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 ($Na_2S_2O_35H_2O$) 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.

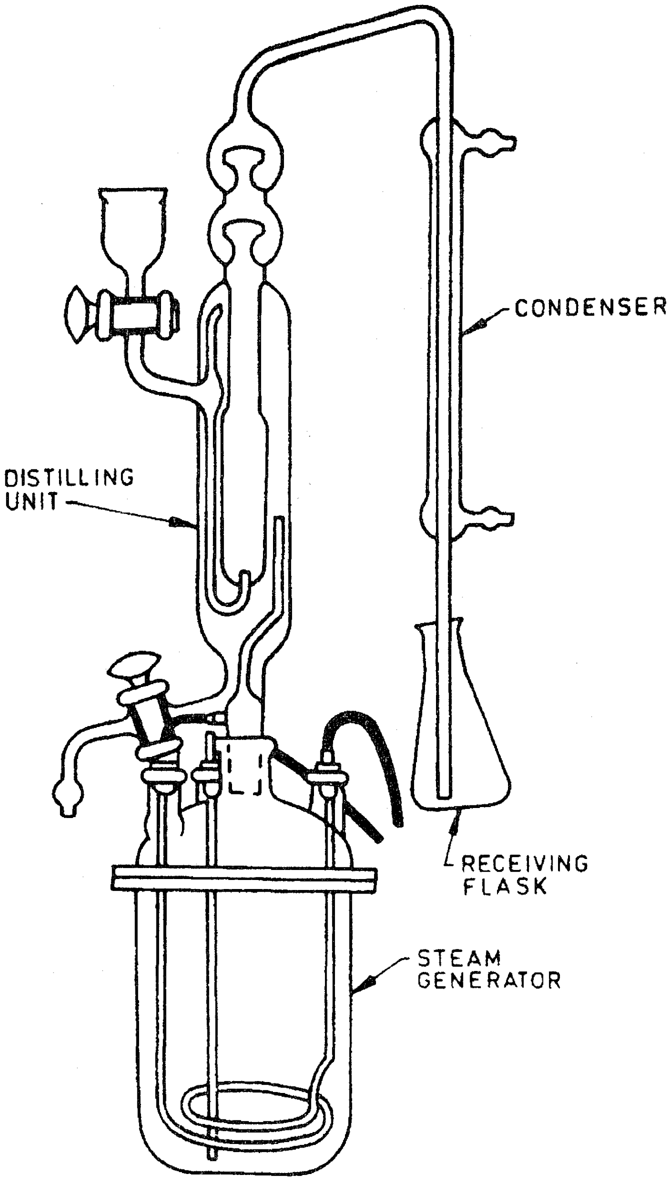

FIG. 2 MICRO-KJELDAHL DISTILLATION APPARATUS

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 nitrógen 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'.

Printed at Dee Kay Printers, New Delhi, India