

Determination of Nitrate (NO_3^- -N)

Introduction:

Nitrate is the oxidized state of nitrogen and in oxygenated surface waters, all organic and inorganic nitrogen should be in the form of nitrate. Major sources of these two forms are industrial waste waters, domestic sewage, fertilizers from run off, soil organic matter etc. It is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth limiting nutrient. In quantities normally found in food or feed, nitrate becomes toxic only under condition when they reduced to nitrites, otherwise, at reasonable concentration, nitrates are rapidly excreted in the urine.

As there is no direct method for nitrate estimation, the method of reducing nitrate to nitrite chemically is being used. Two commonly employed methods are Cadmium reduction method and Overnight reduction method.

Cadmium Reduction Method:

Principle:

Nitrate in the sample is reduced quantitatively to nitrite when a sample is run through a column containing cadmium fillings loosely coated with metallic copper. The nitrite thus produced is determined by diazotizing with sulphanilamide and coupling with NNED to form a highly coloured azo dye, the extinction of which is measured at 543 nm.

Reagents:

1. Conc. Ammonium chloride solution:

Dissolve 125 g of AR quality ammonium chloride in 500 ml of distilled water and store in a glass or plastic bottle.

2. Dilute ammonium chloride solution:

Dilute 50 ml of conc. ammonium chloride solution to 2000 ml with distilled water and store in a glass or plastic bottle.

3. Sulphanilamide solution:

Dissolve 5 g of sulphanilamide in a mixture of 50 ml of conc. HCl and about 300 ml of distilled water. Dilute to 500 ml with water.

2. N-(1-naphthyl) – ethylene diamine dihydrochloride solution (NNED):

Dissolve 0.50 g of NNED in 500 ml of distilled water. Store the solution in a dark bottle.

Experimental Procedure:

Add 1-2 ml of ammonium chloride to the 50 ml sample in the Erlenmeyer flask and mix it. Add the sample to the column and allow it to pass through. Collect 25 ml of the reduced solution in measuring cylinder by placing under the collection tube. As soon as possible after reduction, add 0.50 ml of sulphanilamide and allow the reagent to react for 2 min. but not exceeding 8 minutes. Add 0.5 ml of NED solution and mix immediately. Between 10 minutes and 2 hrs, afterwards measure the extinction of the solution at a wavelength of 543 nm. A correction may be made for any nitrite initially present in the sample by following the same procedure without reduction.

$$\mu\text{g.at} - \text{NO}_3 - \text{N/ltr} = (\text{Extinction} \times F) - C$$

Where, F = Factor value

C = concentration of nitrite present in the sample as $\mu\text{g.at} - \text{NO}_2 - \text{N/ltr}$

Calibration:

As there is a slight salt effect in this method, calibration should be carried out using synthetic sea water or natural sea water.

1. Synthetic sea water:

dissolve 310 g of AR quality sodium chloride, 100 g of AR quality magnesium sulphate and 0.5 g sodium bicarbonate in 10 ltrs of distilled water.

2. Standard nitrate solution:

Dissolve 1.02 g of AR quality potassium nitrate, KNO_3 , in 1000 ml distilled water.

1 ml = 10.0 $\mu\text{g.at.NO}_3\text{-N}$.

Further dilution is made with synthetic or natural sea water.

Procedure:

Prepare four standards of 20.0 $\mu\text{g.at.NO}_3\text{-N}$ concentration in Erlenmeyer flasks and carry out the determination exactly as described above in experimental procedure. Carryout two blanks simultaneously

F is calculated as:

$$F = \frac{20.00}{E_s - E_b}$$

Where, E_s = Mean extinction of four standards.

E_b = Mean extinction of two blanks

Result: