Determination of Reactive Phosphorus

Introduction:

Phosphorus occurs in natural water and in waste waters almost solely as phosphate. These are classified as orthophosphate, condensed phosphate (pyro, meta and polyphosphates) and organically bound phosphate. Phosphate that responds to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample is termed as “reactive phosphorus”. It is largely a measure of orthophosphate and it occurs in both dissolved and suspended forms.

Phosphorus in a water body is not autochthonus and high inflow of phosphorus is due to erosion of soil and other sources are agricultural, domestic and industrial wastes. Waters containing high phosphorus have blooms of algae which are not of any food value and process is known as eutrophication. Although needed in small quantities, phosphorus is one of the important phytoplankton growth limiting nutrient.

There are three methods to estimate concentration of phosphorus. They are:

i) Vanadomolybdo phosphoric acid colorimetric method

ii) Stannous chloride method

iii) Ascorbic acid method

Ascorbic Acid method:

Principle:

Ammonium molybdate and potassium antimonyl tartrate reacts in acid medium with the phosphate to form a heteropoly phosphomolybdic acid – that is reduced to intensively coloured molybdenum blue by ascorbic acid and the extinction is measured at 885nm.

Sample collection and storage:

Since polythene/ plastic bottles are said to contain phosphorus, these should not to be used for sample collection. The sample should be collected in glass containers after washing by using dilute acid and distilled water.

Samples should be kept in cool, dark place and not warmed to room temperature to avoid conversion and loss of phosphate to polythene bottles.

Reagents:

1. Ammonium molybdate:

Dissolve 15 g of ammonium molybdate in 500 ml of distilled water. Keep in plastic container and store in a refrigerator. The solution is stable indefinitely.

2. Sulphuric acid:

Add 140 ml of concentrated sulphuric acid to 900 ml distilled water. (Note: add acid to water)

3. Ascorbic acid:

Dissolve 27 g of ascorbic acid in 500 ml distilled water. Store it in a plastic bottle in a refrigerator.

4. Potassium antimonyl-tartrate:

Dissolve 0.34 g of PAT in 250 ml of distilled water, warm if necessary. The solution is stable for many months.

5. Mixed reagent:

To prepare 500 ml of mixed reagent, mix together 100 ml ammonium molybdate, 250 ml sulphuric acid, 100 ml ascorbic acid and 50 ml potassium- antimonyl- tartrate solutions. Prepare this reagent for use and discard any excess.

Colour – slightly yellowish.

Procedure:

To 100 ml of the sample, add10 ± 0.5 ml of mixed reagent and mix at once. After 5 minutes and preferably within 2-3 hrs, measure the extinction of the solution spectrophotometrically at a wavelength of 885nm.

Calculate the phosphate concentration from the expression,

µg.at – P/litre = Extiction x F (F= Factor)

Calibration:

Dissolve 0.816 g potassium dihydrogen phosphate (anhydrous KH_2PO_4) in 1000 ml distilled water.1 ml = 6.0 µg. at. P

Dilute 10 ml of this solution to1000 ml with distilled water, so 1 ml = 0.06 µg. at. P.

1 ml = 6.0 µg. at. P/L in 100 ml sample.

Prepare four standards consisting of 5 ml equivalent to 3.0 µg. at. P/L made to a volume of exactly 100 ml with distilled water. Carryout the procedure described above. Run two blanks with distilled water simultaneously.

Calculate the factor ‘F’ from the expression,

$$F = \frac{3.00}{E_s - E_b}$$

E_s = Mean extinction of the four standards.

E_b = Mean extinction of two blanks.

Result: