

Chapter 11. The Determination of Phosphorus in Sea Water

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Modified from: Strickland and Parsons (1968)

1.0 Scope and field of application

This procedure describes a method for the determination of reactive phosphorus in seawater suitable for the assay of oceanic concentrations of 0.01- 2.5 $\mu\text{mol l}^{-1}$.

2.0 Definition

The reactive phosphate concentration is given in units of $\mu\text{mol kg}^{-1}$ in seawater.

3.0 Principle of Analysis

The determination of reactive phosphorus in seawater is based on the method proposed by Strickland and Parsons (1968). The seawater sample is allowed to react with a composite reagent containing ammonium molybdate, ascorbic acid and potassium antimonyl-tartrate. The resulting complex is reduced *in situ* to give a blue colored solution, the absorbance of which can be measured spectrophotometrically.

4.0 Apparatus

Spectrophotometer

5.0 Reagents

5.1 *Ammonium molybdate solution*: 15 g of reagent grade ammonium paramolybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, dissolved in 500 ml of Milli-Q water. The solution is stable indefinitely if stored out of direct sunlight in a plastic bottle, but if a precipitate forms the solution is discarded.

5.2 *Sulfuric acid solution*: 140 ml of concentrated sulfuric acid added to 900 ml of Milli-Q water. The solution is cooled and stored in a glass bottle.

- 5.3 *Ascorbic acid solution:* 27 g of ascorbic acid dissolved in 500 ml of Milli-Q water. This solution is stored frozen in a plastic container and thawed as needed.
- 5.4 *Potassium antimonyl-tartrate solution:* 0.34 g of potassium antimonyl- tartrate dissolved in 250 ml of Milli-Q water. This solution is stable for many months.
- 5.5 *Mixed reagent:* 100 ml ammonium molybdate solution, 250 ml sulfuric acid solution, 100 ml ascorbic acid solution and 50 ml potassium antimonyl-tartrate solution. The ammonium molybdate is the last reagent to be added. The mixed reagent should have a yellow color and be used preferably at once, or within 6 hours.

6.0 Preparation for sampling

- 6.1 Samples are collected in 250 ml polyethylene bottles. Contamination is a major problem with nutrient samples, especially near the surface where the ambient concentrations are low. All the nutrient bottles are rigorously cleaned before use. New bottles are soaked for 2-3 days in 5 % Aquet and tap water, rinsed with tap water, then soaked for 2-3 days in 10 % HCl. Bottles are then soaked overnight in Milli-Q water and rinsed 5-6 times with Milli-Q water. After bottles have been seasoned they are cleaned between uses by soaking overnight in 5 % detergent, transferred to 10 % HCl overnight, and rinsed 5-6 times with Milli-Q water.
- 6.2 Polycarbonate filter holders (Gelman) are used in the filtering of samples. Cleaning of these begins with an overnight soak in Aquet, followed by tap water rinsing, a soak in 5 % HCl for 1-2 hours, and 5-6 rinses with Milli-Q water.

7.0 Sampling

- 7.1 Samples are collected at 35 depths between the surface and 4200 m. A polycarbonate filter holder (Gelman) containing a 0.8 μm Nuclepore filter is connected to the OTE bottle with Tygon[®] tubing. The spigot is opened and samples are collected from the filtered water. Each bottle is rinsed three times and then filled to just below the shoulder. Care is taken to avoid overfilling of samples. Samples are transferred to a freezer (-20°C) and kept frozen until analysis.
- 7.2 Prolonged storage of samples is avoided.

8.0 Procedures

8.1 *Sample analysis*

- 8.1.1 Prior to analysis the samples are thawed and brought to a temperature of between 15° and 30°C. Samples should not sit for long periods of time as the polyethylene bottles may absorb phosphate.
- 8.1.2 100 ml of sample is placed into a 200 ml polyethylene bottle.
- 8.1.3 10 ± 0.5 ml of the mixed reagent is added and immediately mixed.
- 8.1.4 After 5 minutes but within 2 hours, the absorbance of the sample at a wavelength of 885nm is measured in a 10 cm cell, against a blank of Milli-Q water.

8.2 *Blank determination*

- 8.2.1 A reagent blank is determined by using Milli-Q water in place of the 100ml seawater sample and carrying out the exact method described in Section 8.1.
- 8.2.2 The reagent blank should not exceed 0.03. If it does, the ammonium molybdate reagent is replaced and the blank determination repeated.

8.3 *Standardization*

- 8.3.1 *Primary phosphate standard:* 0.816 g of anhydrous potassium dihydrogen phosphate, KH_2PO_4 , dissolved in 1000 ml of Milli-Q water. 1 ml = 6 μmol . This solution is stored in a dark bottle and stable for many months.
- 8.3.2 *Secondary standard:* 10.0 ml of the primary standard solution diluted to 1000 ml with Milli-Q water. 1 ml = 0.06 μmol . The standard is stored in a dark bottle and made fresh every 10 days.
- 8.3.3 A standard solution of 3.0 μM is prepared by diluting 5.0 ml of secondary standard to a volume of 100 ml with Milli-Q water. These standards are run as described in section 8.1.

9.0 **Calculation and expression of results**

- 9.1 A standardization factor F can be calculated as:

$$F = \frac{3.0 \mu\text{mol kg}^{-1}}{E_s - E_b}$$

Where:

$3.0 \mu\text{mol kg}^{-1}$ = concentration of the standard
 E_s = mean absorbance of the standards
 E_b = mean absorbance of the blanks

The reactive phosphate concentration is calculated by:

reactive phosphorus ($\mu\text{mol l}^{-1}$) = $F \cdot$ corrected absorbance

Where:

F = standardization factor
corrected absorbance = sample absorbance - reagent blank

9.2 The units of $\mu\text{moles kg}^{-1}$ can be obtained by dividing the calculated phosphate concentration by the density of the seawater at the time of analysis.

10.0 References

Strickland, J.D.H., and Parsons, T.R. (1968). Determination of reactive phosphorus. In: *A Practical Handbook of Seawater Analysis*. Fisheries Research Board of Canada, Bulletin 167, 49–56.