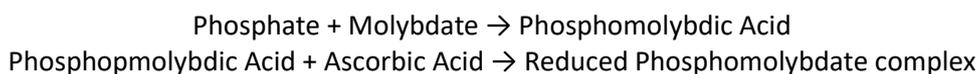


Dissolved phosphorus determination by ascorbic acid-molybdenum blue method

The determination of reactive phosphorus (0.01 to 2.5 μM) 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. In the ascorbic acid-molybdate method orthophosphate reacts with molybdate to form phosphomolybdic acid. Phosphomolybdic acid is reduced by ascorbic acid to form a blue complex:



The intensity of the blue color at 885 nm is proportional to the phosphate concentration in the solution. It has been demonstrated that in dilute, acidic solutions with an excess of the molybdate species present, Beer's Law is obeyed with respect to phosphate.

Use acid-washed volumetric flasks for preparation of all the solutions

Reagents

Ammonium molybdate solution

Dissolve 15 g of reagent grade ammonium paramolybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}\cdot 4\text{H}_2\text{O}$, in 500 mL of Milli-Q water. The solution is stable indefinitely if stored out of direct sunlight in a plastic bottle. Discard if precipitates form.

Sulfuric acid solution

Add 140 mL of concentrated sulfuric acid to 900 mL of Milli-Q water. Cool the solution and store it in a glass bottle. The solution is then stable for many months but should not be kept for more than a week at laboratory temperatures.

Ascorbic acid solution

Dissolve 27 g of ascorbic acid in 500 mL of Milli-Q water. This solution should be frozen in a plastic container. Thaw for use and refreeze at once.

Potassium antimonyl-tartrate solution

Dissolve 0.34 g of potassium antimonyl-tartrate in 250 mL of Milli-Q water. This solution is stable for many months.

Mixed reagent

Mix together 10 mL ammonium molybdate solution, 25 mL sulfuric acid solution, 10 mL ascorbic acid solution and 5 mL potassium antimonyl-tartrate 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.

Standards

Primary phosphate standard (6 mM P)

Dissolve 0.816 g of anhydrous potassium dihydrogen phosphate, KH_2PO_4 , (dried in the oven at 105°C for 1 h) in 1000 mL of Milli-Q water. Store in a dark bottle with 1 mL of chloroform. This solution is stable for many months.

Secondary phosphate standard (60 μ M P)

Dilute 10 mL of the primary standard solution to 1000 mL with Milli-Q water. Store in a dark bottle. Make fresh every 10 days.

Standard solution

Prepare the standard solutions by diluting the secondary standard to a volume of 50 mL with Milli-Q water.

Phosphate standard (μ M)	Volume of 60 μ M phosphate standard (mL)
0.1	0.083
0.25	0.208
0.5	0.417
1	0.833
2	1.667
3	2.5

Sample analysis

1. Switch on the instrument 15 minutes before the measurements and set wavelength to 882 nm. This is to enable the bulbs in the spectrometer to warm up.
2. Prepare the spectrophotometric cuvettes, always hold them from the opaque sides. Cuvettes must be inserted in the same orientation all the time, there is a small arrow at the top to help ensure they are used the same way around.
3. Add 1 mL of blank / standards / sample to the cuvette.
4. Add 0.1 mL of the mixed reagent.
5. After 5 min and within 2 h, measure the absorbance at 882 nm.

Reference:

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.