

4500-P PHOSPHORUS*

4500-P A. Introduction

1. Occurrence

Phosphorus occurs in natural waters and in wastewaters almost solely as phosphates. These are classified as orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, or in the bodies of aquatic organisms.

These forms of phosphate arise from a variety of sources. Small amounts of orthophosphate or certain condensed phosphates are added to some water supplies during treatment. Larger quantities of the same compounds may be added during laundering or other cleaning, because these materials are major constituents of many commercial cleaning preparations. Phosphates are used extensively in the treatment of boiler waters. Orthophosphates applied to agricultural or residential cultivated land as fertilizers are carried into surface waters with storm runoff and to a lesser extent with melting snow. Organic phosphates are formed primarily by biological processes. They are contributed to sewage by body wastes and food residues, and also may be formed from orthophosphates in biological treatment processes or by receiving-water biota.

Phosphorus is essential to the growth of organisms and can be the nutrient that limits the primary productivity of a body of water. In instances where phosphate is a growth-limiting nutrient, the discharge of raw or treated wastewater, agricultural drainage, or certain industrial wastes to that water may stimulate

the growth of photosynthetic aquatic micro- and macroorganisms in nuisance quantities.

Phosphates also occur in bottom sediments and in biological sludges, both as precipitated inorganic forms and incorporated into organic compounds.

2. Definition of Terms

Phosphorus analyses embody two general procedural steps: (a) conversion of the phosphorus form of interest to dissolved orthophosphate, and (b) colorimetric determination of dissolved orthophosphate. The separation of phosphorus into its various forms is defined analytically but the analytical differentiations have been selected so that they may be used for interpretive purposes.

Filtration through a 0.45- μm -pore-diam membrane filter separates dissolved from suspended forms of phosphorus. No claim is made that filtration through 0.45- μm filters is a true separation of suspended and dissolved forms of phosphorus; it is merely a convenient and replicable analytical technique designed to make a gross separation. Prefiltration through a glass fiber filter may be used to increase the filtration rate.

Phosphates that respond to colorimetric tests without preliminary hydrolysis or oxidative digestion of the sample are termed "reactive phosphorus." While reactive phosphorus is largely a measure of orthophosphate, a small fraction of any condensed phosphate present usually is hydrolyzed unavoidably in the procedure. Reactive phosphorus occurs in both dissolved and suspended forms.

Acid hydrolysis at boiling-water temperature converts dissolved and particulate condensed phosphates to dissolved or-

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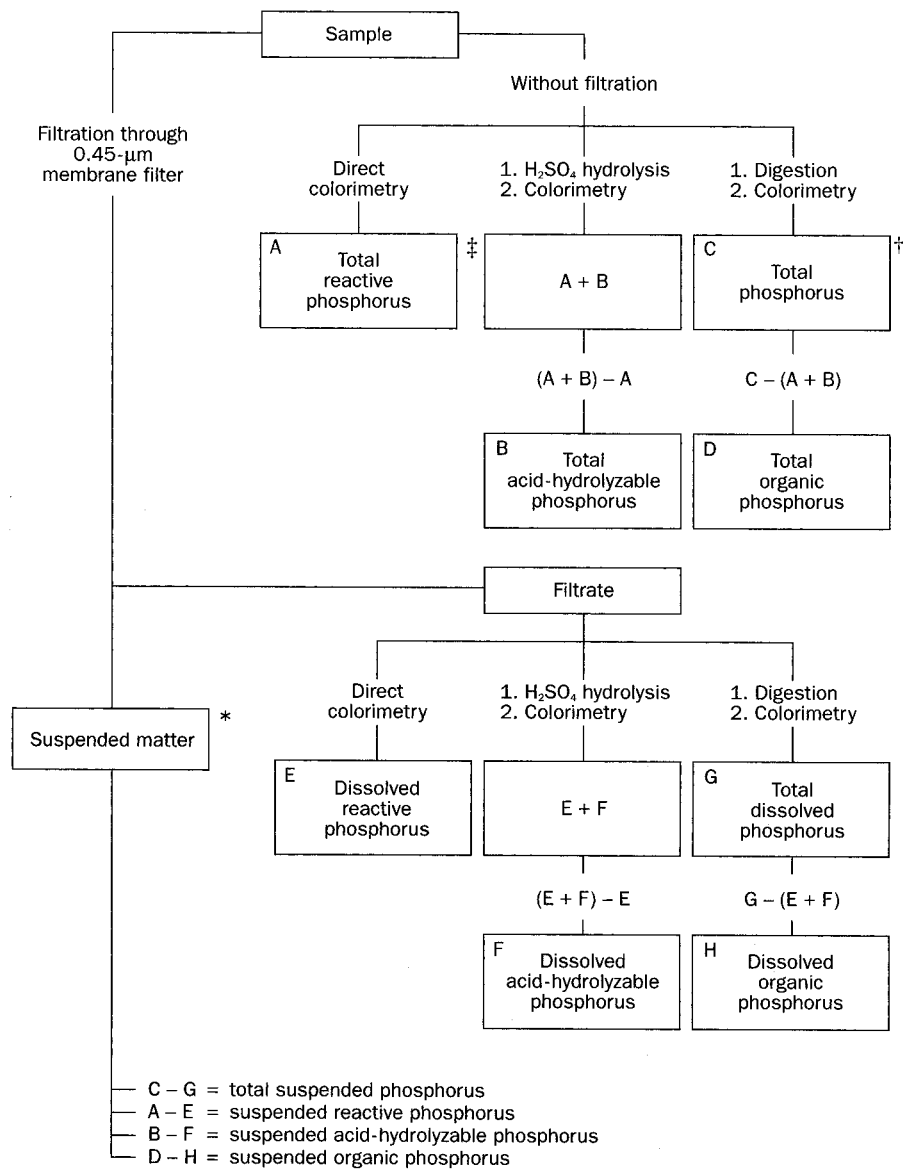


Figure 4500-P:1. Steps for analysis of phosphate fractions.

* Direct determination of phosphorus on the membrane filter containing suspended matter will be required where greater precision than that obtained by difference is desired. Digest filter with HNO_3 and follow by perchloric acid. Then perform colorimetry.

† Total phosphorus measurements on highly saline samples may be difficult because of precipitation of large quantities of salt as a result of digestion techniques that drastically reduce sample volume. For total phosphorus analyses on such samples, directly determine total dissolved phosphorus and total suspended phosphorus and add the results.

‡ In determination of total dissolved or total suspended reactive phosphorus, anomalous results may be obtained on samples containing large amounts of suspended sediments. Very often results depend largely on the degree of agitation and mixing to which samples are subjected during analysis because of a time-dependent desorption of orthophosphate from the suspended particles.

thophosphate. The hydrolysis unavoidably releases some phosphate from organic compounds, but this may be reduced to a

minimum by judicious selection of acid strength and hydrolysis time and temperature. The term “acid-hydrolyzable phosphorus” is preferred over “condensed phosphate” for this fraction.

The phosphate fractions that are converted to orthophosphate only by oxidation destruction of the organic matter present are considered “organic” or “organically bound” phosphorus. The severity of the oxidation required for this conversion depends on the form—and to some extent on the amount—of the organic phosphorus present. Like reactive phosphorus and acid-hydrolyzable phosphorus, organic phosphorus occurs both in the dissolved and suspended fractions.

The total phosphorus as well as the dissolved and suspended phosphorus fractions each may be divided analytically into the three chemical types that have been described: reactive, acid-hydrolyzable, and organic phosphorus. Figure 4500-P:1 shows

TABLE 4500-P.I. PRECISION AND BIAS DATA FOR MANUAL PHOSPHORUS METHODS

| Method | Phosphorus Concentration | | | No. of Laboratories | Relative Standard Deviation % | Relative Error % |
|--|--------------------------------|-------------------------------|-----------------------|---------------------|-------------------------------|------------------|
| | Orthophosphate $\mu\text{g/L}$ | Polyphosphate $\mu\text{g/L}$ | Total $\mu\text{g/L}$ | | | |
| Vanadomolybdophosphoric acid | 100 | | | 45 | 75.2 | 21.6 |
| | 600 | | | 43 | 19.6 | 10.8 |
| | 7000 | | | 44 | 8.6 | 5.4 |
| Stannous chloride | 100 | | | 45 | 25.5 | 28.7 |
| | 600 | | | 44 | 14.2 | 8.0 |
| | 7000 | | | 45 | 7.6 | 4.3 |
| Ascorbic acid | 100 | | | 3 | 9.1 | 10.0 |
| | 600 | | | 3 | 4.0 | 4.4 |
| | 7000 | | | 3 | 5.2 | 4.9 |
| Acid hydrolysis + vanadomolybdophosphoric acid | | 80 | | 37 | 106.8 | 7.4 |
| | | 300 | | 38 | 66.5 | 14.0 |
| | | 3000 | | 37 | 36.1 | 23.5 |
| Acid hydrolysis + stannous chloride | | 80 | | 39 | 60.1 | 12.5 |
| | | 300 | | 36 | 47.6 | 21.7 |
| | | 3000 | | 38 | 37.4 | 22.8 |
| Persulfate + vanadomolybdophosphoric acid | | | 210 | 32 | 55.8 | 1.6 |
| | | | 990 | 32 | 23.9 | 2.3 |
| | | | 10 230 | 31 | 6.5 | 0.3 |
| Sulfuric-nitric acids + vanadomolybdophosphoric acid | | | 210 | 23 | 65.6 | 20.9 |
| | | | 990 | 22 | 47.3 | 0.6 |
| | | | 10 230 | 20 | 7.0 | 0.4 |
| Perchloric acid + vanadomolybdophosphoric acid | | | 210 | 4 | 33.5 | 45.2 |
| | | | 990 | 5 | 20.3 | 2.6 |
| | | | 10 230 | 6 | 11.7 | 2.2 |
| Persulfate + stannous chloride | | | 210 | 29 | 28.1 | 9.2 |
| | | | 990 | 30 | 14.9 | 12.3 |
| | | | 10 230 | 29 | 11.5 | 4.3 |
| Sulfuric-nitric acids + stannous chloride | | | 210 | 20 | 20.8 | 1.2 |
| | | | 990 | 17 | 8.8 | 3.2 |
| | | | 10 230 | 19 | 7.5 | 0.4 |

the steps for analysis of individual phosphorus fractions. As indicated, determinations usually are conducted only on the unfiltered and filtered samples. Suspended fractions generally are determined by difference; however, they may be determined directly by digestion of the material retained on a glass-fiber filter.

3. Selection of Method

a. Digestion methods: Because phosphorus may occur in combination with organic matter, a digestion method to determine total phosphorus must be able to oxidize organic matter effectively to release phosphorus as orthophosphate. Three digestion methods are given in Section 4500-P.B.3, 4, and 5. The perchloric acid method, the most drastic and time-consuming method, is

recommended only for particularly difficult samples such as sediments. The nitric acid-sulfuric acid method is recommended for most samples. By far the simplest method is the persulfate oxidation technique. Persulfate oxidation is coupled with ultraviolet light for a more efficient digestion in an automated in-line digestion/determination by flow injection analysis (4500-P.I).

The persulfate oxidation method in Section 4500-P.J renders a digestate that can be analyzed for both total nitrogen and total phosphorus. This procedure can be used for both parameters because it occurs over a broad pH range. During the initial stage of the digestion, sample pH is alkaline (pH > 12); during the final stage, sample pH becomes acidic. As a result, nitrogenous compounds are oxidized to nitrate and phosphorus compounds to orthophosphate.

It is recommended that persulfate oxidation methods be checked against one or more of the more drastic digestion techniques and be adopted if identical recoveries are obtained.

b. Colorimetric method: Three methods of orthophosphate determination are described. Selection depends largely on the concentration range of orthophosphate. The vanadomolybdophosphoric acid method (C) is most useful for routine analysis in the range of 1 to 20 mg P/L. The stannous chloride method (D) or the ascorbic acid method (E) is more suited for the range of 0.01 to 6 mg P/L. An extraction step is recommended for the lower levels of this range and when interferences must be overcome. Automated versions of the ascorbic acid method (F, G, and H) also are presented. Careful attention to procedure may allow application of these methods to very low levels of phosphorus, such as those found in unimpaired fresh-water systems.

Ion chromatography (4110) and capillary ion electrophoresis (4140) are useful for determination of orthophosphate in undigested samples.

4. Precision and Bias

To aid in method selection, Table 4500-P:I presents the results of various combinations of digestions, hydrolysis, and colorimetric techniques for three synthetic samples of the following compositions:

Sample 1: 100 μg orthophosphate phosphorus (PO_4^{3-} -P/L), 80 μg acid-hydrolyzable phosphate phosphorus/L (sodium hexametaphosphate), 30 μg organic phosphorus/L (adenylic acid), 1.5 mg NH_3 -N/L, 0.5 mg NO_3^- -N/L, and 400 mg Cl^- /L.

Sample 2: 600 μg PO_4^{3-} -P/L, 300 μg acid-hydrolyzable phosphate phosphorus/L (sodium hexametaphosphate), 90 μg organic phosphorus/L (adenylic acid), 0.8 mg NH_3 -N/L, 5.0 mg NO_3^- -N/L, and 400 mg Cl^- /L.

Sample 3: 7.00 mg PO_4^{3-} -P/L, 3.00 μg acid-hydrolyzable phosphate phosphorus/L (sodium hexametaphosphate), 0.230

mg organic phosphorus/L (adenylic acid), 0.20 mg NH_3 -N/L, 0.05 mg NO_3^- -N/L, and 400 mg Cl^- /L.

5. Sampling and Storage

If dissolved phosphorus forms are to be differentiated, filter sample immediately after collection. Preserve by freezing at or below -10°C . In some cases 40 mg HgCl_2 /L may be added to the samples, especially when they are to be stored for long periods before analysis. CAUTION: *HgCl₂ is a hazardous substance; take appropriate precautions in disposal; use of HgCl₂ is not encouraged.* Do not add either acid or CHCl_3 as a preservative when phosphorus forms are to be determined. If total phosphorus alone is to be determined, add H_2SO_4 or HCl to $\text{pH}<2$ and cool to 4°C , or freeze without any additions.

Do not store samples containing low concentrations of phosphorus in plastic bottles unless kept in a frozen state because phosphates may be adsorbed onto the walls of plastic bottles.

Rinse all glass containers with hot dilute HCl , then rinse several times in reagent water. Never use commercial detergents containing phosphate for cleaning glassware used in phosphate analysis. More strenuous cleaning techniques may be used.

6. Bibliography

- BLACK, C.A., D.D. EVANS, J.L. WHITE, L.E. ENSMINGER & F.E. CLARK, eds. 1965. *Methods of Soil Analysis, Part 2, Chemical and Microbiological Properties*. American Soc. Agronomy, Madison, Wisc.
- JENKINS, D. 1965. A study of methods suitable for the analysis and preservation of phosphorus forms in an estuarine environment. SERL Rep. No. 65-18, Sanitary Engineering Research Lab., Univ. California, Berkeley.
- LEE, G.F. 1967. Analytical chemistry of plant nutrients. *In Proc. Int. Conf. Eutrophication*, Madison, Wisc.
- FITZGERALD, G.P. & S.L. FAUST. 1967. Effect of water sample preservation methods on the release of phosphorus from algae. *Limnol. Oceanogr.* 12:332.

4500-P B. Sample Preparation

For information on selection of digestion method (¶s 3 through 5 below), see 4500-P.A.3a.

1. Preliminary Filtration

Filter samples for determination of dissolved reactive phosphorus, dissolved acid-hydrolyzable phosphorus, and total dissolved phosphorus through 0.45- μm membrane filters. A glass fiber filter may be used to prefilter hard-to-filter samples.

Wash membrane filters by soaking in distilled water before use because they may contribute significant amounts of phosphorus to samples containing low concentrations of phosphate. Use one of two washing techniques: (a) soak 50 filters in 2 L distilled water for 24 h; (b) soak 50 filters in 2 L distilled water for 1 h, change distilled water, and soak filters an additional 3 h. Membrane filters also may be washed by running several 100-mL portions of distilled water through them. This procedure

requires more frequent determination of blank values to ensure consistency in washing and to evaluate different lots of filters.

2. Preliminary Acid Hydrolysis

The acid-hydrolyzable phosphorus content of the sample is defined operationally as the difference between reactive phosphorus as measured in the untreated sample and phosphate found after mild acid hydrolysis. Generally, it includes condensed phosphates such as pyro-, tripoly-, and higher-molecular-weight species such as hexametaphosphate. In addition, some natural waters contain organic phosphate compounds that are hydrolyzed to orthophosphate under the test conditions. Polyphosphates generally do not respond to reactive phosphorus tests but can be hydrolyzed to orthophosphate by boiling with acid.

After hydrolysis, determine reactive phosphorus by a colorimetric method (C, D, or E). Interferences, precision, bias, and sensitivity will depend on the colorimetric method used.

a. Apparatus:

Autoclave or pressure cooker, capable of operating at 98 to 137 kPa.

b. Reagents:

- 1) *Phenolphthalein indicator aqueous solution.*
- 2) *Strong acid solution:* Slowly add 300 mL conc H_2SO_4 to about 600 mL distilled water. When cool, add 4.0 mL conc HNO_3 and dilute to 1 L.
- 3) *Sodium hydroxide*, NaOH, 6*N*.

c. Procedure: To 100-mL sample or a portion diluted to 100 mL, add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add strong acid solution dropwise, to just discharge the color. Then add 1 mL more.

Boil gently for at least 90 min, adding distilled water to keep the volume between 25 and 50 mL. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa. Cool, neutralize to a faint pink color with NaOH solution, and restore to the original 100-mL volume with distilled water.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see colorimetric method C, D, or E) through the hydrolysis step. Do not use orthophosphate standards without hydrolysis, because the salts added in hydrolysis cause an increase in the color intensity in some methods.

Determine reactive phosphorus content of treated portions, using Method C, D, or E. This gives the sum of polyphosphate and orthophosphate in the sample. To calculate its content of acid-hydrolyzable phosphorus, determine reactive phosphorus in a sample portion that has not been hydrolyzed, using the same colorimetric method as for treated sample, and subtract.

3. Perchloric Acid Digestion

a. Apparatus:

- 1) *Hot plate:* A 30- × 50-cm heating surface is adequate.
- 2) *Safety shield.*
- 3) *Safety goggles.*
- 4) *Erlenmeyer flasks*, 125-mL, acid-washed and rinsed with distilled water.

b. Reagents:

- 1) *Nitric acid*, HNO_3 , conc.
- 2) *Perchloric acid*, $\text{HClO}_4 \cdot 2\text{H}_2\text{O}$, purchased as 70 to 72% HClO_4 , reagent-grade.
- 3) *Sodium hydroxide*, NaOH, 6*N*.
- 4) *Methyl orange indicator solution.*
- 5) *Phenolphthalein indicator aqueous solution.*

c. Procedure: CAUTION—Heated mixtures of HClO_4 and organic matter may explode violently. Avoid this hazard by taking the following precautions: (a) Do not add HClO_4 to a hot solution that may contain organic matter. (b) Always initiate digestion of samples containing organic matter with HNO_3 . Complete digestion using the mixture of HNO_3 and HClO_4 . (c) Do not fume with HClO_4 in ordinary hoods. Use hoods especially constructed for HClO_4 fuming or a glass fume eradicator* connected to a water pump. (d) Never let samples being digested with HClO_4 evaporate to dryness.

Measure sample containing the desired amount of phosphorus (this will be determined by whether Method C, D, or E is to be

used) into a 125-mL erlenmeyer flask. Acidify to methyl orange with conc HNO_3 , add another 5 mL conc HNO_3 , and evaporate on a steam bath or hot plate to 15 to 20 mL.

Add 10 mL each of conc HNO_3 and HClO_4 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 HClO_4 just appear. If solution is not clear, cover neck of flask with a watch glass and keep solution barely boiling until it clears. If necessary, add 10 mL more HNO_3 to aid oxidation.

Cool digested solution and add 1 drop aqueous phenolphthalein solution. Add 6*N* NaOH 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.

Determine the PO_4^{3-} -P content of the treated sample by Method C, D, or E.

Prepare a calibration curve by carrying a series of standards containing orthophosphate (see Method C, D, or E) through digestion step. Do not use orthophosphate standards without treatment.

4. Sulfuric Acid-Nitric Acid Digestion

a. Apparatus:

1) *Digestion rack:* An electrically or gas-heated digestion rack with provision for withdrawal of fumes is recommended. Digestion racks typical of those used for micro-kjeldahl digestions are suitable.

2) *Micro-kjeldahl flasks.*

b. Reagents:

- 1) *Sulfuric acid*, H_2SO_4 , conc.
- 2) *Nitric acid*, HNO_3 , conc.
- 3) *Phenolphthalein indicator aqueous solution.*
- 4) *Sodium hydroxide*, NaOH, 1*N*.

c. Procedure: Into a micro-kjeldahl flask, measure a sample containing the desired amount of phosphorus (this is determined by the colorimetric method used). Add 1 mL conc H_2SO_4 and 5 mL conc HNO_3 .

Digest to a volume of 1 mL and then continue until solution becomes colorless to remove HNO_3 .

Cool and add approximately 20 mL distilled water, 0.05 mL (1 drop) phenolphthalein indicator, and as much 1*N* NaOH solution as required to produce a faint pink tinge. Transfer neutralized solution, filtering if necessary to remove particulate material or turbidity, into a 100-mL volumetric flask. Add filter washings to flask and adjust sample volume to 100 mL with distilled water.

Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the acid digestion procedure.

5. Persulfate Digestion Method

a. Apparatus:

- 1) *Hot plate:* A 30- × 50-cm heating surface is adequate.
- 2) *Autoclave:* An autoclave or pressure cooker capable of developing 98 to 137 kPa may be used in place of a hot plate.
- 3) *Glass scoop*, to hold required amounts of persulfate crystals.

b. Reagents:

* GFS Chemical Co., Columbus, OH, or equivalent.

1) *Phenolphthalein indicator aqueous solution.*
 2) *Sulfuric acid solution:* Carefully add 300 mL conc H_2SO_4 to approximately 600 mL distilled water and dilute to 1 L with distilled water.

3) *Ammonium persulfate*, $(\text{NH}_4)_2\text{S}_2\text{O}_8$, solid, or potassium persulfate, $\text{K}_2\text{S}_2\text{O}_8$, solid.

4) *Sodium hydroxide*, NaOH, 1N.

c. *Procedure:* Use 50 mL or a suitable portion of thoroughly mixed sample. Add 0.05 mL (1 drop) phenolphthalein indicator solution. If a red color develops, add H_2SO_4 solution dropwise to just discharge the color. Then add 1 mL H_2SO_4 solution and either 0.4 g solid $(\text{NH}_4)_2\text{S}_2\text{O}_8$ or 0.5 g solid $\text{K}_2\text{S}_2\text{O}_8$.

Boil gently on a preheated hot plate for 30 to 40 min or until a final volume of 10 mL is reached. Organophosphorus compounds such as AMP may require as much as 1.5 to 2 h for complete digestion. Cool, dilute to 30 mL with distilled water, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. Alternatively, heat for 30 min in an autoclave or pressure cooker at 98 to 137 kPa.

Cool, add 0.05 mL (1 drop) phenolphthalein indicator solution, and neutralize to a faint pink color with NaOH. 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 subdividing of the sample, shake well. The precipitate (which is possibly a calcium phosphate) redissolves under the acid conditions of the colorimetric reactive phosphorus test. Determine phosphorus by Method C, D, or E, for which a separate calibration curve has been constructed by carrying standards through the persulfate digestion procedure.

6. Bibliography

- LEE, G.F., N.L. CLESCERI & G.P. FITZGERALD. 1965. Studies on the analysis of phosphates in algal cultures. *J. Air Water Pollut.* 9:715.
 SHANNON, J.E. & G.F. LEE. 1966. Hydrolysis of condensed phosphates in natural waters. *J. Air Water Pollut.* 10:735.
 GALES, M.E., JR., E.C. JULIAN & R.C. KRONER. 1966. Method for quantitative determination of total phosphorus in water. *J. Amer. Water Works Assoc.* 58:1363.

4500-P C. Vanadomolybdophosphoric Acid Colorimetric Method

1. General Discussion

a. *Principle:* In a dilute orthophosphate solution, ammonium molybdate reacts under acid conditions to form a heteropoly acid, molybdophosphoric acid. In the presence of vanadium, yellow vanadomolybdophosphoric acid is formed. The intensity of the yellow color is proportional to phosphate concentration.

b. *Interference:* Positive interference is caused by silica and arsenate only if the sample is heated. Negative interferences are caused by arsenate, fluoride, thorium, bismuth, sulfide, thiosulfate, thiocyanate, or excess molybdate. Blue color is caused by ferrous iron but this does not affect results if ferrous iron concentration is less than 100 mg/L. Sulfide interference may be removed by oxidation with bromine water. Ions that do not interfere in concentrations up to 1000 mg/L are Al^{3+} , Fe^{3+} , Mg^{2+} , Ca^{2+} , Ba^{2+} , Sr^{2+} , Li^+ , Na^+ , K^+ , NH_4^+ , Cd^{2+} , Mn^{2+} , Pb^{2+} , Hg^+ , Hg^{2+} , Sn^{2+} , Cu^{2+} , Ni^{2+} , Ag^+ , U^{4+} , Zr^{4+} , AsO_3^- , Br^- , CO_3^{2-} , ClO_4^- , CN^- , IO_3^- , SiO_4^{4-} , NO_3^- , NO_2^- , SO_4^{2-} , SO_3^{2-} , pyrophosphate, molybdate, tetraborate, selenate, benzoate, citrate, oxalate, lactate, tartrate, formate, and salicylate. If HNO_3 is used in the test, Cl^- interferes at 75 mg/L.

c. *Minimum detectable concentration:* The minimum detectable concentration is 200 $\mu\text{g P/L}$ in 1-cm spectrophotometer cells.

2. Apparatus

a. *Colorimetric equipment:* One of the following is required:

- 1) *Spectrophotometer*, for use at 400 to 490 nm.
- 2) *Filter photometer*, provided with a blue or violet filter exhibiting maximum transmittance between 400 and 470 nm.

The wavelength at which color intensity is measured depends on sensitivity desired, because sensitivity varies tenfold with wavelengths 400 to 490 nm. Ferric iron causes interference at

low wavelengths, particularly at 400 nm. A wavelength of 470 nm usually is used. Concentration ranges for different wavelengths are:

| P Range mg/L | Wavelength nm |
|-----------------|------------------|
| 1.0–5.0 | 400 |
| 2.0–10 | 420 |
| 4.0–18 | 470 |

b. *Acid-washed glassware:* Use acid-washed glassware for determining low concentrations of phosphorus. Phosphate contamination is common because of its absorption on glass surfaces. Avoid using commercial detergents containing phosphate. Clean all glassware with hot dilute HCl and rinse well with distilled water. Preferably, reserve the glassware only for phosphate determination, and after use, wash and keep filled with water until needed. If this is done, acid treatment is required only occasionally.

c. *Filtration apparatus and filter paper.**

3. Reagents

a. *Phenolphthalein indicator aqueous solution.*

b. *Hydrochloric acid*, HCl, 1 + 1. H_2SO_4 , HClO_4 , or HNO_3 may be substituted for HCl. The acid concentration in the determination is not critical but a final sample concentration of 0.5N is recommended.

c. *Activated carbon.*† Remove fine particles by rinsing with distilled water.

* Whatman No. 42 or equivalent.

† Darco G60 or equivalent.

d. Vanadate-molybdate reagent:

1) *Solution A:* Dissolve 25 g ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, in 300 mL distilled water.

2) *Solution B:* Dissolve 1.25 g ammonium metavanadate, NH_4VO_3 , by heating to boiling in 300 mL distilled water. Cool and add 330 mL conc HCl. Cool Solution B to room temperature, pour Solution A into Solution B, mix, and dilute to 1 L.

e. Standard phosphate solution: Dissolve in distilled water 219.5 mg anhydrous KH_2PO_4 and dilute to 1000 mL; 1.00 mL = 50.0 $\mu\text{g PO}_4^{3-}\text{-P}$.

4. Procedure

a. Sample pH adjustment: If sample pH is greater than 10, add 0.05 mL (1 drop) phenolphthalein indicator to 50.0 mL sample and discharge the red color with 1 + 1 HCl before diluting to 100 mL.

b. Color removal from sample: Remove excessive color in sample by shaking about 50 mL with 200 mg activated carbon in an erlenmeyer flask for 5 min and filter to remove carbon. Check each batch of carbon for phosphate because some batches produce high reagent blanks.

c. Color development in sample: Place 35 mL or less of sample, containing 0.05 to 1.0 mg 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 distilled water is substituted for the sample. After 10 min or more, measure absorbance of sample versus a blank at a wavelength of 400 to 490 nm, depending on sensitivity desired (see ¶ 2a above). The color is stable for days and its intensity is unaffected by variation in room temperature.

d. Preparation of calibration curve: Prepare a calibration curve by using suitable volumes of standard phosphate solution and proceeding as in ¶ 4c. When ferric ion is low enough not to interfere, plot a family of calibration curves of one series of standard solutions for various wavelengths. This permits a wide

latitude of concentrations in one series of determinations. Analyze at least one standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in 50 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

See Table 4500-P:I.

7. Bibliography

- KITSON, R.E. & M.G. MELLON. 1944. Colorimetric determination of phosphorus as molybdovanadophosphoric acid. *Ind. Eng. Chem., Anal. Ed.* 16:379.
- BOLTZ, D.F. & M.G. MELLON. 1947. Determination of phosphorus, germanium, silicon, and arsenic by the heteropoly blue method. *Ind. Eng. Chem., Anal. Ed.* 19:873.
- GREENBERG, A.E., L.W. WEINBERGER & C.N. SAWYER. 1950. Control of nitrite interference in colorimetric determination of phosphorus. *Anal. Chem.* 22:499.
- YOUNG, R.S. & A. GOLLEDGE. 1950. Determination of hexametaphosphate in water after threshold treatment. *Ind. Chem.* 26:13.
- GRISWOLD, B.L., F.L. HUMOLLER & A.R. MCINTYRE. 1951. Inorganic phosphates and phosphate esters in tissue extracts. *Anal. Chem.* 23:192.
- BOLTZ, D.F., ed. 1958. *Colorimetric Determination of Nonmetals*. Interscience Publishers, New York, N.Y.
- AMERICAN WATER WORKS ASSOCIATION. 1958. Committee report. Determination of orthophosphate, hydrolyzable phosphate, and total phosphate in surface waters. *J. Amer. Water Works Assoc.* 50:1563.
- JACKSON, M.L. 1958. *Soil Chemical Analysis*. Prentice-Hall, Englewood Cliffs, N.J.
- ABBOT, D.C., G.E. EMSDEN & J.R. HARRIS. 1963. A method for determining orthophosphate in water. *Analyst* 88:814.
- PROFT, G. 1964. Determination of total phosphorus in water and wastewater as molybdovanadophosphoric acid. *Limnologica* 2:407.

4500-P D. Stannous Chloride Method

1. General Discussion

a. Principle: Molybdophosphoric acid is formed and reduced by stannous chloride to intensely colored molybdenum blue. This method is more sensitive than Method C and makes feasible measurements down to 7 $\mu\text{g P/L}$ by use of increased light path length. Below 100 $\mu\text{g P/L}$ an extraction step may increase reliability and lessen interference.

b. Interference: See Section 4500-P.C.1b.

c. Minimum detectable concentration: The minimum detectable concentration is about 3 $\mu\text{g P/L}$. The sensitivity at 0.3010 absorbance is about 10 $\mu\text{g P/L}$ for an absorbance change of 0.009.

2. Apparatus

The same apparatus is required as for Method C, except that a pipetting bulb is required for the extraction step. Set spectrophotometer at 625 nm in the measurement of benzene-isobutanol ex-

tracts and at 690 nm for aqueous solutions. If the instrument is not equipped to read at 690 nm, use a wavelength of 650 nm for aqueous solutions, with somewhat reduced sensitivity and precision.

3. Reagents

a. Phenolphthalein indicator aqueous solution.

b. Strong-acid solution: Prepare as directed in Section 4500-P.B.2b2).

c. Ammonium molybdate reagent I: Dissolve 25 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in 175 mL distilled water. Cautiously add 280 mL conc H_2SO_4 to 400 mL distilled water. Cool, add molybdate solution, and dilute to 1 L.

d. Stannous chloride reagent I: Dissolve 2.5 g fresh $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 100 mL 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.

e. Standard phosphate solution: Prepare as directed in Section 4500-P.C.3e.

f. Reagents for extraction:

1) *Benzene-isobutanol solvent*: Mix equal volumes of benzene and isobutyl alcohol. (CAUTION—This solvent is highly flammable.)

2) *Ammonium molybdate reagent II*: Dissolve 40.1 g $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ in approximately 500 mL distilled water. Slowly add 396 mL ammonium molybdate reagent I. Cool and dilute to 1 L.

3) *Alcoholic sulfuric acid solution*: Cautiously add 20 mL conc H_2SO_4 to 980 mL methyl alcohol with continuous mixing.

4) *Dilute stannous chloride reagent II*: Mix 8 mL stannous chloride reagent I with 50 mL glycerol. This reagent is stable for at least 6 months.

4. Procedure

a. Preliminary sample treatment: To 100 mL sample containing not more than 200 μg P and free from color and turbidity, add 0.05 mL (1 drop) phenolphthalein indicator. If sample turns pink, add strong acid solution dropwise to discharge the color. If more than 0.25 mL (5 drops) is required, take a smaller sample and dilute to 100 mL with distilled water after first discharging the pink color with acid.

b. Color development: Add, with thorough mixing after each addition, 4.0 mL molybdate reagent I and 0.5 mL (10 drops) stannous chloride reagent I. Rate of color development and intensity of color depend on temperature of the final solution, each 1°C increase producing about 1% increase in color. Hence, hold samples, standards, and reagents within 2°C of one another and in the temperature range between 20 and 30°C.

c. Color measurement: After 10 min, but before 12 min, using the same specific interval for all determinations, measure color photometrically at 690 nm and compare with a calibration curve, using a distilled water blank. Light path lengths suitable for various concentration ranges are as follows:

| Approximate P Range mg/L | Light Path cm |
|--------------------------------|------------------|
| 0.3–2 | 0.5 |
| 0.1–1 | 2 |
| 0.007–0.2 | 10 |

Always run a blank on reagents and distilled water. Because the color at first develops progressively and later fades, maintain

equal timing conditions for samples and standards. Prepare at least one standard with each set of samples or once each day that tests are made. The calibration curve may deviate from a straight line at the upper concentrations of the 0.3 to 2.0-mg/L range.

d. Extraction: When increased sensitivity is desired or interferences must be overcome, extract phosphate as follows: Pipet a 40-mL sample, or one diluted to that volume, into a 125-mL separatory funnel. Add 50.0 mL benzene-isobutanol solvent and 15.0 mL molybdate reagent II. Close funnel at once and shake vigorously for exactly 15 s. If condensed phosphate is present, any delay will increase its conversion to orthophosphate. Remove stopper and withdraw 25.0 mL of separated organic layer, using a pipet with safety bulb. Transfer to a 50-mL volumetric flask, add 15 to 16 mL alcoholic H_2SO_4 solution, swirl, add 0.50 mL (10 drops) dilute stannous chloride reagent II, swirl, and dilute to the mark with alcoholic H_2SO_4 . Mix thoroughly. After 10 min, but before 30 min, read against the blank at 625 nm. Prepare blank by carrying 40 mL distilled water through the same procedure used for the sample. Read phosphate concentration from a calibration curve prepared by taking known phosphate standards through the same procedure used for samples.

5. Calculation

Calculate as follows:

a. Direct procedure:

$$\text{mg P/L} = \frac{\text{mg P (in approximately 104.5 mL final volume)} \times 1000}{\text{mL sample}}$$

b. Extraction procedure:

$$\text{mg P/L} = \frac{\text{mg P (in 50 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

See Table 4500-P:I.

4500-P E. Ascorbic Acid Method

1. General Discussion

a. Principle: Ammonium molybdate and antimony potassium tartrate react in acid medium with orthophosphate to form a heteropoly acid—phosphomolybdic acid—that is reduced to intensely colored molybdenum blue by ascorbic acid.

b. Interference: Arsenates react with the molybdate reagent to produce a blue color similar to that formed with phosphate.

Concentrations as low as 0.1 mg As/L interfere with the phosphate determination. Hexavalent chromium and NO_2^- interfere to give results about 3% low at concentrations of 1 mg/L and 10 to 15% low at 10 mg/L. Sulfide (Na_2S) and silicate do not interfere at concentrations of 1.0 and 10 mg/L.

c. Minimum detectable concentration: Approximately 10 μg P/L. P ranges are as follows:

| Approximate P Range mg/L | Light Path cm |
|--------------------------------|------------------|
| 0.30–2.0 | 0.5 |
| 0.15–1.30 | 1.0 |
| 0.01–0.25 | 5.0 |

2. Apparatus

a. Colorimetric equipment: One of the following is required:

1) *Spectrophotometer*, with infrared phototube for use at 880 nm, providing a light path of 2.5 cm or longer.

2) *Filter photometer*, equipped with a red color filter and a light path of 0.5 cm or longer.

b. Acid-washed glassware: See Section 4500-P.C.2b.

3. Reagents

a. Sulfuric acid, H₂SO₄, 5N: Dilute 70 mL conc H₂SO₄ to 500 mL with distilled water.

b. Antimony potassium tartrate solution: Dissolve 1.3715 g K(SbO)C₄H₄O₆ · ½H₂O in 400 mL distilled water in a 500-mL volumetric flask and dilute to volume. Store in a glass-stoppered bottle.

c. Ammonium molybdate solution: Dissolve 20 g (NH₄)₆Mo₇O₂₄ · 4H₂O in 500 mL distilled water. Store in a glass-stoppered bottle.

d. Ascorbic acid, 0.1M: Dissolve 1.76 g ascorbic acid in 100 mL distilled water. The solution is stable for about 1 week at 4°C.

e. Combined reagent: Mix the above reagents in the following proportions for 100 mL of the combined reagent: 50 mL 5N H₂SO₄, 5 mL antimony potassium tartrate solution, 15 mL ammonium molybdate solution, and 30 mL ascorbic acid solution. *Mix after addition of each reagent.* Let all reagents reach room temperature before they are mixed and mix in the order given. If turbidity forms in the combined reagent, shake and let stand for a few minutes until turbidity disappears before proceeding. The reagent is stable for 4 h.

f. Stock phosphate solution: See Section 4500-P.C.3e.

g. Standard phosphate solution: Dilute 50.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 2.50 µg P.

4. Procedure

a. Treatment of sample: Pipet 50.0 mL sample into a clean, dry test tube or 125-mL erlenmeyer flask. Add 0.05 mL (1 drop) phenolphthalein indicator. If a red color develops add 5N H₂SO₄ solution dropwise to just discharge the color. Add 8.0 mL combined reagent and mix thoroughly. After at least 10 min but no more than 30 min, measure absorbance of each sample at 880 nm, using reagent blank as the reference solution.

b. Correction for turbidity or interfering color: Natural color of water generally does not interfere at the high wavelength used. For highly colored or turbid waters, prepare a blank by adding all reagents except ascorbic acid and antimony potassium tartrate to the sample. Subtract blank absorbance from absorbance of each sample.

c. Preparation of calibration curve: Prepare individual calibration curves from a series of six standards within the phosphate ranges indicated in ¶ 1c above. Use a distilled water blank with the combined reagent to make photometric readings for the calibration curve. Plot absorbance vs. phosphate concentration to give a straight line passing through the origin. Test at least one phosphate standard with each set of samples.

5. Calculation

$$\text{mg P/L} = \frac{\text{mg P (in approximately 58 mL final volume)} \times 1000}{\text{mL sample}}$$

6. Precision and Bias

The precision and bias values given in Table 4500-P:I are for a single-solution procedure given in the 13th edition. The present procedure differs in reagent-to-sample ratios, no addition of solvent, and acidity conditions. It is superior in precision and bias to the previous technique in the analysis of both distilled water and river water at the 228-µg P/L level (Table 4500-P:II).

7. References

- EDWARDS, G.P., A.H. MOLOF & R.W. SCHNEEMAN. 1965. Determination of orthophosphate in fresh and saline waters. *J. Amer. Water Works Assoc.* 57:917.

TABLE 4500-P:II. COMPARISON OF PRECISION AND BIAS OF ASCORBIC ACID METHODS

| Ascorbic Acid Method | Phosphorus Concentration, Dissolved Orthophosphate µg/L | No. of Labora- tories | Relative Standard Deviation % | | Relative Error % | |
|-----------------------------|---|-----------------------------|--|----------------|------------------------|----------------|
| | | | Distilled Water | River Water | Distilled Water | River Water |
| 13th Edition ¹ | 228 | 8 | 3.87 | 2.17 | 4.01 | 2.08 |
| Current method ² | 228 | 8 | 3.03 | 1.75 | 2.38 | 1.39 |

2. MURPHY, J. & J. RILEY. 1962. A modified single solution method for the determination of phosphate in natural waters. *Anal. Chim. Acta* 27:31.

8. Bibliography

- SLETTEN, O. & C.M. BACH. 1961. Modified stannous chloride reagent for orthophosphate determination. *J. Amer. Water Works Assoc.* 53: 1031.

- STRICKLAND, J.D.H. & T.R. PARSONS. 1965. *A Manual of Sea Water Analysis*, 2nd ed. Fisheries Research Board of Canada, Ottawa.

4500-P F. Automated Ascorbic Acid Reduction Method

1. General Discussion

a. Principle: Ammonium molybdate and antimony potassium tartrate react with orthophosphate in an acid medium to form an antimony-phosphomolybdate complex, which, on reduction with ascorbic acid, yields an intense blue color suitable for photometric measurement.

b. Interferences: As much as 50 mg Fe³⁺/L, 10 mg Cu/L, and 10 mg SiO₂/L can be tolerated. High silica concentrations cause positive interference.

In terms of phosphorus, the results are high by 0.005, 0.015, and 0.025 mg/L for silica concentrations of 20, 50, and 100 mg/L, respectively. Salt concentrations up to 20% (w/v) cause an error of less than 1%. Arsenate (AsO₄³⁻) is a positive interference.

Eliminate interference from NO₂⁻ and S²⁻ by adding an excess of bromine water or a saturated potassium permanganate (KMnO₄) solution. Remove interfering turbidity by filtration before analysis. Filter samples for total or total hydrolyzable phosphorus only after digestion. Sample color that absorbs in the photometric range used for analysis also will interfere. See also Section 4500-P.E.1b.

c. Application: Orthophosphate can be determined in potable, surface, and saline waters as well as domestic and industrial

wastewaters over a range of 0.001 to 10.0 mg P/L when photometric measurements are made at 650 to 660 or 880 nm in a 15-mm or 50-mm tubular flow cell. Determine higher concentrations by diluting sample. Although the automated test is designed for orthophosphate only, other phosphorus compounds can be converted to this reactive form by various sample pre-treatments described in Section 4500-P.B.1, 2, and 5.

2. Apparatus

a. Automated analytical equipment: An example of the continuous-flow analytical instrument consists of the interchangeable components shown in Figure 4500-P:2. A flow cell of 15 or 50 mm and a filter of 650 to 660 or 880 nm may be used.

b. Hot plate or autoclave.

c. Acid-washed glassware: See Section 4500-P.C.2b.

3. Reagents

a. Antimony potassium tartrate solution: Dissolve 0.3 g K(SbO)C₄H₄O₆ · ½H₂O in approximately 50 mL distilled water and dilute to 100 mL. Store at 4°C in a dark, glass-stoppered bottle.

b. Ammonium molybdate solution: Dissolve 4 g (NH₄)₆Mo₇O₂₄ · 4H₂O in 100 mL distilled water. Store in a plastic bottle at 4°C.

c. Ascorbic acid solution: See Section 4500-P.E.3d.

d. Combined reagent: See Section 4500-P.E.3e.

e. Dilute sulfuric acid solution: Slowly add 140 mL conc H₂SO₄ to 600 mL distilled water. When cool, dilute to 1 L.

f. Ammonium persulfate, (NH₄)₂S₂O₈, crystalline.

g. Phenolphthalein indicator aqueous solution.

h. Stock phosphate solution: Dissolve 439.3 mg anhydrous KH₂PO₄, dried for 1 h at 105°C, in distilled water and dilute to 1000 mL; 1.00 mL = 100 µg P.

i. Intermediate phosphate solution: Dilute 100.0 mL stock phosphate solution to 1000 mL with distilled water; 1.00 mL = 10.0 µg P.

j. Standard phosphate solutions: Prepare a suitable series of standards by diluting appropriate volumes of intermediate phosphate solution.

4. Procedure

Set up manifold as shown in Figure 4500-P:2 and follow the general procedure described by the manufacturer.

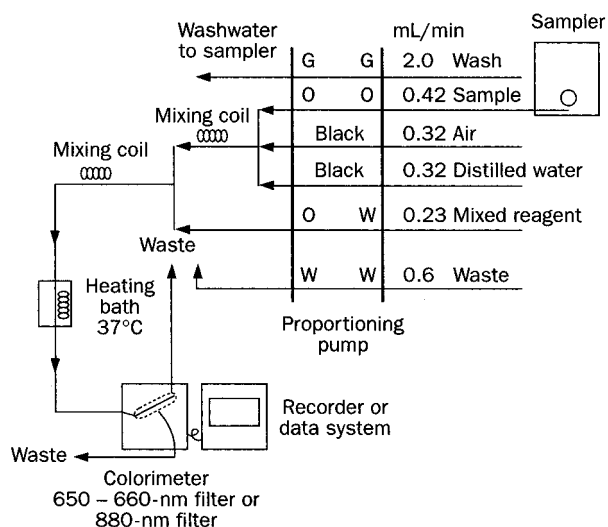


Figure 4500-P:2. Phosphate manifold for automated analytical system.

Add 0.05 mL (1 drop) phenolphthalein indicator solution to approximately 50 mL sample. If a red color develops, add H_2SO_4 (¶ 3e) dropwise to just discharge the color.

5. Calculation

Prepare standard curves by plotting response of standards processed through the manifold against P concentration in standards. Compute sample P concentration by comparing sample response with standard curve.

6. Precision and Bias

Six samples were analyzed in a single laboratory in septuplicate. At an average PO_4^{3-} concentration of 0.340 mg/L, the average deviation was 0.015 mg/L. The coefficient of variation

was 6.2%. In two samples with added PO_4^{3-} , recoveries were 89 and 96%.

7. Bibliography

- HENRIKSEN, A. 1966. An automatic method for determining orthophosphate in sewage and highly polluted waters. *Analyst* 91:652.
- LOBRING, L.B. & R.L. BOOTH. 1973. Evaluation of the AutoAnalyzer II; A progress report. In *Advances in Automated Analysis: 1972 Technicon International Congress*. Vol. 8, p. 7. Mediad, Inc., Tarrytown, N.Y.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Methods for Chemical Analysis of Water and Wastes. EPA-600/4-79-020, National Environmental Research Center, Cincinnati, Ohio.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. MDQARL Method Study 4, Automated Methods. National Environmental Research Center, Cincinnati, Ohio.

4500-P G. Flow Injection Analysis for Orthophosphate

1. General Discussion

a. Principle: The orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of orthophosphate in the sample.

Also see Sections 4500-P.A, B, and F, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: Remove large or fibrous particulates by filtering sample through glass wool. Guard against contamination from reagents, water, glassware, and the sample preservation process.

Silica forms a pale blue complex that also absorbs at 880 nm. This interference is generally insignificant because a silica concentration of approximately 30 mg/L would be required to produce a 0.005 mg P/L positive error in orthophosphate.

Concentrations of ferric iron greater than 50 mg/L cause a negative error due to competition with the complex for the reducing agent ascorbic acid. Treat samples high in iron with sodium bisulfite to eliminate this interference, as well as the interference due to arsenates.

Glassware contamination is a problem in low-level phosphorus determinations. Wash glassware with hot dilute HCl and rinse with reagent water. Commercial detergents are rarely needed but, if they are used, use special phosphate-free preparations.

Also see Section 4500-P.F.

2. Apparatus

Flow injection analysis equipment consisting of:

- FIA injection valve* with sample loop or equivalent.
- Multichannel proportioning pump*.
- FIA manifold* (Figure 4500-P:3) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:3. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

- Absorbance detector*, 880 nm, 10-nm bandpass.
- Injection valve control and data acquisition system*.

3. Reagents

Use reagent water (>10 megohm) to prepare carrier and all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Stock ammonium molybdate solution: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate $[(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}]$ and 983 g water. Mix with a magnetic stirrer for at least 4 h. Store in plastic and refrigerate.

b. Stock antimony potassium tartrate solution: To a 1-L dark, tared container add 3.0 g antimony potassium tartrate (potassium antimony tartrate hemihydrate), $\text{K}(\text{SbO})\text{C}_4\text{H}_4\text{O}_6 \cdot \frac{1}{2}\text{H}_2\text{O}$, and 995 g water. Mix with a magnetic stirrer until dissolved. Store in a dark bottle and refrigerate.

c. Working molybdate color reagent: To a tared 1-L container add 680 g water, then add 64.4 g conc sulfuric acid. CAUTION: *This solution becomes very hot!* Swirl to mix. When mixture can be handled comfortably, add 213 g stock ammonium molybdate

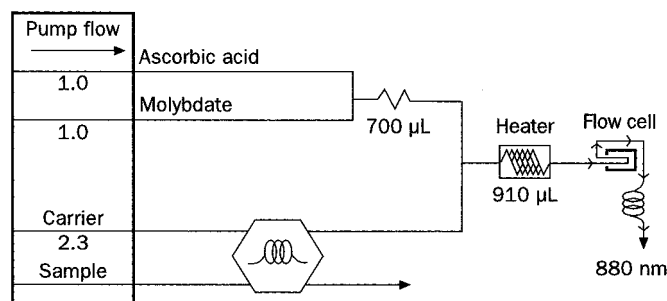


Figure 4500-P:3. FIA orthophosphate manifold.

solution (¶ 3a) and 72.0 g stock antimony potassium tartrate solution (¶ 3b). Shake and degas with helium.

d. *Ascorbic acid solution*: To a tared 1-L container, add 60.0 g granular ascorbic acid and 975 g water. Stir or shake until dissolved. Degas this reagent with helium, then add 1.0 g dodecyl sulfate, $\text{CH}_3(\text{CH}_2)_{11}\text{OSO}_3\text{Na}$, stirring gently to mix. Prepare fresh weekly.

e. *Stock orthophosphate standard*, 25.00 mg P/L: In a 1-L volumetric flask dissolve 0.1099 g primary standard grade anhydrous potassium phosphate monobasic (KH_2PO_4) that has been dried for 1 h at 105°C in about 800 mL water. Dilute to mark with water and invert to mix.

f. *Standard orthophosphate solutions*: Prepare orthophosphate standards in desired concentration range, using stock standard (¶ 3e) and diluting with water.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:3 and follow method supplied by manufacturer or laboratory standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus orthophosphate concentration. The calibration curve is linear.

6. Precision and Bias

a. *Recovery and relative standard deviation*: Table 4500-P:III gives results of single-laboratory studies.

b. *MDL*: A 700- μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 5.0- μg P/L standard. These gave a mean of 5.26 μg P/L, a standard deviation of 0.264 μg P/L, and MDL of 0.67 μg P/L.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

TABLE 4500-P:III. RESULTS OF SINGLE-LABORATORY STUDIES WITH SELECTED MATRICES

| Matrix | Sample/Blank Designation | Known Addition mg P/L | Recovery % | Relative Standard Deviation % |
|-------------------------------------|--------------------------|-----------------------|------------|-------------------------------|
| Wastewater treatment plant influent | Reference sample* | — | 101 | — |
| | Blank† | 0.05 | 96 | — |
| | | 0.1 | 95 | — |
| | Site A‡§ | 0 | — | 0.7 |
| | | 0.05 | 98 | — |
| | | 0.1 | 101 | — |
| | Site B‡§ | 0 | — | 5 |
| | | 0.05 | 75 | — |
| | | 0.1 | 91 | — |
| | Site C‡§ | 0 | — | 0.6 |
| | | 0.05 | 88 | — |
| | | 0.1 | 97 | — |
| Wastewater treatment plant effluent | Reference sample* | — | 100 | — |
| | Blank† | 0.05 | 96 | — |
| | | 0.1 | 96 | — |
| | Site A‡ | 0 | — | 0.7 |
| | | 0.05 | 94 | — |
| | | 0.1 | 96 | — |
| | Site B‡ | 0 | — | 0.3 |
| | | 0.05 | 94 | — |
| | | 0.1 | 99 | — |
| | Site C‡ | 0 | — | 0.5 |
| | | 0.05 | 109 | — |
| | | 0.1 | 107 | — |
| Landfill leachate | Reference sample* | — | 98 | — |
| | Blank† | 0.05 | 94 | — |
| | | 0.1 | 95 | — |
| | Site A‡# | 0 | — | 0.9 |
| | | 0.05 | 105 | — |
| | | 0.1 | 106 | — |
| | Site B‡# | 0 | — | 6.7 |
| | | 0.05 | 89 | — |
| | | 0.1 | 94 | — |
| | Site C‡# | 0 | — | 0.9 |
| | | 0.05 | 110 | — |
| | | 0.1 | 109 | — |

* U.S. EPA QC sample, 0.109 mg P/L.

† Determined in duplicate.

‡ Samples without known additions determined four times; samples with known additions determined in duplicate.

§ Sample dilutions: A - 5-fold; B - 100-fold; C - 10-fold. Typical relative difference between duplicates 0.5%.

|| Sample dilutions: A - 5-fold; B - 20-fold; C - 10-fold. Typical relative difference between duplicates 0.3%.

Sample dilutions: A - 20-fold; B - 10-fold; C - 20-fold. Typical relative difference between duplicates 1%.

4500-P H. Manual Digestion and Flow Injection Analysis for Total Phosphorus

1. General Discussion

a. Principle: Polyphosphates are converted to the orthophosphate form by a sulfuric acid digestion and organic phosphorus is converted to orthophosphate by a persulfate digestion. When the resulting solution is injected onto the manifold, the orthophosphate ion (PO_4^{3-}) reacts with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex that absorbs light at 880 nm. The absorbance is proportional to the concentration of total phosphorus in the sample.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: See 4500-P.G.1b.

2. Apparatus

Digestion and flow injection analysis equipment consisting of:

- Hotplate or autoclave.
- FIA injection valve with sample loop or equivalent.
- Multichannel proportioning pump.
- FIA manifold (Figure 4500-P:4) with tubing heater and flow cell. Relative flow rates only are shown in Figure 4500-P:4. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE.

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and buffer with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He

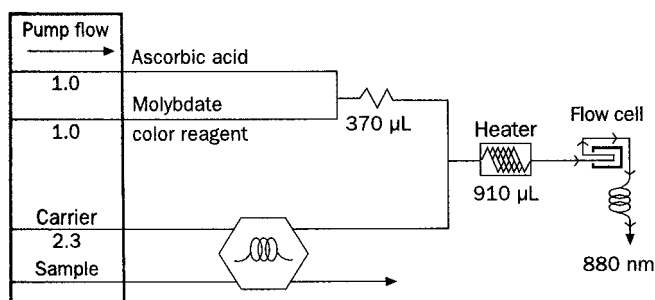


Figure 4500-P:4. FIA total phosphorus manifold.

through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

Prepare reagents listed in 4500-P.G.3a, b, d, e, and f, and in addition:

a. Sulfuric acid carrier, H_2SO_4 , 0.13M: To a tared 1-L container add 993 g water, then add 13.3 g conc H_2SO_4 . Shake carefully to mix. Degas daily. Prepare fresh weekly.

b. Molybdate color reagent: To a tared 1-L container add 694 g water, then add 38.4 g conc H_2SO_4 . CAUTION: *The solution becomes very hot!* Swirl to mix. When mixture can be handled comfortably, add 72.0 g stock antimony potassium tartrate (¶ G.3b) and 213 g stock ammonium molybdate (¶ G.3a). Shake to mix, and degas.

4. Procedure

See Section 4500-P.B.4 or 5 for digestion procedures. Carry both standards and samples through the digestion. The resulting solutions should be about 0.13M in sulfuric acid to match the concentration of the carrier. If the solutions differ more than 10% from this concentration, adjust concentration of carrier's sulfuric acid to match that of digested samples.

Set up a manifold equivalent to that in Figure 4500-P:4 and analyze digested samples and standards by following method supplied by manufacturer or laboratory's standard operating procedure. Use quality control protocols outlined in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through the manifold versus phosphorus concentration. The calibration curve is linear.

6. Precision and Bias

a. MDL: A 780- μL sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 3.5- μg P/L standard. These gave a mean of 3.53 μg P/L, a standard deviation of 0.82 μg P/L, and MDL of 2.0 μg P/L. The MDL is limited mainly by the precision of the digestion.

b. Precision study: Ten injections of a 100.0- μg P/L standard gave a percent relative standard deviation of 0.3%.

7. Reference

- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

4500-P I. In-line UV/Persulfate Digestion and Flow Injection Analysis for Total Phosphorus

1. General Discussion

a. Principle: Organic phosphorus is converted in-line to orthophosphate by heat, ultraviolet radiation, and persulfate digestion. At the same time, inorganic polyphosphates are converted to orthophosphate by in-line sulfuric acid digestion. The digestion processes occur before sample injection. A portion of the digested sample is then injected and its orthophosphate concentration determined by the flow injection method described in Section 4500-P.H.1.

See Section 4500-P.A for a discussion of the various forms of phosphorus found in waters and wastewaters, Section 4500-P.B for a discussion of sample preparation and digestion, and Section 4130, Flow Injection Analysis (FIA).

b. Interferences: See 4500-P.G.1b.

2. Apparatus

Flow injection analysis equipment consisting of:

- a. FIA injection valve* with sample loop or equivalent.
- b. Multichannel proportioning pump.*
- c. FIA manifold* (Figure 4500-P:5) with tubing heater, in-line ultraviolet digestion fluidics including a debubbler consisting of a gas-permeable TFE membrane and its holder, and flow cell. Relative flow rates only are shown in Figure 4500-P:5. Tubing volumes are given as an example only; they may be scaled down proportionally. Use manifold tubing of an inert material such as TFE. The block marked "UV" should consist of TFE tubing irradiated by a mercury discharge ultraviolet lamp emitting radiation at 254 nm.
- d. Absorbance detector*, 880 nm, 10-nm bandpass.
- e. Injection valve control and data acquisition system.*

3. Reagents

Use reagent water (>10 megohm) for all solutions. To prevent bubble formation, degas carrier and all reagents with helium. Pass He at 140 kPa (20 psi) through a helium degassing tube. Bubble He through 1 L solution for 1 min. As an alternative to preparing reagents by weight/weight, use weight/volume.

a. Digestion reagent 1: To a tared 1-L container, add 893.5 g water, then slowly add 196.0 g sulfuric acid, H₂SO₄. CAUTION: This solution becomes very hot! Prepare weekly. Degas before using.

b. Digestion reagent 2: To a tared 1-L container, add 1000 g water, then add 26 g potassium persulfate, K₂S₄O₈. Mix with a magnetic stirrer until dissolved. Prepare weekly. Degas before using.

c. Sulfuric acid carrier, 0.71M: To a tared 1-L container, slowly add 70 g H₂SO₄ to 962 g water. Add 5 g sodium chloride, NaCl. Let cool, then degas with helium. Add 1.0 g sodium dodecyl sulfate. Invert to mix. Prepare weekly.

d. Stock ammonium molybdate: To a tared 1-L container add 40.0 g ammonium molybdate tetrahydrate, (NH₄)₆Mo₇O₂₄ · 4H₂O, and 983 g water. Mix with a magnetic stirrer for at least 4 h. The solution can be stored in plastic for up to 2 months if refrigerated.

e. Stock antimony potassium tartrate: To a 1-L dark, plastic, tared container add 3.0 g antimony potassium tartrate (potassium antimony tartrate trihydrate), C₈H₄K₂O₁₂Sb₂ · 3H₂O, and 995 g water. Mix with a magnetic stirrer until dissolved. The solution can be stored in a dark plastic container for up to 2 months if refrigerated.

f. Molybdate color reagent: To a tared 1-L container add 715 g water, then 213 g stock ammonium molybdate (¶ 3e) and 72.0 g stock antimony potassium tartrate (¶ 3f). Add and dissolve 22.8 g sodium hydroxide, NaOH. Shake and degas with helium. Prepare weekly.

g. Ascorbic acid: To a tared 1-L container add 70.0 g ascorbic acid and 975 g water. Mix with a magnetic stirrer until dissolved. Degas with helium. Add 1.0 g sodium dodecyl sulfate. Mix with a magnetic stirrer. Prepare fresh every 2 d.

h. Stock orthophosphate standard, 1000 mg P/L: In a 1-L volumetric flask dissolve 4.396 g primary standard grade anhydrous potassium phosphate monobasic, KH₂PO₄ (dried for 1 h at

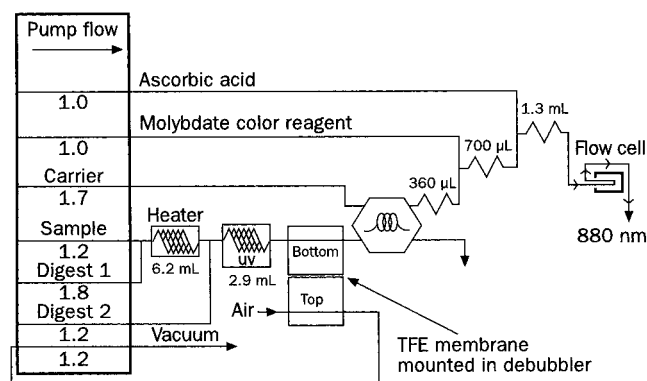


Figure 4500-P:5. FIA in-line total phosphorus manifold.

TABLE 4500-P:IV. RECOVERIES OF TOTAL PHOSPHORUS

| Compound | Known | Mean | Relative | |
|-------------------------|---------------|---------------|----------|-----------|
| | Concentration | Concentration | Recovery | Standard |
| | mg P/L | mg P/L | % | Deviation |
| Sodium pyrophosphate | 10 | 8.99 | 90.3 | 0.5 |
| | 2 | 1.81 | 90.2 | 0.6 |
| Phenylphosphate | 0.2 | 0.19 | 93.4 | 1.0 |
| | 10 | 10.10 | 101.5 | 0.2 |
| Trimethylphosphate | 2 | 2.12 | 105.0 | 5.6 |
| | 0.2 | 0.20 | 101.3 | 1.0 |
| Sodium tripolyphosphate | 10 | 8.99 | 90.3 | 0.2 |
| | 2 | 1.86 | 92.7 | 0.3 |
| | 0.2 | 0.18 | 95.3 | 1.1 |
| | 10 | 10.61 | 106.7 | 1.0 |
| | 2 | 2.14 | 106.6 | 0.2 |
| | 0.2 | 0.22 | 108.9 | 0.9 |

TABLE 4500-P:V. COMPARISON OF MANUAL AND IN-LINE TOTAL PHOSPHORUS METHODS

| Samples | Concentration by Manual Persulfate Digestion Method mg P/L | Concentration by In-Line Digestion Method mg P/L | Relative Difference % |
|-------------------------|---|---|-----------------------|
| Influent (I2) | 5.93 | 5.52 | -6.9 |
| Influent (I3) | 5.03 | 4.50 | -10.5 |
| Influent (I5) | 2.14 | 2.11 | -1.4 |
| Influent (I6) | 1.88 | 1.71 | -9.0 |
| Effluent (E1) | 3.42 | 2.87 | -16.1 |
| Effluent (E2) | 3.62 | 3.55 | -1.9 |
| Effluent (E3) | 3.26 | 3.34 | +2.4 |
| Effluent (E4) | 8.36 | 8.16 | -2.4 |
| Effluent (E5) | 0.65 | 0.71 | +9.2 |
| Effluent (E6) | 0.74 | 0.81 | +9.5 |
| Phenylphosphate | 1.95 | 1.91 | -2.1 |
| Trimethylphosphate | 1.87 | 1.80 | -3.7 |
| Sodium pyrophosphate | 1.90 | 1.73 | -8.9 |
| Sodium tripolyphosphate | 1.84 | 1.73 | -6.0 |

105°C), in about 800 mL water. Dilute to mark with water and invert to mix. Prepare monthly.

i. Standard solutions: Prepare orthophosphate standards in desired concentration range, using stock orthophosphate standards (§ 3i), and diluting with water. If the samples are preserved with sulfuric acid, ensure that stock standard and diluted standards solutions are of the same concentration.

4. Procedure

Set up a manifold equivalent to that in Figure 4500-P:5 and follow method supplied by manufacturer or laboratory's standard operating procedure. Use quality control procedures described in Section 4020.

5. Calculations

Prepare standard curves by plotting absorbance of standards processed through manifold versus phosphorus concentration. The calibration curve is linear.

Verify digestion efficiency by determining tripolyphosphate and trimethylphosphate standards at regular intervals. In the concentration range of the method, the recovery of either of these compounds should be >95%.

4500-P J. Persulfate Method for Simultaneous Determination of Total Nitrogen and Total Phosphorus

1. General Discussion

a. Principle: The oxidation of nitrogenous compounds for determining total nitrogen must occur in an alkaline medium. Conversely, the oxidation of phosphorus compounds for deter-

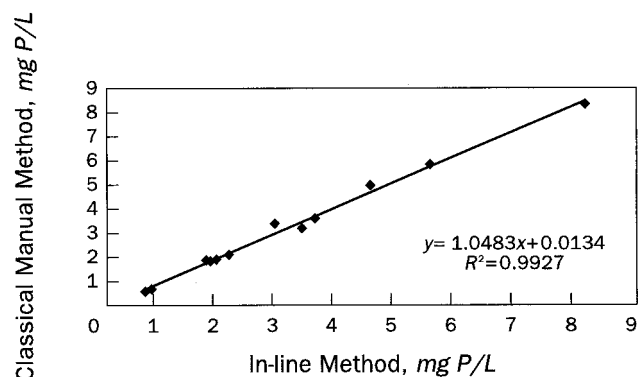


Figure 4500-P:6. Correlation between manual and in-line total phosphorus methods.

6. Precision and Bias

a. MDL: A 390- μ L sample loop was used in the method described above. Using a published MDL method,¹ analysts ran 21 replicates of a 0.10-mg P/L orthophosphate standard. These gave a mean of 0.10 mg P/L, a standard deviation of 0.003 mg P/L, and MDL of 0.007 mg P/L.

b. Precision of recovery study: Ten injections of a 10.0-mg P/L trimethylphosphate standard gave a mean percent recovery of 98% and a percent relative standard deviation of 0.8%.

c. Recovery of total phosphorus: Two organic and two inorganic complex phosphorus compounds were determined in triplicate at three concentrations. The results are shown in Table 4500-P:IV.

d. Comparison of in-line digestion with manual digestion method: Samples from a wastewater treatment plant influent and effluent and total phosphorus samples at 2.0 mg P/L were determined in duplicate with both manual persulfate digestion followed by the method in Section 4500-P:H and in-line digestion method. Table 4500-P:V gives the results of this comparison, and Figure 4500-P:6 shows the correlation between manual and in-line total phosphorus methods.

7. Reference

1. U.S. ENVIRONMENTAL PROTECTION AGENCY. 1984. Definition and procedure for the determination of method detection limits. Appendix B to 40 CFR 136 Rev. 1.11 amended June 30, 1986. 49 CFR 43430.

mining total phosphorus must occur under acidic conditions. Methods determining total nitrogen have used a persulfate-sodium hydroxide system to oxidize nitrogenous compounds to nitrate. Accordingly, methods determining total phosphorus have used persulfate in an acidic medium.

During the initial stage of the digestion, sample pH is alkaline (pH > 12). In the final stage of the digestion, the sodium hydroxide is consumed, causing sample pH to become acidic (pH < 2). By means of this broad pH range, the method allows for the oxidation of both nitrogen and phosphorus compounds. The digested sample is analyzed for nitrate and orthophosphate, yielding total nitrogen and total phosphorus results.

b. Selection of nitrate/orthophosphate measurement methods: Using a dual-channel autoanalyzer that performs nitrate-nitrite by the cadmium reduction method and orthophosphate by the ascorbic acid reduction method, total nitrogen and total phosphorus can be measured simultaneously. Alternatively, other methods for orthophosphate and nitrate can be used.

2. Apparatus

Clean all glassware with HCl before use.

a. Autoclave, capable of achieving a temperature of 120°C for a minimum of 120 min.

b. Glass culture tubes, 13-mm-OD × 100-mm-long with autoclavable caps.

c. Autopipettor, capable of pipetting a 6.0-mL portion.

d. Repeating pipettor, capable of pipetting 1.25-mL portion.

e. Erlenmeyer flask, 3000-mL.

f. Aluminum foil.

g. Automated continuous-flow instrument system for nitrate and phosphate determination: The suggested analytical instruments are described in Sections 4500-NO₃⁻.F and 4500-P.F.

3. Reagents

a. Deionized water, high-quality, free of phosphorus and nitrogen compounds. Prepare by ion-exchange or distillation methods as directed in 4500-NH₃.B.3a and 4500-NO₃⁻.B.3a.

b. Sodium hydroxide, 3N: Dissolve 120 g low-nitrogen NaOH in 800 mL deionized water in a 1000-mL volumetric flask. Cool and dilute to volume.

c. Oxidizing reagent: Dissolve 64 g low-nitrogen (<0.001% N) potassium persulfate, K₂S₂O₈, in 500 mL deionized water. Use low heat if necessary. Add 80 mL 3N NaOH, prepared from low-nitrogen sodium hydroxide, and dilute to 1000 mL. Store in a brown bottle at room temperature.

d. All of the reagents listed for determining nitrate + nitrite as indicated in Section 4500-NO₃⁻.F.3.

e. All of the reagents listed for determining phosphate as indicated in Section 4500-P.F.3.

f. Nicotinic acid p-toluenesulfonate stock and working standards: Dry nicotinic acid p-toluenesulfonate in an oven at 105°C for 24 h. Dissolve 2.1084 g in deionized water and dilute to 100 mL; 1 mL = 1 mg N. To prepare a working standard, dilute 2.0 mL stock solution to 1000 mL; 1 mL = 2 µg N.

g. Adenosine triphosphate stock and working standards: Dissolve 0.6514 g adenosine triphosphate in deionized water and dilute to 1000 mL; 1 mL = 0.1 mg P. To prepare a working standard, dilute 20.0 mL stock solution to 1000 mL; 1 mL = 2 µg P. To prepare a low-range working standard, dilute 1.0 mL stock solution to 1000 mL; 1 mL = 0.1 µg P.

4. Procedure

a. Calibration curve: Prepare a minimum of five standards over the desired calibration ranges using a stock calibration standard containing both nitrate and orthophosphate. Treat standards in the same manner as samples. Include blanks in calibration curves.

b. Sample preparation: If necessary, dilute sample with deionized water so that expected nitrogen and phosphorus concentrations fall within the range of the calibration standards. Samples preserved with acid cannot be analyzed by this digestion method.

c. Digestion check standards: Analyze quality-control standards containing organic nitrogen and phosphorus on each analytical run (see ¶s 3f and g for suggested standards and preparation procedures). These standards provide reference checks on the calibration and test the efficiency of the digestion.

d. Digestion: Pipet 6.0 mL of sample or standard into the culture tubes. Add 1.25 mL oxidizing reagent to each tube using a repeating pipettor. Cover the tubes with loose-fitting plastic caps. Prepare an autoanalyzer wash water in an erlenmeyer flask by adding oxidation reagent to deionized water in the same proportion as was added to the samples. Cover flask with foil. Autoclave samples and wash water for 55 min at 120°C. Cool to room temperature. Add 0.05 mL of 3N NaOH to each tube before proceeding to nitrate + nitrite and phosphate analyses. Shake to mix. Add same proportion of 3N NaOH to digested wash water.

e. Final nitrate + nitrite measurement: Use the automated cadmium reduction method for the determination of nitrate-nitrite after digestion. See Section 4500-NO₃⁻.F. Other nitrate analysis methods may be applicable; however, precision and bias data do not exist for these methods on this matrix at this time.

f. Final phosphate measurement: Use the automated ascorbic acid reduction method for the determination of phosphate after digestion. See Section 4500-P.F. Other phosphate analysis methods may be applicable; however, precision and bias data do not exist for these methods at this time.

5. Calculation

Prepare nitrogen and phosphorus standard curves by plotting the instrument response of standards against standard concentrations. Compute the nitrogen and phosphorus concentrations by comparing the sample response with the standard curve. Where necessary, multiply sample concentration by the appropriate dilution factor to determine final concentration.

6. Quality Control

Use protocols specified in Section 4020 to verify performance. These include daily use of reagent blanks, laboratory-fortified blanks, and known additions. Regulatory analysis may require additional quality control procedures

7. Precision and Bias

a. Total nitrogen:

1) Nitrogen digestion check standards — Four different organic nitrogen standards (2 mg N/L) were analyzed by a single

laboratory on three separate analytical runs yielding the following results:

| Nitrogen Compound | Mean mg N/L | Recovery of N % | Standard Deviation mg N/L | Relative |
|---|----------------|-----------------------|---------------------------------|----------------------------|
| | | | | Standard Deviation % |
| Urea | 2.03 | 102 | 0.021 | 1.0 |
| Ammonium <i>p</i> -toluenesulfonate | 1.93 | 97 | 0.037 | 1.9 |
| Glycine <i>p</i> -toluenesulfonate | 1.94 | 97 | 0.034 | 1.8 |
| Nicotinic acid <i>p</i> -toluenesulfonate | 1.86 | 93 | 0.044 | 2.4 |

2) Mixed ammonia-nitrate solution — A mixed ammonia-nitrate sample (0.55 mg N/L) was analyzed nine times, yielding the following results:

| Nitrogen Compounds | Mean mg/L | Recovery of N % | Standard Deviation mg/L | Relative |
|--------------------------------------|--------------|-----------------------|-------------------------------|----------------------------|
| | | | | Standard Deviation % |
| Ammonium sulfate & potassium nitrate | 0.557 | 101 | 0.012 | 2.1 |

3) Samples — A single laboratory analyzed five samples in quadruplicate on three separate occasions. Samples analyzed for total nitrogen included surface water and diluted wastewater samples. At an average concentration of 15.0 mg N/L, the average standard deviation was 0.143 mg/L.

b. Phosphorus:

1) Adenosine triphosphate solutions — Two concentrations (2 mg P/L and 100 μ g P/L) adenosine triphosphate were analyzed by a single laboratory on two separate analytical runs yielding the following results:

| Phosphorus Compound | Mean mg P/L | Recovery |
|---|----------------|-----------|
| | | of P % |
| Adenosine triphosphate, 2 mg P/L | 2.05 | 103 |
| Adenosine triphosphate, 0.100 μ g/L | 0.103 | 103 |

2) Samples — A single laboratory analyzed five samples in quadruplicate on three separate occasions. Samples were analyzed for total phosphorus over two different calibration ranges. Surface water and diluted wastewater samples were analyzed utilizing a low calibration range (0 to 250 μ g P/L, method detection level (MDL) = 2 μ g P/L). At an average concentration of 1670 μ g P/L, the average standard deviation was 29.4 μ g/L. Surface water and undiluted wastewater samples were analyzed using a high calibration range (0 to 6 mg P/L, MDL = 0.05 mg P/L). At an average concentration of 1.97 mg P/L, the average standard deviation was 0.028 mg/L.

8. Bibliography

- KOROLEFF, F. 1972. Determination of total nitrogen in natural waters by means of persulfate oxidation. In S.R. Carlberg, ed. *New Baltic Montreal. ICES Cooperative Res. Rep., Ser. A, No. 29, p. 73.*
- KOROLEFF, F. 1977. Simultaneous persulfate oxidation of phosphorus and nitrogen compounds in water. In K. Grasshoff, Report of the Baltic Intercalibration Workshop. Annex. Interim Commission for the Protection of the Environment of the Baltic Sea, p. 52.
- NYDAHL, F. 1978. On the peroxodisulphate oxidation of total nitrogen in waters to nitrate. *Water Res.* 12: 1123.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1979. Method 365.1 in *Methods for Chemical Analysis of Water and Wastes. EPA 600/4-79/020*, U.S. Environmental Protection Agency, Cincinnati, Ohio.
- VALDERRAMA, J.C. 1981. The simultaneous analysis of total nitrogen and total phosphorus in natural waters. *Marine Chemistry* 10:109.
- U.S. ENVIRONMENTAL PROTECTION AGENCY. 1987. *Handbook of Methods for Acid Deposition Studies: Laboratory Analyses for Surface Water Chemistry. EPA 600/4-87/026*, U.S. Environmental Protection Agency, Washington, D.C., p. 1811.
- AMEEL, J.J., R.P. AXLER & C.J. OWEN. 1993. Persulfate Digestion for Determination of Total Nitrogen and Phosphorus in Low Nutrient Waters. Center for Water and the Environmental, Natural Resources Research Inst. *Amer. Environ. Lab.* Oct 1993:2.