Indian Standard (Reaffirmed 1999)

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

PART 34 NITROGEN

(First Revision)

Water Sectional Committee, CDC 26; Panel for Methods of Test for Water, Wastewater and Effluents, CDC 26 : P1 [Ref : Doc : CDC 26 (9302)]

1. Scope — Prescribes methods for determination of various types of nitrogen like ammoniacal, nitrate, nitrite and organic in water and wastewater.

2. Ammoniacal Nitrogen

2.1 *General* — Prescribes four methods as follows for determination of ammoniacal nitrogen:

a) Nesslerization method,

b) Phenate method,

c) Titrimetric method, and

d) Ammonia selective electrode method.

2.1.1 Nesslerization method shall be the refree method.

2.2 *Preliminary Distillation Step*

2.2.1 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.

2.2.2 Add 500 ml water and 20 ml of borate buffer solution (*see* **3.4.4.2**) to a distillation flask and adjust *p*H to 9·5 with 6N 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.

2.2.3 Use 500 ml of dechlorinated sample or a portion diluted to 500 ml with water. Remove residual chlorine by adding, 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 6N sodium hydroxide solution using a *p*H meter.

2.2.4 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 to 10 ml/minute with the tip of the delivery tube below the surface of acid receiving solution. Collect distillate in a 500-ml Erlenmeyer flask containing 50 ml plain boric acid solution for nesslerization method. Use 50 ml indicating boric acid (*see* **2.5.3.2**) 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 1N sodium hydroxide solution.

2.3 *Nesslerization Method*

2.3.1 *Principle* — The sample is buffered and distilled. The ammonia in the distillate or in the sample is treated with Nessler's reagent and the colour developed is matched with that of a series of standard ammonia solutions or measured photometrically at 400 to 425 nm.

2.3.2 *Apparatus*

2.3.2.1 *Spectrophotometer* — for use at 400 to 500 nm.

2.3.2.2 *Filter photometer* — equipped with violet filter and having maximum absorbance at 400-425 nm.

2.3.2.3 *Nessler tubes*

2.3.2.4 *p*H *meter*

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2.3.3 *Reagents*

2.3.3.1 *Zinc sulphate solution* — Dissolve 100 g of zinc sulphate $ZnSO_4 7H_2O$ and dilute to 1 litre with water.

2.3.3.2 *Stabilizer reagent* — Use EDTA or Rochelle salt to prevent calcium or magnesium precipitation in undistilled samples:

a) *EDTA reagent* — Dissolve 50 g of EDTA in 60 ml water containing 10 g of sodium hydroxide. Heat gently to complete dissolution. Cool to room temperature and dilute to 100 ml.

b) *Rochelle salt solution* — Dissolve 50 g of potassium sodium tartrate tetrahydrate in 100 ml of water.

2.3.3.3 *Nessler's reagent* — Dissolve 100 g of mercuric iodide and 70 g of potassium iodide in a small quantity of water and add this mixture slowly, with stirring, to a cool solution of 160 g of sodium hydroxide dissolved in 500 ml of water. Dilute to 1 litre. Store in brown rubber stopper glass bottle. Reagent is stable up to one year. It is toxic and so avoid ingestion.

2.3.3.4 *Stock ammonia solution* — Dissolve 3·819 g of anhydrous ammonium chloride in water and dilute to 1 litre. (1·00 ml = 1·00 mg of nitrogen = 1·22 mg of ammonia).

2.3.3.5 *Standard ammonia solution* — Dilute 10·00 ml of stock solution to 1 000 ml with water (1·00 ml = 12·2 μg of ammonia = 10·0 μg of N).

2.3.3.6 *Permanent colour solutions*

a) *Potassium chloroplatinate solution* — Dissolve 2·0 g of potassium chloroplatinate in 300 to 400 ml of water, add 100 ml of concentrated hydrochloric acid and dilute to 1 litre.

b) *Cobaltous chloride solution* — Dissolve 12·0 g of cobaltous chloride ($CoCl_2.6H_2O$) in 200 ml of water. Add 100 ml of concentrated hydrochloric acid and dilute to 1 litre.

2.3.4 *Procedure*

2.3.4.1 *Treatment of undistilled samples* — If necessary, remove residual chlorine. Add 1 ml of zinc sulphate solution to 100 ml of sample and mix thoroughly. Add 0·4 to 0·5 ml of 6 N sodium hydroxide solution to obtain a *p*H of 10·5 and mix well. Let treated sample stand for a few minutes, whereupon a heavy flocculent precipitate should fall, leaving a clear and colourless supernate. Clarify by centrifuging or filtering. Pretest any filter paper used to be sure no ammonia is present as a contaminant. Do this by running water through the filter and testing the filtrate by nesslerization. Filter sample, discarding first 25 ml of filtrate.

2.3.4.2 *Colour development*

a) *Undistilled samples* — Use 50 ml of sample or a portion diluted to 50 ml with water. If undistilled portion contains sufficient concentrations of calcium, magnesium or other ions that produce turbidity or precipitate with nessler reagent, add 1 drop of EDTA reagent or 1 or 2 drops of Rochelle salt solution. Mix well. Add 2·0 ml of nessler reagent if EDTA is used or 1·0 ml of nessler reagent if Rochelle salt is used.

b) *Distilled samples* — Neutralize the boric acid used for absorbing ammonia distillate by adding either 2 ml of nessler's reagent, an excess that raises the *p*H to the desired high level or alternatively, neutralizing the boric acid with sodium hydroxide before adding 1 ml nessler's reagent.

2.3.4.3 Mix samples by capping nessler tubes with clean rubber stoppers and then inverting the tubes at least 6 times. Keep such conditions as temperature and reaction time the same in blank, samples and standards. Let reaction proceed for at least 10 minutes after adding nessler reagent. Measure colour in sample and standards. If ammoniacal nitrogen is very low, use a 30 minute contact time for sample, blank and standards. Measure colour either by photometry or visually as given in **2.3.4.4** or **2.3.4.5**.

2.3.4.4 *Photometric measurement* — Measure absorbance or transmittance with spectrophotometer or filter photometer. Prepare calibration curve at the same temperature and reaction time used for samples. Measure transmittance readings against a reagent blank and run parallel checks frequently against standards in the nitrogen range of the samples. Redetermine complete calibration curve for each new batch of nessler reagent.

For distilled samples, prepare standard curve under the same conditions as the samples. Distill reagent blank and appropriate standards, each diluted to 500 ml, in the same manner as the samples. Dilute 300 ml of distillate plus 50 ml of boric acid absorbent to 500 ml with water and take a 50 ml portion for nesslerization.

2.3.4.5 *Visual comparison* — Compare colours produced in sample against those of ammonia standards. Prepare temporary or permanent standards as follows:

a) *Temporary standards* — Prepare a series of visual standards in nessler tubes by adding the following volumes of standard ammonium chloride solution and diluting to 50 ml with water: 0, 0·2, 0·4, 0·7, 1·0, 1·4, 1·7, 2·0, 2·5, 3·0, 3·5, 4·0, 4·5, 5·0 and 6·0 ml. Nesslerize standards and portions of distillate by adding 1·0 ml nessler reagent to each tube and mixing well.

b) *Permanent standards* — Measure into 50-ml nessler tube, the volumes of potassium chloroplatinate and cobaltous chloride solutions indicated in Table 1, dilute to mark and mix well. The values given in the table are approximate; actual equivalents of the ammonium standards will differ with the quantity of nessler reagent, the kind of illumination used and the colour sensitivity of analyst's eye. Therefore, compare colour standards with nesslerized temporary ammonia standards and modify the tints as necessary. Make such comparisons for each newly prepared nessler reagent and satisfy each analyst as to the aptness of the colour match. Protect standards from dust to extend their usefulness for several months. Compare either 10 or 30 minutes after nesslerization, depending upon reaction time used in preparing nesslerized ammonium standards against which they were matched.

TABLE 1 PREPARATION OF PERMANENT COLOUR STANDARDS FOR VISUAL DETERMINATION OF AMMONIACAL NITROGEN

[*Clause* 2.3.4.5(b)]

Value in Ammoniacal Nitrogen, µg	Approximate Volume of Platinum Solution, ml (in matched 50 ml nessler tubes)	Approximate Volume of Cobalt Solution, ml (in matched 50 ml nessler tubes)
0	1·2	0·0
2	2·8	0·0
4	4·7	0·1
7	5·9	0·2
10	7·7	0·5
14	9·9	1·1
17	11·4	1·7
20	12·7	2·2
25	15·0	3·3
30	17·3	4·5
35	19·0	5·7
40	19·7	7·1
45	19·9	8·7
50	20·0	10·4
60	20·0	15·0

2.3.5 *Calculation*

2.3.5.1 Deduct the amount of ammoniacal nitrogen in water used for diluting original sample before computing final nitrogen value.

2.3.5.2 Deduct also reagent blank for volume of borate buffer and 6 N sodium hydroxide solutions used with sample.

2.3.5.3 Compute total ammoniacal nitrogen by the following equation:

$$\text{Nitrogen, ammoniacal, mg/l (51 ml of final volume)} = \frac{A}{V} \times \frac{B}{C}$$

where

A = µg of ammoniacal nitrogen (51 ml of final volume);

B = total volume of distillate collected, in ml, including acid absorbent;

C = volume distillate taken for nesslerization, in ml; and

V = volume in ml of sample taken.

Note — The ratio B/C applies only to distilled samples, and should be ignored in direct nesslerization.

2.4 *Phenate Method*

2.4.1 *Principle* — An intensely blue compound, indophenol, is formed by the reaction of ammonia, hypochlorite and phenol catalyzed by a manganous salt.

2.4.2 *Interference* — Alkalinity over 500 mg/litre as calcium carbonate or acidity over 100 mg/l as calcium carbonate or turbidity interfere. Remove these by preliminary distillation.

2.4.3 *Apparatus*

2.4.3.1 *Spectrophotometer or filter photometer* — for use at 630 nm. The photometer is equipped with a red-orange filter. The light path of these photometers should be 1 cm, approximately.

2.4.3.2 *Magnetic stirrer*

2.4.4 *Reagents*

2.4.4.1 *Ammonia-free water* — Prepare ammonia free water by passing distilled water through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin. Alternatively, redistil distilled water by adding 0·1 ml of concentrated sulphuric acid to 1 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 to 5 mg/l. Redistil after standing at least for 1 hour. Discard the first 100 ml distillate.

2.4.4.2 *Hypochlorous acid reagent* — Add to 40 ml of water, 10 ml of 5 percent solution of sodium hypochlorite prepared from commercial bleaching powder. Adjust *p*H to 6·5 to 7·0 with hydrochloric acid.

2.4.4.3 *Manganese sulphate solution* — 0·006 N. Dissolve 50 mg of manganous sulphate monohydrate in 100 ml of water.

2.4.4.4 *Phenate reagent* — Dissolve 2·5 g of sodium hydroxide and 10 g of phenol in 100 ml of water. Prepare weekly. Since phenol is corrosive, handle with care.

2.4.4.5 *Stock ammonium solution* — Dissolve 381·9 mg of anhydrous ammonium chloride in water and dilute to 1 000 ml (1·00 ml = 122 µg as NH_3 or 100 µg as N).

2.4.4.6 *Standard ammonia 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).

2.4.5 *Procedure*

2.4.5.1 *Treatment of sample* — To a 10·0 ml of sample in a 50 ml beaker, add 1 drop of manganous sulphate solution. Place on a magnetic stirrer and add 0·5 ml of hypochlorous acid reagent. Immediately add, a drop at a time, 0·6 ml of phenate reagent. Add reagent without delay using a bulb pipette or burette for convenient delivery. Mark pipette for hypochlorous acid at the 0·5 ml level and deliver the phenate reagent from a pipette or burette that has been calibrated by counting the drops previously found to be equivalent to 0·6 ml. Stir vigorously during addition of reagents. Because colour intensity is affected by the age of reagents, carry a blank and a standard through the procedure with each batch of samples. Measure absorbance using reagent blank to zero in the spectrophotometer. Colour formation is complete in 10 minutes and is stable for at least 24 hours. Although the blue colour has a maximum absorbance at 630 nm, satisfactory measurements can be made in 600 to 660 nm region.

2.4.5.2 *Preparation of standards* — Prepare a calibration curve in the ammoniacal nitrogen range of 0·1 to 5 μg, treating standards exactly as the sample.

2.4.6 *Calculation*

2.4.6.1 Calculate ammonia concentration as follows:

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

where

A = absorbance of sample;

B = ammoniacal nitrogen in the standard, μg;

C = absorbance of standard;

S = volume in ml of sample;

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

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

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

2.5 *Titrimetric Method*

2.5.1 *Principle* — The method is used only on samples that have been carried through preliminary distillation (*see* **2.2**). Use the following values for selecting sample volume for the distillation and titration method:

NH_3-N in Sample, mg/l	*Sample Volume,* ml
5 - 10	250
10 - 20	100
20 - 50	50.0
50 - 100	25·0

The ammonia in distillate is titrated with standard sulphuric acid.

2.5.2 *Apparatus*

2.5.2.1 *Distillation assembly* — Borosilicate glass flask of 800 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.

2.5.3 *Reagents*

2.5.3.1 *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.

2.5.3.2 *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.

2.5.3.3 *Standard sulphuric acid titrant* — 0·02 N (1 ml = 280 μg of nitrogen).

2.5.4 *Procedure*

2.5.4.1 Proceed as prescribed in **2.2** using indicating boric acid solution as absorbent for distillate.

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

2.5.5 *Calculation*

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

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.

2.6 *Ammonia Selective Electrode Method*

2.6.1 *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 (aq) by raising *p*H to above 11 with a strong base. NH_3 (aq) diffuses through the membrane and changes the 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.

2.6.2 *Interference* — Amines are a positive interference. Mercury and silver interfere by complexing with ammonia.

2.6.3 *Apparatus*

2.6.3.1 *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.

2.6.3.2 *Ammonia-selective electrode*

2.6.3.3 *Magnetic stirrer*

2.6.4 *Reagents*

2.6.4.1 *Ammonia free water* — *See* **2.4.4.1**.

2.6.4.2 *Sodium hydroxide solution* — 10 N.

2.6.4.3 *Stock ammonium chloride solution* — *See* **2.4.4.5**.

2.6.4.4 *Standard ammonium chloride solution* — *See* **2.4.4.6**.

2.6.5 *Procedure*

2.6.5.1 *Preparation of standards* — Prepare a series of standard solutions covering the concentrations of 1 000, 100, 10, 1 and 0·1 mg of nitrogen (ammoniacal) by making decimal dilutions of stock ammoniacal chloride solution with water.

2.6.5.2 *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 11. 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 minutes before recording millivolts for standards and samples containing $\leqslant$1 mg of nitrogen (ammoniacal) per litre.

2.6.5.3 *Preservation of standard curve* — Using semilogarithmic 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 tenfold change of ammoniacal nitrogen concentration produces a potential change of 59 mV.

2.6.5.4 *Calibration of specific ion meter* — Following manufacturer's instructions, follow steps given in **2.6.5.1** and **2.6.5.2**.

2.6.5.5 *Measurement of samples* — Dilute, if necessary, to bring ammonical nitrogen to within calibration curve range. Place 100 ml sample in 150 ml beaker and follow procedure given in **2.6.5.2**. Record the volume of 10 N of sodium hydroxide added in excess of 1 ml. Read ammoniacal nitrogen concentration from standard curve.

2.6.6 *Calculation*

$$\text{Ammoniacal nitrogen, mg/l} = A \times B \times \left[\frac{101 + C}{101}\right]$$

where

A = dilution factor;

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

C = volume in ml of added 10 N sodium hydroxide in excess of 1 ml.

3. Nitrate Nitrogen

3.1 Three methods for determination of nitrate, nitrogen in waters and wastewaters are prescribed:

a) Cadmium reduction method,

b) Chromotropic acid method, and

c) Devarda's alloy reduction method.

3.1.1 For concentration below 0·1 mg per litre of nitrate nitrogen, cadmium reduction method is suitable. For concentration from 0·1 to 5·0 mg/l, chromotropic acid method may be made applicable and Devarda's alloy reduction method may be used for concentrations more than 2 mg/l or for total nitrogen. Chromotropic acid method shall be the referee method.

3.2 *Cadmium Reduction Method*

3.2.1 *Principle* — Nitrate is reduced to nitrite in presence of cadmium. The nitrite produced is determined by diazotizing with sulphanilamide and coupling with N-(1 naphthyl) ethylenediamine to form a highly coloured azo dye which is measured colorimetrically.

3.2.2 *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.

3.2.3 *Apparatus*

3.2.3.1 *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 (*see* Fig. 1).

3.2.3.2 *Colorimeter* — One of the following:

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

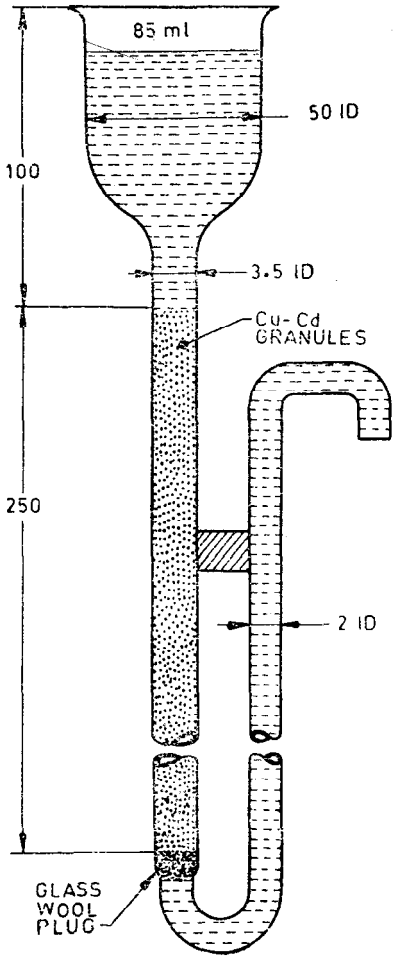
b) *Filter photometer* — provided with a yellow green filter having maximum transmittance near 540 nm and a light path of 1 cm or longer.

3.2.4 *Reagents*

3.2.4.1 *Nitrate free water* — The absorbance of a reagent blank prepared with this water should not exceed 0·01. Use for all solutions and dilution.

3.2.4.2 *Copper-cadmium granules* — Wash 25 g of 40-60 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 minutes 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.

3.2.4.3 *Sulphanilamide reagent* — Dissolve 5 g of sulphanilamide in a mixture of 50 ml concentrated hydrochloric acid and 300 ml of water. Dilute to 500 ml with water. The reagent is stable for many months.



All dimensions in millimetres.

FIG. 1 REDUCTION COLUMN

3.2.4.4 *N-(1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride) solution* — Dissolve 500 mg of NED dihydrochloride in 500 ml of water. Store in dark coloured bottle. Replace as soon as a brown colour develops.

3.2.4.5 *Ammonium chloride — EDTA solution* — Dissolve 13 g of ammonium chloride and 1·7 g of disodium ethylenediamine tetraacetate in 900 ml of water. Adjust *p*H to 8·5 with liquor ammonia and dilute to 1 litre.

3.2.4.6 Dilute 300 ml of the above solution to 500 ml with water to get a dilute solution.

3.2.4.7 *Hydrochloric acid* — 6 N.

3.2.4.8 *Copper sulphate solution* — 2 percent (m/v).

3.2.4.9 *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 per litre (1 ml = 100 μg of nitrate nitrogen).

3.2.4.10 Dilute 50 ml of stock nitrate solution to 500 ml with water to get standard solution 1·00 ml equal to 10·0 μg nitrate nitrogen.

3.2.4.11 *Stock nitrite solution* — Dissolve 0·607 2 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.

3.2.4.12 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).

3.2.5 *Procedure*

3.2.5.1 *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 to 10 ml/minute, 100 ml of a solution comprising 25 ml of a 1·0 mg nitrogen (nitrate) per litre standard and 75 ml of ammonium chloride EDTA solution.

3.2.5.2 *Treatment of sample* — If turbidity or suspended solids are present, remove by filtering through a 0·45 µm 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 to 10 ml/minute. Discard first 25 ml. Collect the rest in original sample flask. There is no need to wash the column between samples but if 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.

3.2.5.3 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 to 8 minutes. Add 2 ml of NED dihydrochloric acid solution and mix immediately. Between 10 min and 2 h afterwards, measure absorbance at 540 nm against a distilled water reagent blank. Using the standard nitrate nitrogen solution, prepare standards in the range of 0·05 to 1·0 mg of nitrate nitrogen per litre by diluting the following volumes of standards to 100 ml in volumetric flasks: 0·5, 1·0, 2·0, 5·0 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.

3.2.6 *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.

3.3 *Chromotropic Acid Method*

3.3.1 *Principle* — Two moles of nitrate nitrogen react with one mole of chromotropic acid to form a yellow reaction product having maximum absorbance at 410 nm.

3.3.2 *Interferences* — Residual chlorine, certain oxidants, and nitrites yield yellow colour with chromotropic acid. Addition of sulphite removes interference from residual chlorine and oxidants. Urea converts nitrites to nitrogen gas. The minimum detectable quantity is 50 µg of nitrate nitrogen per litre.

3.3.3 *Apparatus*

3.3.3.1 *Spectrophotometer* — for use of 410 nm and with a light path of 1 cm or longer.

3.3.3.2 *Photometer* — having maximum transmittance at 410 nm and having a light path of 1 cm or longer and equipped with a violet filter.

3.3.4 *Reagents*

3.3.4.1 *Nitrate-free water — See* **3.2.4.1.**

3.3.4.2 *Stock nitrate solution — See* **3.2.4.9.**

3.3.4.3 *Standard nitrate solution — See* **3.2.4.10.**

3.3.4.4 *Sulphite urea reagent* — Dissolve 5 g of urea and 4 g of anhydrous sodium sulphite in water and dilute to 1 000 ml.

3.3.4.5 *Antimony reagent* — Dissolve 500 mg antimony metal by heating in 80 ml concentrated sulphuric acid. Cool and cautiously add to 20 ml of iced water. If crystals form upon standing overnight, redissolve by heating.

3.3.4.6 *Chromotropic acid reagent* — Dissolve 100 mg of purified chromotropic acid crystals in 100 ml of concentrated sulphuric acid and store in a brown bottle. Prepare every 2 weeks. A colourless reagent solution signifies the absence of nitrate contamination from sulphuric acid.

3.3.4.7 *Sulphuric acid* — concentrated, nitrate free.

3.3.5 *Procedure* — Prepare nitrate standards in the range of 0·10 to 5·0 mg/l by diluting 0, 1·0, 5·0, 10, 25, 40 and 50 ml of standard nitrate solution to 100 ml with water. If appreciable amount of suspended matter is present, filter suitably. Pipette 2·0 ml portions of the standard nitrate solutions, samples and a water blank into dry 10 ml volumetric flasks. To each flask, add 1 drop of sulphite-urea reagent. Place flasks in tray of cold water (10 to 20°C) and add 2 ml of antimony reagent. Swirl flasks during addition of each reagent. After about 4 minutes in the bath, add 1 ml of chromotropic acid reagent, swirl and let stand in cooling bath for 3 minutes. Add concentrated sulphuric acid to bring volume near the 10 ml mark. Stopper the flasks and mix by inverting each flask four times. Let it stand for 45 minutes at room temperature and adjust volume to 10 ml with concentrated sulphuric acid. Perform final mixing very carefully and gently to avoid introducing gas bubbles. Read absorbance at 410 nm between 15 minutes and 24 hours after last volume adjustment. Use nitrate free water in the reference cell of the spectrophotometer.

3.3.6 *Calculation*

$$\text{Nitrate nitrogen (as } NO_3\text{), mg/l} = \frac{\mu\text{g of nitrate nitrogen in 10 ml final volume}}{\text{Volume in ml of sample taken for test}}$$

3.4 *Devarda's Alloy Reduction Method*

3.4.1 *Principle* — The nitrate and nitrite is reduced to ammonia under hot alkaline conditions in the presence of the reducing agent (Devarda's alloy). The ammonia formed distils and is trapped in a receiving flask containing boric acid. The ammonia can be determined either by direct nesslerization or acidimetrically. This method is recommended for nitrate nitrogen and nitrite nitrogen.

3.4.2 *Interference* — Ammonia is to be removed from sample by preliminary distillation. Nitrite also gets reduced to ammonia by this method. Therefore, a separate determination is made for nitrite and substract the result. This method is not recommended for levels of nitrate nitrogen below 2 mg/l.

3.4.3 *Apparatus*

3.4.3.1 *Distillation assembly* — Kjeldahl assembly is suitable.

3.4.3.2 *Measuring scoop* — to contain 1 g of Devarda's alloy.

3.4.3.3 *Spectrophotometer or photometer* — suitable for use at 400-425 nm. The photometer should be equipped with a blue filter.

3.4.4 *Reagents*

3.4.4.1 *Ammonia free water* — *See* **2.4.4.1**.

3.4.4.2 *Borate buffer solution* — Add 88 ml of 0·1 N sodium hydroxide to 500 ml of 0·025 M sodium tetraborate (5·0 g $Na_2B_4O_7$ or 9·5 g $Na_2B_4O_7.H_2O$) and make up to 1 litre.

3.4.4.3 *Sodium hydroxide* — 6 N.

3.4.4.4 *Devarda's alloy* (An alloy of 50 percent Cu, 45 percent Al and 5 percent Zn) — 20 mesh or smaller containing less than 0·005 percent nitrogen.

3.4.4.5 *Reagents for acidimetric titration* — *See* **2.5.3**.

3.4.4.6 *Reagents for colorimetric estimation* — *See* **2.3.3.3, 2.3.3.4** and **2.3.3.5**.

3.4.5 *Procedure* — If ammonia has not been determined by a method involving preliminary distillation, dilute a portion of the sample to 500 ml with ammonia free water. Add 25 ml of borate buffer and adjust to *p*H 9·5 with 6 N sodium hydroxide using a *p*H meter or short range *p*H paper. Distil 250 to 300 ml into a dry receiving flask and discard. Make sure that the last part of the distillation is conducted with condenser tip out of the liquid in receiving flask. To the residue after removing ammonia, add 1 g of Devarda's alloy and sufficient ammonia-free distilled water to bring total volume to 350 ml. Place in a receiving flask 50 ml boric acid absorbent for each milligram of nitrate nitrogen in sample. Immerse the end of condenser in the absorbent. Heat distillation flask until boiling or vigorous bubbling occurs. Reduce heat and distil at the rate of 5 to 10 ml/min until at least 150 ml distillate have been collected. Lower receiver so that liquid is below the end of the condenser and continue distillation for 1 to 2 minutes to cleanse condenser. Determine ammoniacal nitrogen either by nesslerization or titration with standard strong acid as given in **2.3** or **2.5**.

3.4.6 *Calculation*

3.4.6.1 *Nesslerization method*

$$\text{Ammoniacal nitrogen } (NH_3\text{-N}),\ \text{mg/l} = \frac{A \times B}{V \times C}$$

where

A = μg of ammoniacal nitrogen in 51 ml of final volume;

B = total volume distillate collected, in ml, including acid absorbents;

V = volume in ml of sample taken for test; and

C = volume distillate taken for nesslerization.

3.4.6.2 *Titrimetric method*

$$\text{Ammoniacal nitrogen } (NH_3\text{-N}),\ \text{mg/l} = \frac{(A-B) \times 280}{V}$$

where

A = volume in ml of sulphuric acid titrated for sample,

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

V = volume in ml of sample taken for test.

The above two (**3.4.6.1** and **3.4.6.2**) represent the ammonia produced from reduction of nitrate and nitrite. To get nitrate nitrogen, determine nitrite separately and subtract.

4. Nitrite, Nitrogen

4.1 *General* — Prescribes a method for determination of nitrite nitrogen using Nessler's cylinders or spectrophotometer. Spectrophotometric method shall be the refree method.

4.2 *Principle* — Nitrite is determined through formation of a reddish purple azo dye produced at *p*H 2·0 to 2·5 by coupling diazotized sulphanalic acid with N-(1 naphthyl)-ethylene diamine dihydrochloride (NED dihydrochloride). The colour obeys Beers' law up to 180 μg/l with 1 cm path length at 543 nm.

4.3 *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 dihydrochloride 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.

4.4 *Apparatus*

4.4.1 *Spectrophotometer or photometer* — for use at 543 nm in case of spectrophotometer or photometer having a green filter and having maximum absorbance near 540 nm.

4.4.2 *Nessler tubes* — matched, 50 ml capacity.

4.5 *Reagents*

4.5.1 *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. Redistil in an all borosilicate glass bottle.

b) Add 1 ml of concentrated sulphuric acid and 0·2 ml of manganese sulphate (36·48 g $MnSO_4 \cdot H_2O$/100 ml) solution to each 1 litre of distilled water and make pink with 1 to 3 ml of potassium permanganate solution (400 mg/l). Redistil as in (a) above. Use this water in making all reagents and dilutions.

4.5.2 *Sulphanilamide reagent* — Dissolve 5 g of the material in a mixture of 50 ml of concentrated hydrochloric acid and 300 ml of water. Dilute to 500 ml with water. The reagent is stable for several months.

4.5.3 *NED dihydrochloride* — Dissolve 500 mg of the material in 500 ml of water. Store in coloured bottle in dark. Replace monthly or when it turn dark brown in colour.

4.5.4 *Hydrochloric acid* — 1 : 3.

4.5.5 *Sodium oxalate* — 0·05 N. Dissolve 3·350 g of sodium oxalate (primary standard grade) in 1 000 ml of water.

4.5.6 *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 with standard dichromate.

4.5.7 *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 with 1 ml of chloroform. Standardize using sodium oxalate (**4.5.5**) and standard potassium permanganate solution.

4.5.7.1 *Intermediate nitrite solution* — Calculate the volume, G, of stock nitrite solution required for intermediate nitrite solution from $G = 12{\cdot}5/A$, where A is the stock solution in mg/l. Dilute the volume G to 250 ml with water (1·00 ml = 50·0 μg N).

4.5.7.2 *Standard nitrite solution* — Dilute 10·00 ml of intermediate nitrite solution to 1 000 ml with water (1·00 ml = 0·500 μg N).

4.6 *Procedure*

4.6.1 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 1 ml of sulphanilamide solution. Let the reagent react for 2 to 8 minutes. Add 1·0 ml of NED dihydrochloride solution and mix immediately. Let stand for at least 10 minutes but not more than 2 hours. Measure absorbance at 543 nm. As a guide, use the following light paths for the indicated nitrite nitrogen concentrations:

Light Path Length, cm	*Nitrite Nitrogen*, μg/l
1	2 - 25
5	2 - 6
10	2

Run parallel checks frequently against nitrite standards.

4.6.1.1 *Colour standards for visual comparison* — Prepare a suitable series of visual colour standards in Nessler tubes by adding the following volumes of standard nitrite solutions and diluting to 50 ml with water: 0, 0·1, 0·2, 0·4, 0·7, 1·0, 1·4, 1·7, 2·0 and 2·5 ml, corresponding, respectively to 0, 1·0, 2·0, 4·0, 7·0, 10, 14, 17, 20 and 25 μg of nitrite per litre. Develop colour as described in **4.6.1**. Compare samples to visual standards in matched Nessler tubes between 10 and 120 minutes after adding NED dihydrochloride reagent. Select the concentration where the sample tube colour matches the standard tube colour.

4.7 *Calculation*

4.7.1 Calculate nitrite nitrogen from the following:

$$\text{Nirite nitrogen (as } NO_2\text{-N) per litre} = \frac{\mu g\ NO_2\text{-N (in 52 ml final volume)}}{\text{ml of sample}}$$

5. Organic Nitrogen

5.1 *General* — Prescribes two methods for determination of organic nitrogen 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 refree method.

5.2 *Macro-Kjeldahl Method*

5.2.1 *Principle* — In the presence of sulphuric acid, potassium sulphate, and mercuric sulphate catalyst, aminonitrogen 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. T e ammonia is determined colorimetrically or by titration with a standard mineral acid.

5.2.2 *Apparatus*

5.2.2.1 *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 to 370°C for effective digestion.

5.2.2.2 *Distillation apparatus — See* **2.5.2.1.**

5.2.2.3 *Apparatus for ammonia determination*

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

5.2.3 *Reagents*

5.2.3.1 *Zinc sulphate solution — See* **2.3.3.1.**

5.2.3.2 *EDTA reagent — See* **2.3.3.2 (a).**

5.2.3.3 *Rochelle salt solution — See* **2.3.3.2 (b).**

5.2.3.4 *Nessler reagent — See* **2.3.3.3.**

5.2.3.5 *Stock ammonium solution — See* **2.3.3.4.**

5.2.3.6 *Standard ammonium solution — See* **2.3.3.5.**

5.2.3.7 *Potassium chloroplatinate solution — See* **2.3.3.6 (a).**

5.2.3.8 *Cobaltous chloride solution — See* **2.3.3.6 (b).**

5.2.3.9 *Mercuric sulphate solution* — 8 g of red mercuric oxide dissolved in 100 ml of 6 N sulphuric acid.

5.2.3.10 *Digestion reagent* — Dissolve 134 g of potassium sulphate in 650 ml of water and 200 ml of concentrated sulphuric acid. Add, with stirring, 25 ml of mercuric sulphate solution. Dilute the combined solution to 1 litre with water. Keep at a temperature close to 20°C to prevent crystallization.

5.2.3.11 *Sodium hydroxide - sodium thiosulphate* — Dissolve 500 g of sodium hydroxide and 25 g of sodium thiosulphate ($Na_2S_2O_3 5H_2O$) in water and dilute to 1 litre.

5.2.3.12 *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.

5.2.3.13 *Sodium hydroxide* — 6 N.

5.2.4 *Procedure*

5.2.4.1 *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:

Organic Nitrogen in Sample, mg/l	*Sample Size*, ml
0 -1	500
1 -10	250
10 -20	100
20 -50	50·0
50 -100	25·0

If necessary, dilute sample to 300 ml, neutralize to *p*H 7, and dechlorinate as given in **2.2.3**.

5.2.4.2 *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.

5.2.4.3 *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 flask bottom. 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.

5.2.4.4 *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 or 2 minutes to cleanse condenser.

5.2.4.5 *Final ammonia measurement* — Use the nesslerization, manual phenate, titration or ammonia selective electrode method as given in **2**.

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

5.2.5 *Calculation*

5.2.5.1 Calculate ammoniacal nitrogen (NH_3-N) in mg per litre as given in the relevant methods in **2**.

5.3 *Semi-Micro-Kjeldahl Method*

5.3.1 *Apparatus*

5.3.1.1 *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 to 380°C for effective digestion.

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

5.3.1.3 *p*H *meter*

5.3.2 *Reagents* — All reagents listed for determination of nitrogen by the various methods given in **2** and **5.2.3** are required.

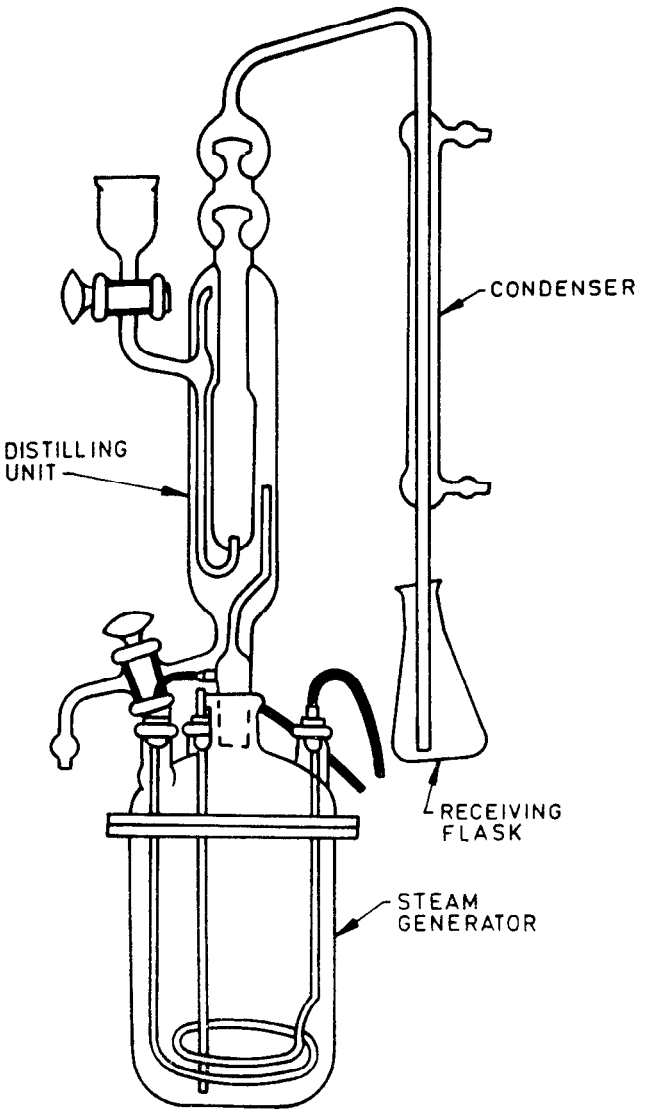


FIG. 2 MICRO-KJELDAHL DISTILLATION APPARATUS

5.3.3 *Procedure*

5.3.3.1 *Selection of sample volume* — Determine the sample size from the following table:

Organic Nitrogen in the Sample, mg/l	*Sample Size,* ml
4 - 40	50
8 - 80	25
20 - 200	10
40 - 400	5

5.3.3.2 *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 **5.3.3.3.** Distillation following this direct digestion yields Kjeldahl nitrogen concentration rather than organic nitrogen.

5.3.3.3 *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.

5.3.3.4 *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 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 or 2 minutes to cleanse condenser.

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

5.3.3.6 *Final ammonia measurement* — Determine ammonia by any of the methods prescribed in **2.**

5.3.4 *Calculation* — Calculate nitrogen, organic as per calculations given under different methods prescribed in **2.**

EXPLANATORY NOTE

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 interconvertible 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 trinegative state. Analytically organic and ammoniacal nitrogen can be determined together and called as Kjeldahl nitrogen. In the preparation of this standard, considerable assistance has been derived from 'Standard methods for the examination of water and wastewater', 16th edition 1988 published by the American Public Health Association, Washington, USA. This standard supersedes clauses **47**, **48**, **49** of IS : 3025-1964 'Methods of sampling (physical and chemical) for water used in industry' and clause **5** of IS : 2488 (Part 4)-1974 'Methods of sampling and test for industrial effluents, Part 4'.

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