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Determination of Phosphate in Seawater by an Isobutyl-Acetate-Extraction Procedure

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ABSTRACT

This paper describes methods for determining the quantity of inorganic phosphate and total phosphorus in seawater by extracting the molybdophosphoric acid with isobutyl acetate and by then reducing the solution to heteropoly blue. Arsenic and silicate in amounts ordinarily encountered in seawater do not interfere.

Introduction. The amount of inorganic phosphate in seawater can be determined by direct methods, but Martin (1968) has reported that, for phosphate concentrations below 5 µg PO₄-P/L, direct determination is not satisfactory. Inorganic phosphate in seawater can also be determined by using isobutanol-extraction procedure (Proctor and Wood 1954). Jones and Spencer (1963), who compared this extraction procedure with four direct methods, found that the reproducibility of the extraction method was comparatively poor. Strickland and Parsons (1968) modified Proctor and Wood’s (1954) isobutanol-extraction procedure for determining the amount of phosphorus in oligotrophic areas and in many freshwater lakes, but their method was unsatisfactory when used on water stored in iodine-treated containers.

Traces of phosphorus in metals and salts have been determined by extracting the yellow molybdophosphoric acid with isobutyl acetate derived from sulphate solutions and by then reducing the solution to heteropoly blue (Pakalns 1968). This procedure allows phosphorus to be determined without preliminary separation from silicon and arsenic and does not require washing of the organic phase, thus eliminating a source of considerable variation in the sensitivity. Pakalns and McAllister (1971) extended the method to a determination of phosphorus at trace levels in nitric, perchloric, and hydrochloric acid and in mixed acids. From these results a nitric-perchloric-acid system was selected for determining the amount of phosphate in seawater; although the procedure was designed

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specifically for such a determination, a standard addition procedure allows phosphate to be determined for all types of potable and industrial waters.

**Materials.** For storage of the seawater samples, use polyethylene bottles that have been treated with a solution of iodine-potassium iodide. Heron (1962) has recommended that the sample bottles be filled with such a solution (5 g of iodine and 8 g of potassium iodide per 100 ml of distilled water), be permitted to stand for a week, and then be drained and washed well with distilled water.

**Apparatus and Reagents.** Use a Unicam SP-600 Spectrophotometer with 2-cm and 4-cm cuvettes.

Throughout the determinations, use a demineralized water, further purified by means of a single distillation from permanganate.

Standard phosphorus solution: for a stock solution of 0.1 mg P/ml, dissolve 0.4395 g of KH$_2$PO$_4$ in 1000 ml of distilled water; for a working solution of 1 µg or 2 µg P/ml, dilute 5 or 10 ml of the stock solution to 500 ml.

Ammonium molybdate solutions (5% and 10%): prepare from (NH$_4$)$_6$Mo$_7$O$_{24}$·4H$_2$O and store in a polyethylene bottle.

Perchloric acid, 72% (Merck): check the purity of perchloric acid by preparing a blank solution and taking it through Method A (p. 307). The absorbance must be less than 0.030.

Tin (II) chloride solution: dissolve 4 g of SnCl$_2$·2H$_2$O in 10 ml of hydrochloric acid. Prepare a fresh solution each week.

Reducing solution: dissolve 1.3 g of ascorbic acid in 20 ml of water; add 1.3 ml of tin (II) chloride solution and 15 ml of 9N sulphuric acid; dilute to 100 ml.

Isobutyl acetate (May and Baker Ltd.): boiling range 115°-120°C.

**Methods.** Filter seawater samples through a 0.45-µ membrane filter and store in iodine-treated polyethylene bottles in a temperature of -20°C. According to B. Newell (1970; private communication), samples may be stored indefinitely in this manner prior to determining the inorganic phosphate content.

The stability of the seawater samples was ascertained as follows. Samples were put in iodine-treated polyethylene bottles and stored in a deep freeze for three weeks. The samples were then thawed to room temperature and analyzed for inorganic phosphate. The solutions were then returned to the original polyethylene containers, stored at room temperature, and again analyzed for inorganic phosphate at 10-day and 20-day intervals. Increases or decreases in the inorganic phosphate content do show up over a period of time (see Table I).

Two methods for determining inorganic phosphate are proposed below. Method A describes the determination of inorganic phosphate in seawater that contains less than 40 µg PO$_4$-P/L, using a 100-ml sample. A modified proced-
Table I. The effect of standing in iodine-treated polyethylene containers and of reaction time between phosphate and molybdate on \( \text{PO}_4^- \)-P results.

<table>
<thead>
<tr>
<th>Source of seawater</th>
<th>Total P (µg/L)</th>
<th>( \text{PO}_4^- )-P (µg/L after standing*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 days</td>
<td>10 days</td>
</tr>
<tr>
<td></td>
<td>3 min**</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td>3 min</td>
<td>10 min</td>
</tr>
<tr>
<td></td>
<td>3 min</td>
<td>10 min</td>
</tr>
<tr>
<td>Murray’s Beach . . . .</td>
<td>11.0</td>
<td>4.3</td>
</tr>
<tr>
<td>Bowen Island . . .</td>
<td>9.9</td>
<td>4.5</td>
</tr>
<tr>
<td>Cronulla Beach . . . .</td>
<td>16.5</td>
<td>7.2</td>
</tr>
</tbody>
</table>

* Averages of 3 analyses.  ** Reaction time in minutes after adding molybdate.

ure is included for seawater samples containing more than 40 µg \( \text{PO}_4^- \)-P/L. Method B is recommended for use when the \( \text{PO}_4^- \)-P concentration is below 5 µg/L; it can also be used to determine inorganic phosphate in oligotrophic areas and freshwater lakes.

**METHOD A.** Transfer two 100-ml aliquots of seawater that have been filtered through a 0.45-µ membrane filter and contain less than 4 µg of phosphorus per aliquot into two 250-ml separating funnels. To one aliquot add 4 ml of 1 µg P/ml solution and mix. To both aliquots add 1 ml of 9M nitric acid and 1 ml of 10% hydroxylammonium chloride, mixing after each addition. After 10 min, add to both aliquots 1 ml of 9M nitric acid and 8 ml of 72% perchloric acid, mixing after each addition. To both aliquots add 10 ml of 5% ammonium molybdate solution, mix, and let stand for 3 min.

Add 13 ml of isobutyl acetate to solutions in 250-ml separating funnels, shake for 1 min, and let stand for 5 min. Transfer the organic phase to a 50-ml separating funnel and let stand for 5 min; swirl the organic extract to dislodge any water droplets, then remove the aqueous phase, taking care that it is also removed from the bore of the stopcock. Add 3 ml of the reducing solution to the organic phase, shake for 45 sec, and let stand for 5 min.

Run the contents of the funnel into a dry 25-ml volumetric flask, wash the funnel with methanol, and add the washings to the flask. Mix and dilute to volume with methanol. Mix and then measure the absorbance at 12 ± 3 min against a blank at 725 nm in 4-cm cells.

Prepare a blank using distilled water in place of the sample.

If the inorganic phosphorus content of the seawater is above 40 µg/L, make a standard addition of 8 µg of P and measure the absorbance in 2-cm cells. In case the inorganic phosphate or total phosphorus is very high, use a smaller sample size, but always dilute the sample to 100 ml, using distilled water.

**METHOD B.** Transfer two 200-ml aliquots of seawater that have been filtered through a 0.45-µ membrane filter and contain less than 4 µg of phosphorus per aliquot into two 250-ml separatory funnels. To one aliquot add 4 ml of 1 µg P/ml solution and mix. To both aliquots, add 2 ml of 9M nitric acid and 2 ml of 10% hydroxylammonium chloride, mixing after each addition. After 10 min, add to both aliquots 2 ml of 9M nitric acid and 17 ml of 72% perchloric acid,
mixing after each addition. To both aliquots add 10 ml of 10% ammonium molybdate solution, mix, and let stand for 3 min. Add 13 ml of isobutyl acetate to solutions in 250-ml separating funnels and continue as in Method A.

In order to determine the Total Phosphorus, filter the seawater through a fast filter paper (Whatman 541) to remove organically combined phosphorus in suspension (Armstrong 1965). To two 50-ml aliquots of the filtrate in 125-ml wide-mouth conical flasks, add 3 ml of 72% perchloric acid. Evaporate the samples on a hot plate until the first fumes of perchloric acid are observed. Place a watch glass on the flasks and heat the contents for 5 min at a temperature just below the temperature required for the fuming (Martin 1968). Remove the flasks from the hot plate and cautiously add 30 ml of distilled water. Heat to boiling and cool. Transfer the solutions to 250-ml separating funnels and dilute to 100 ml with distilled water. To one aliquot, add 4 ml of 1 µg P/ml solution and continue as in Method A (see page 307).

Calculate the microgram of PO₄-P/L as follows:

$$\text{PO}_4^-\text{P}, \mu g/L = \frac{A \times C \times 1000}{(B - C) \times D},$$

where \(A\) = microgram of phosphorus used in the standard addition, \(B\) = absorbance of sample containing standard addition, \(C\) = absorbance of sample, \(D\) = milliliters of seawater sample.

Modifications of Method for Freshwater Samples. After a 50-ml aliquot of seawater having a 35.5°/oo salinity is fumed with 3 ml of 72% perchloric acid, the free acid amounts to approximately 0.5 ml of perchloric; small changes in the acidity do not affect the formation or extraction of the yellow molybdophosphoric acid. In the case of freshwater samples, the free acid that occurs after fuming consists of approximately 2 ml of the 72% perchloric acid; therefore only 6 ml of perchloric acid must be added before the ammonium molybdate addition.

Discussion. Solvent Extraction. Pakalns (1968) observed that only 13 ml of isobutyl acetate can be used for the extraction procedure because larger amounts are not miscible with 3 ml of the reducing solution when it is diluted to 25 ml with methanol to form a single phase. Only one extraction with 13 ml of isobutyl acetate was possible, because two extractions with smaller amounts of solvent formed emulsions that were hard to break down. However, a single extraction of 13 ml of solvent extracted only 95% of the yellow complex.

Extraction from Various Acids, and Stability. Data for the formation and extraction of the yellow molybdophosphoric acid in various acids were obtained by using 30-ml and 50-ml samples (Pakalns and McAllister 1971).
In the present work we found that, for a 100-ml sample, 10 ml of 9N sulphuric acid or 12 ml of either nitric acid or perchloric acid had to be added to develop maximum color for each acid. The absorbance for 10 µg of phosphorus in 2-cm cells was 0.280, 0.420, and 0.500 for sulphuric, perchloric, and nitric acid, respectively.

Standard solutions, prepared with various acids, were taken through the whole procedure to determine the stability of the heteropoly blue complex in the isobutyl acetate-methanol solution. The absorbance decreased rapidly when sulphuric or nitric acid solutions were used, but there was good stability with extracts from perchloric acid.

Pakalns and McAllister (1971) have reported that a standard prepared for solutions that contain the same amount of phosphorus from nitric, perchloric, or sulphuric acids gave various absorbances. It was found that at least 1 ml of the acid that gives the highest absorbance, nitric acid, must be added to obtain the highest absorbance in mixed-acid solutions; however, the total amount of 9N acids must fall within the recommended range of both of the acids used. When this combination effect was checked for 100-ml seawater samples, it was found that the addition of 2 ml of 9N nitric acid in perchloric acid solution gave absorbances that are equivalent to those obtained for nitric acid solutions alone. The absorbance of the heteropoly blue complex in the isobutyl acetate-methanol solution decreases slowly following the formation of a single phase, approximately 0.005 absorbance units in 30 min; for measuring the absorbance, 12 ± 3 min was selected as a convenient standard time following mixing.

A standard curve, prepared for solutions that contain between 0 and 4 µg of phosphorus from nitric-perchloric acid solution, showed that the absorbance-concentration curve is linear and goes through zero. Beer's law is obeyed to at least 1.2 µg P/ml in the final solution.

Iodine interferes with the extraction procedure by destroying the heteropoly blue complex, but the interference can be minimized by reducing the iodine with hydroxylammonium chloride. It was found that 1 ml of 9M nitric acid had to be added to a 100-ml sample prior to the reduction with hydroxylammonium chloride and that a waiting time of 10 min was required for a complete reduction.

**Standard Addition and Recoveries.** The color of the heteropoly blue complex varies with the temperature, with the salinity of the sample, with the concentration of other dissolved salts, and with the development time for the formation of the yellow molybdophosphoric acid. Since a standard curve could not be used, a standard addition was made for each determination (Pakalns 1968). Harvey (1955) also used a standard addition to correct for changes in the experimental conditions that affect the quantity of heteropoly blue formed or the rate of formation.

It was found that phosphorus in seawater gave higher absorbances than phosphorus in distilled water and that the increase was proportional to the
Table II. Absorbance on standard addition in PO$_4$-P determination.

<table>
<thead>
<tr>
<th>Water</th>
<th>$I_3$ µg per sample</th>
<th>Absorbance</th>
<th>100-ml sample</th>
<th>200-ml sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Sample</td>
<td>After standard</td>
<td>Difference for</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>addition for $4 \mu g$ P</td>
<td>$4 \mu g$ P</td>
</tr>
<tr>
<td>Distilled (Blank)*</td>
<td>-</td>
<td>0.024</td>
<td>0.431</td>
<td>0.407</td>
</tr>
<tr>
<td>Sea</td>
<td>-</td>
<td>0.068</td>
<td>0.495</td>
<td>0.427</td>
</tr>
<tr>
<td>Sea (I$_2$ treated)</td>
<td>2</td>
<td>0.068</td>
<td>0.485</td>
<td>0.417</td>
</tr>
<tr>
<td>Sea (I$_2$ treated)</td>
<td>5</td>
<td>0.090</td>
<td>0.494</td>
<td>0.404</td>
</tr>
<tr>
<td>Sea**</td>
<td>-</td>
<td>0.066</td>
<td>0.478</td>
<td>0.412</td>
</tr>
</tbody>
</table>

* Measured against water. ** Total phosphorus (50-ml sample).

salinity. When seawater sampled in iodine-treated polyethylene bottles was frozen, various amounts of free iodine were found in the sample; the amount of iodine varied between 0.6 and 5 mg per 100-ml sample, depending on the pretreatment and the porosity of the polyethylene bottles. In all cases, the absorbance obtained following the standard addition of phosphate to water stored in iodine-treated bottles was lower than that for water in nontreated bottles (Table II).

**FORMATION OF YELLOW MOLYBDOPHOSPHORIC ACID.** Jones and Spencer (1963) have suggested that the type and concentration of acid may affect the release of phosphate. They concluded that, although the acidity (1.2N) used by Proctor and Hood (1954) is much higher than that used by other workers, it is unlikely that rapid hydrolysis of a combined-but-labile phosphate will occur during its short contact time with the acid. Therefore it can be expected that rapid hydrolysis of combined-but-labile phosphate will not occur in 0.9N acid solution as proposed in our method. Table I shows that the rate of reaction between phosphate and molybdate depends on the source of seawater. It is apparent that seawater collected from the beach contains more combined-but-labile phosphate, which hydrolyzes on contact with the acid, than does seawater from the open sea. Therefore, following Martin’s (1968) recommendations, three minutes were selected for the formation of the yellow molybdophosphoric acid.

It was found that 100 µg AsO$_4$-As/L and 5000 µg of Si/L do not interfere with the procedure described here, since they produce errors of less than 0.1 µg P/ml.

**EXTRACTION FROM A 200-ml SAMPLE.** Extraction from a sample of 200-ml makes possible the determination of inorganic phosphate to an amount as low as 0.25 µg P/L. To reduce iodine, it was necessary to add 2 ml of 9M nitric acid and 2 ml of 10% hydroxylammonium chloride. A total amount of 4 ml of 9M nitric and 22 ml of 9M perchloric acid was added to obtain the maximum absorbance for the heteropoly blue complex.
The temperature of the seawater samples taken for analysis ranged between 20° and 21.5°C. After 8 ml of 72% perchloric acid was added, the temperature of a sample in the separating funnel increased by 3°C, and the temperature during formation of the molybdophosphoric acid was between 23° and 24.5°C. The absorbance of the extracted blue complex does not change in the investigated temperature range of 20° to 25°C.

The absorbance of the blanks was low—about 0.020 and 0.045 for 100-ml and 200-ml volumes, respectively. Some reagent grades of perchloric acid produce blanks as high as 0.100 absorbance.

Reproducibility of the Method. The absorbance of blanks varied by ±0.002. The variation in absorbances for phosphorus between 0.05 and 4 µg per 100 ml was ±0.005. For a series of 12 100-ml and 200-ml aliquots, the standard deviation (using in each case a sample and a standard 4 µg P addition) at the 6.2 µg P/L level was ±0.3 and ±0.15 µg P, respectively.

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