Determination of phosphorus: Ascorbic acid procedure

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Abstract

This procedure is suitable for very low to high concentration of phosphorus (0.00001- 6 mg/l).

What happens to this procedure: Ammonium molybdate and potassium antimonyl tartrate react in acidic solution with orthophosphate to form a heteropolyic acid (phosphomopydbic acid) which can be reduced by ascorbic acid to form an intense blue color, the absorbance of which is then measured at 885 nm spectrums.

Forms of Phosphorus: In the natural water phosphorus exists solely in three basic forms: orthophosphates, condensed phosphates (pyro-, meta- and polyphosphates) and organically bound phosphate. All of which combined together to form total phosphorus. From the sample water, orthophosphate can be analyzed directly by spectrophotometry. But for measuring total phosphorus-phosphate, condensed phosphate and, organically bound phosphate need to be digested to orthophosphate prior to the final procedure.

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Protocol

Sampling procedure

Step 1.

List of the Equipment:

1. Niskin bottle

2. Nylon mesh filter (500 um)

3. Acid washed plastic bottle

4. Cooler

5. Dry ice

6. 0.7 um filter

Acid Wash Procedure for Preparing Sampling Containers and equipment:

1. Wash each sample bottle or piece of glassware with a brush and phosphate-free detergent.

2. Rinse three times with the cold tap water.

3. Rinse with 10 percent hydrochloric acid.

4. Rinse three times with deionized water.

For phosphorus analysis, sample water should be either grabbed or composite and, collected from the well-mixed areas of the water body so that they are representative of the total flow. Niskin bottles should be used to collect the sample water which will then filtered through a 300 um nylon filter and transferred to a 100 ml HDPE plastic bottle either made of high-density polyethylene or polypropylene (accepted by EPA). If the containers are to be reused, they must be washed repeatedly with HCl and, de-ionized water as the phosphorus molecule has the tendency to absorb (attach) to the inside surface of the container. The samples bottles will be then placed on a cooler having dry ice on it and transported immediately to the lab for further processing. Prior to final analysis or preservation, sample water should be filtered through a 0.7 um filter.

Digestion of condensed and organically bound phosphate into ortho- phosphate (applicable only for total phosphate determination)

Step 2.

Equipment:

1. Hot plate

2. Gloves

3. Scoop (0.4 g capacity)

4. 125 mL Erlenmeyer flasks (acid washed)

5. 50 mL graduated cylinders (acid washed)

6. Phenolphthalein indicator

7. Sulfuric acid solution: 300 ml concentrated sulfuric acid and 1 L distilled water

8. Ammonium persulfate, crystal 9. Sodium hydroxide solution (1N): 40 g sodium hydroxide crystals and 1 L distilled water

Procedure:

1. Sample water (50 ml) will be taken on an acid washed Erlenmeyer flask and diluted into 50 ml distilled water.

2. One drop of phenolphthalein indicator will be added and if a red color develops, then sulfuric acid solution (1 ml) will be added until color just disappears. If the color disappears, 0.4 g ammonium persulfate will be added.

3. The solution will then boil gently for 30 to 40 minutes or until the total volume is 10 mL.

4. Cool the solution and add 1 drop of phenolphthalein to neutralize to a faint pink color with 1 N sodium hydroxide.

5. Finally, the solution will be diluted with distilled water to make 50 mL solution and ready for total phosphate analysis.

Preservation:

If total Phosphorus is the only determination to be made, acidify the sample to less than pH 2 with sulfuric acid and cool to 4°C. Acidified and cooled samples may be held for up to 28 days.

Determination of orthophosphate/ total phosphate:

Step 3.

Equipment and reagents:

1. Acid washed glassware
2. Analytical balance
3. Spectrophotometer with an infrared phototube for use at 885 nm with a light path of 10 cm
4. Ammonium molybdate solution: 15 g of reagent grade ammonium paramolybdate, (NH4)6Mo7O24 .4H2O and 500 ml of deionized water.
5. Ascorbic acid solution: 27 g of ascorbic acid and 500 ml of deionized water.
6. Sulfuric acid solution: 140 ml of concentrated sulfuric acid and 900 ml of deionized water.
7. Potassium antimonyl-tartrate solution: 0.34 g of potassium antimonyl- tartrate and 250 ml of deionized water.
8. Final reagent: Final reagent can be made by mixing together 250 ml sulfuric acid solution, 100 ml ascorbic acid solution, 50 ml potassium antimonyl-tartarate solution and 100 ml ammonium molybdate solution. Use at once and discard any excess. Do not store for more than 6 hours. Add molybdate last. Solution should have a yellow color.

Sample analysis: Prior to analysis, previously preserved samples should be thawed and brought to a temperature of between 15° and 30°. Do not let the samples sit for long periods of time as the polyethylene bottles may absorb phosphate. Place 50 ml of sample into a 100 ml (or 60) polyethylene bottle. To each sample add 5 ± 0.25 ml of the mixed reagent and mix immediately. After 5 minutes and within 2 hours, measure the absorbance of the sample in a 10 cm cell against de-ionized water at a wavelength of 885 nm.

Blank determination: A reagent blank is determined by using de-ionized water in place of the 50 ml seawater sample and carrying out the exact method described in above (sample analysis). The reagent blank should not exceed 0.03. If it does, the ammonium molybdate reagent should be replaced and the blank determination repeated.

Standardization:
Primary phosphate standard: Dissolve 0.816 g of anhydrous potassium dihydrogen phosphate, KH2 PO4 , in 1000 ml of de-ionized water. 1 ml = 6 umol. Store in a dark bottle. This solution is stable for many months. Secondary standard: Dilute 10.0 ml of the primary standard solution to 1000 ml with de-ionized water. 1 ml = 0.06 mmol. Store in a dark bottle. Make fresh every 10 days.
A standard solution of 3.0 uM by diluting 5.0 ml of secondary standard to a volume of 100 ml with deionized water. Run these standards as described in sample analysis portion.

Calculation:

Step 4.

The phosphate concentration can be calculated by the following equation:
Concentration of orthophosphate or total phosphate (umol l -1) = F * corrected absorbance
Where:
Corrected absorbance = sample absorbance - reagent blank
F = standardization factor
A standardization factor F can be calculated as:
F = 3.0 umol/kg / (Es- Eb)
Where:
3.0 umol/kg = concentration of the standard
Es = mean absorbance of the standards
Eb = mean absorbance of the blanks

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