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 methoa 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.

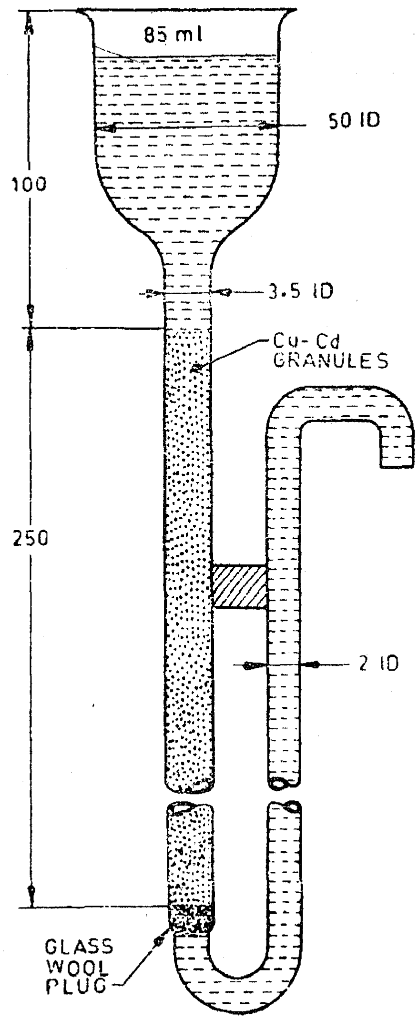
b) *Filter photometer* — provided with a yellow green filter having maximum transmittance near 540 nm and a light path of 1 cm or longer.

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.