or straw coloured. After digestion, let flask and contents cool, dilute to 300 ml with water and mix. Tilt flask and carefully add 50 ml of hydroxide thiosulphate to form an alkaline layer at flaskbottom. Connect flask to steamed-out distillation apparatus and shake flask to insure complete mixing. A black precipitate of mercuric sulphide will form and the *p*H should exceed 11.0.

8.2.4.4 *Distillation*

Distil and collect 200 ml of distillate below surface of 50 ml absorbent solution. Use plain boric acid solution when ammonia is to be determined by nesslerization and use indicating boric acid for titrimetric finish. Use 50 ml of 0.04 N sulphuric acid for collecting distillate for manual phenate, nesslerization or electrode methods. Extend tip of condenser well below the level of absorbent solution and do not let temperature in condenser rise above 29 °C. Lower collected distillate free of contact with delivery tube and continue during last 1 min or 2 min to cleanse condenser.

8.2.4.5 *Final ammonia measurement*

Use the manual phenate, titration or ammonia selective electrode method as given in **5**.

8.2.4.6 *Blank*

Carry out a reagent blank through all steps of the procedure and apply necessary corrections to results.

8.2.4.7 *Calculation*

Calculate ammoniacal nitrogen (NH_3–N) in mg per litre as given in the relevant methods inthe relevant methods in **5**.

8.3 Semi-Micro-Kjeldahl Method

8.3.1 *Apparatus*

8.3.1.1 *Digestion apparatus*

Use Kjeldahl flasks with a capacity of 100 ml in a semi-micro-Kjeldahl digestion apparatus equipped with heating elements to accommodate Kjeldahl flasks and a suction outlet to vent fumes. The heating elements should provide the temperature range of 365 °C to 380 °C for effective digestion.

8.3.1.2 *Distillation apparatus*

Use an all glass unit equipped with a steam-generating vessel containing an immersion heater as shown in Fig. 2.

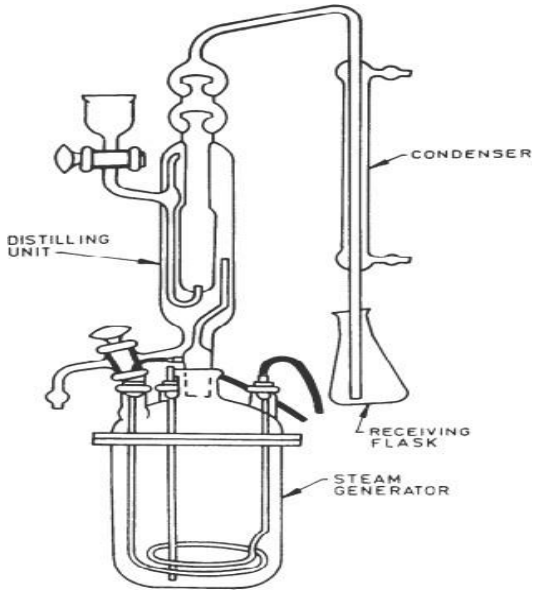

FIG. 2 MICRO-KJEDAHL DISTILLATION APPARATUS

8.3.1.3 *p*H *meter*

8.3.2 *Reagents*

All reagents listed for determination of nitrogen by the various methods given in **5** and **8.2.3** are required.

8.3.3 *Procedure*

8.3.3.1 *Selection of sample volume*

Determine the sample size from the following table:

Sl No.	*Organic Nitrogen in the Sample, mg/l*	*Sample Size, ml*
(1)	(2)	(3)
i)	4 to 40	50
ii)	8 to 80	25
iii)	20 to 200	10
iv)	40 to 400	5

8.3.3.2 *Ammonia removal*

Pipette 50 ml or appropriate volume of the sample diluted to 50 ml. with water into a 100 ml beaker. Add 3 ml of borate buffer and adjust to *p*H 9.5 with 6 N sodium hydroxide, using a *p*H meter. Quantitatively transfer sample to a 100 ml Kjeldahl flask and boil off 30 ml. Alternatively, if ammonia removal is not required, digest samples directly as described in **8.3.3.3**.

Distillation following this direct digestion yields Kjeldahl nitrogen concentration rather than organic nitrogen.

8.3.3.3 *Digestion*

Carefully add 10 ml of digestion reagent to Kjeldahl flask containing sample. Add 5 or 6 glass beads to prevent bumping. Set each heating unit on micro Kjeldahl digestion apparatus to its medium setting and digest for an additional 30 minutes. Cool quantitatively transfer digestion sample by diluting and rinsing several times into micro Kjeldahl distillation apparatus so that total volume in distillation apparatus does not exceed 30 ml. Add 10 ml of hydroxide-thiosulphate reagent and turn on steam.

8.3.3.4 *Distillation*

Control rate of steam generation to boil contents in distillation unit so that neither the escape of steam from tip of the condenser nor the bubbling of contents in the receiving flask occurs. Distil and collect 30 ml to 40 ml distillate below surface of 10 ml boric acid solution contained in 125 ml Erlenmeyer flask. Use plain boric acid solution when ammonia is to be determined by nesslerization and use indicating boric acid for a titrimetric finish. Use 10 ml of 0.24 N sulphuric acid solution for collecting distillate for the phenate, Nessler or electrode methods. Extend tip of condenser well below the level of boric acid solution and do not let temperature in condenser rise above 29 °C. Lower collected distillate free of contact with delivery tube and continue distillation during last 1 min or 2 min to cleanse condenser.

8.3.3.5 *Blank*

Carry out a reagent blank through all steps of procedure and apply necessary correction to results.

8.3.3.6 *Final ammonia measurement*

Determine ammonia by any of the methods prescribed in **5**.

8.3.3.7 *Calculation*

Calculate nitrogen, organic as per calculations given under different methods prescribed in **5**.

ANNEX A

(*Foreword*)

COMMITTEE COMPOSITION

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This Indian Standard has been developed from Doc No.:CHD 36 (17012).

Amendments Issued Since Publication

Amend No.	Date of Issue	Text Affected

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Published by BIS, New Delhi