Indian Standard

(Reaffirmed 1999)

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

PART 31 PHOSPHORUS

(First Revision)

1. Scope — Prescribes two methods for determination of phosphorus, namely (a) vanadomolybdo — phosphoric acid method; and (b) stannous chloride method. In case of difference of opinion, vanadomolybdo — phosphoric acid method shall be the referee method.

2. Preliminary Digestion Steps

2.1 Phosphorus analysis involves the conversion of the phosphorus present in water in different forms to dissolved orthophosphate which is then estimated colorimetrically.

2.1.1 *For acid hydrolyzable phosphates* — To 100 ml of sample or a portion diluted to 100 ml, add 0·05 ml phenolphthalein. If a red colour develops, add strong acid dropwise to just discharge the colour, then add 1 ml more. Boil gently for 90 minutes at least, adding distilled water to keep the volume between 25 and 50 ml. Alternatively, heat for 30 minutes in an autoclave or pressure cooker at 98 to 137 kPa. Cool, neutralize to a faint pink colour with sodium hydroxide solution and restore to original 100 ml volume with distilled water.

2.1.2 *For total phosphorus*

2.1.2.1 *Perchloric acid digestion* — Measure sample containing the desired amount of phosphorus into a 125-ml Erlenmeyer flask. Acidify methyl orange with concentrated nitric acid, add 5 ml excess of nitric acid and evaporate on a steam bath or hot plate to 15 to 20 ml. Add 10 ml each of concentrated nitric acid and perchloric acid to the 125-ml conical flask, cooling the flask between additions. Add a few boiling chips, heat on a hot plate, and evaporate gently until dense white fumes of perchloric acid just appear. If solution is not clear, cover neck of the flask with a watch glass and keep solution barely boiling until it clears. If necessary, add 10 ml more of nitric acid to aid oxidation. Cool digested solution and add one drop of aqueous phenolphthalein solution. Add 6 N sodium hydroxide solution until the solution just turns pink. If necessary, filter neutralized solution and wash filter liberally with distilled water. Make up to 100 ml with distilled water.

2.1.2.2 *Sulphuric acid-nitric acid digestion* — Into a micro kjeldahl flask, measure a sample containing the desired amount of phosphorus. Add 1 ml of concentrated sulphuric acid and 5 ml of concentrated nitric acid. Digest to a volume of 1 ml and then continue until solution becomes colourless to remove nitric acid. Cool and add approximately 20 ml of distilled water, 0·05 ml of phenolphthalein indicator and as much 1 N sodium hydroxide solution as required to produce a faint pink tinge. Transfer neutralized solution, filtering, if necessary, to remove turbidity or particulate matter, into a 100 ml volumetric flask. Add filter washings to the flask and adjust sample volume to 100 ml with distilled water.

2.1.2.3 *Persulphate digestion method* — Use 50 ml or a suitable portion of thoroughly mixed sample. Add 0·05 ml of phenolphthalein indicator solution. If a red colour develops, add sulphuric acid solution dropwise to just discharge the colour. Then add 1 ml of sulphuric acid solution and either 0·4 g solid ammonium persulphate or 0·5 g of solid potassium persulphate. Boil gently on a preheated hot plate for 30 to 40 minutes or until a final volume of 10 ml is reached. Cool, dilute to 30 ml with distilled water, add 0·05 ml of phenolphthalein indicator solution, and neutralize to a faint pink colour with 1 N sodium hydroxide solution. Make up to 100 ml with distilled water. In some samples, a precipitate may form at this stage, but do not filter. For any subsequent subdivision of the sample, shake well. The precipitate redissolves under the acidic conditions of the colorimetric reactive phosphorus test.

Adopted 29 February 1988 | | Gr 2

BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

3. Vanadomolybdo — Phosphoric Acid Method

3.1 *Principle* — In dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a hetropoly acid, molybdo — phosphoric acid. In presence of vanadium, yellow vanadomolybdo — phosphoric acid is formed. The intensity of yellow colour is proportional to phosphate concentration.

3.2 *Interference* — Positive interference is caused by silica and arsenate only, if the sample is heated. Arsenate, fluoride, thorium, bismuth, sulphide, thiosulphate, thiocyanate or excess molybdate cause negative interference. Ferrous iron results in blue colour and does not affect the results, if the concentration is less than 100 mg/l.

3.2.1 *Minimum detectable concentration* — 200 μg of phosphorus per litre in 1 cm spectrophotometer cells.

3.3 *Apparatus*

3.3.1 *Spectrophotometer* — For use at 400 to 490 nm. Wavelength of 470 nm is generally used for determination.

3.3.2 *Acid washed glassware*

3.3.3 *Filteration apparatus*

3.3.4 *Filter paper* — Whatman No. 42 or equivalent.

3.4 *Reagents*

3.4.1 *Phenolphthalein solution*

3.4.2 *Hydrochloric acid* — 1:1.

3.4.3 *Activated carbon* — Phosphate free. Remove fine particles by rinsing with distilled water.

3.4.4 *Vanadate-molybdate reagent* — (Solution A) Dissolve 25 g of ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$] in 300 ml of distilled water. Dissolve 1·25 g of ammonium metavanadate (NH_4VO_3) by heating to boiling in 300 ml of distilled water. Cool and add 330 ml of concentrated hydrochloric acid. (Solution B) Cool to room temperature. Pour solution A to solution B, mix and dilute to 1 litre.

3.4.5 *Standard phosphate solution* — Dissolve 219·5 mg of anhydrous potassium dihydrogen phosphate (KH_2PO_4) in distilled water and dilute to 1 000 ml, 1 ml = 50 μg orthophosphate phosphorus.

3.5 *Procedure* — If the sample *p*H is greater than 10, add 0·05 ml of phenolphthalein indicator to 50·0 ml of sample and discharge the red colour with 1 : 1 hydrochloric acid before diluting to 100 ml. Remove excessive colour in sample by shaking about 50 ml with 200 mg of activated carbon in an Erlenmeyer flask for 5 minutes and filter to remove carbon. Place 35 ml or less of sample, containing 0·05 to 1·0 mg of phosphorus (P), in a 50-ml volumetric flask. Add 10 ml vanadate-molybdate reagent and dilute to the mark with distilled water. Prepare a blank in which 35 ml of distilled water is substituted for the sample. After 10 minutes or more, measure absorbance of sample *versus* blank at a wavelength of 470 nm. The colour is stable for days and is not affected by variation in room temperature.

Prepare a calibration curve by using suitable volumes of standard phosphate solution and proceeding as given above. Read the concentration of the sample from the calibration curve for the given absorbance.

3.6 *Calculation*

$$\text{Phosphorus (P), mg/l} = \frac{m}{v} \times 1\,000$$

where

m = mg of phosphorus (in 50 ml of final volume), and

v = volume in ml of sample.

4. Stannous Chloride Method

4.1 *Principle* — The molybdo—phosphoric acid formed is reduced to an intensely coloured complex molybdenum blue by stannous chloride. This method is significantly sensitive and the reliability of the method increases at concentrations below 0·1 mg/l of phosphorus with minimum interference.

4.2 *Interference* — Silica and arsenic interfere positively, if the sample is heated. Arsenate, fluoride, sulphide, thiosulphate, thiocyanate or excess molybdate cause negative interference. Ferrous iron which causes blue colour does not affect the result if the cencentration is below 100 mg/l. If nitric acid is used in the test, chloride interferes at 75 mg/l.

4.2.1 The minimum detectable concentration by this method is about 3 μg/l of phosphorus.

4.3 *Apparatus*

4.3.1 *Spectrophotometer* — Suitable for use at 690 nm for aqueous solution, provided with a light path of 1 to 10 cm.

4.3.2 *Acid washed glassware* — This is of great importance when determination of low concentration of phosphorus is done. Avoid commercial detergents containing phosphates. Clean all glassware with hot dilute hydrochloric acid and rinse thoroughly with distilled water. It is advisable to reserve glassware only for phosphate determination and to keep them filled with water till needed. If this is practised, only occasional acid treatment is required.

4.4 *Reagents*

4.4.1 *Phenolphthalein indicator solution*

4.4.2 *Strong acid solution* — Add slowly 300 ml of sulphuric acid to about 600 ml of distilled water, cool and add 4 ml of nitric acid and dilute to 1 litre.

4.4.3 *Ammonium molybdate reagent* — Dissolve 25 g of ammonium molybdate [$(NH_4)_6 Mo_7O_{24}.4 H_2O$] in 175 ml of distilled water. Continuously add 280 ml of concentrated sulphuric acid to 400 ml of distilled water in a separate beaker, cool and add the molybdate solution to this acid solution and dilute to 1 litre.

4.4.4 *Standard phosphate solution* — Dissolve 219·5 mg of anhydrous potassium dihydrogen phosphate (KH_2PO_4) and dilute to 1 litre. 1 ml = 50 μg orthophosphate phosphorus.

4.4.5 *Stannous chloride solution* — Dissolve 2·5 g of a fresh stannous chloride ($SnCl_2.2H_2O$) in 100 ml of glycerol. Heat in a water bath and stir with a glass rod to hasten dissolution. This reagent is stable and requires neither preservatives nor special storage.

4.4.6 *Activated carbon* — Analytical grade, free from phosphates.

4.5 *Procedure*

4.5.1 If coloured, decolourize the sample by shaking about 200 ml sample with 250 mg of activated carbon in an Erlenmeyer flask for 5 minutes. Filter the solution through filter paper (Whatman No. 42 or equivalent) to remove carbon.

Take 100 ml of clear and colourless sample containing not more than 0·2 mg of phosphorus, and add 1 drop of phenolphthalein indicator. If pink colour develops, discharge the colour with strong acid solution. If the acid requirement exceeds 5 drops, take a smaller sample and dilute to 100 ml with distilled water, after first discharging the pink colour with acid.

4.5.2 Add, with thorough mixing after each addition, 4·0 ml of molybdate reagent and 0·5 ml of stannous chloride reagent. The range of colour development and the intensity of colour are dependent on the temperature of the final solution. The increase in colour for each degree rise in temperature is about one percent. Hence samples, standards and reagents should be within 2°C of one another at a temperature between 20 and 30°C.

After 10 minutes, but before 12 minutes, allowing the uniform specific intervals for all estimations, measure the colour spectrophotometrically at 690 nm and compare with calibration curve using distilled water blank. Suitable light paths for various concentration ranges are as follows:

Approximate Phosphorus Range mg/l	*Light Path,* cm
0·3 — 2	0·5
0·1 — 1	2
0·07 — 0·2	10

Always run a blank on the reagents and distilled water. In as much as the colour at first develops progressively and later fades, it is essential to maintain equal timing conditions for samples as well as for standards. Standards should be prepared with each set of samples. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards and following the same procedural steps as the sample.

4.6 *Calculation*

$$\text{Phosphorus, mg/l} = \frac{\text{mg of phosphorus corresponding to control standard}}{\text{volume in ml of sample}} \times 1\,000$$

EXPLANATORY NOTE

Phosphorus occurs in natural waters and wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro, meta and other polyphosphates) and organically bound phosphates. Phosphorus analyses embody two general procedural steps, namely, conversion of the phosphorus form of interest to dissolved orthophosphate and the colorimetric determination of dissolved phosphorus. Separation of phosphorus into its various forms is defined analytically. Filtration through a 0·45 μm pore diameter filter separates dissolved phosphorus from suspended forms of phosphorus. Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the samples are known as reactive phosphorus. Reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present may also be hydrolyzed unavoidably. Reactive phosphorus occurs in both dissolved and suspended forms. Acid hydrolysis at boiling water temperature converts dissolved and particulate condensed phosphates to dissolved orthophosphate. The phosphate fractions that are converted to orthophosphate only by oxidation destruction of organic matter present are considered as organic or organically bound phosphorus. Like reactive and acid hydrolyzable phosphorus, organic phosphorus occurs in both dissolved and suspended fractions. Total phosphorus as well as the dissolved and suspended phosphorus fractions each may be divided analytically into three chemical types, namely, reactive, acid hydrolyzable and organic phosphorus. In the preparation of this standard, considerable assistance has been derived from Standard Methods for the examination of water and wastewater published by American Public Health Association, Washington, USA, 16th edition, 1985.