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# THE DETERMINATION OF ORGANIC PHOSPHORUS IN SEA WATER WITH PERCHLORIC ACID OXIDATION<sup>1</sup>

BY

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## ABSTRACT

A method is proposed for the determination of organic phosphorus in sea water; in this procedure the organic material is oxidized with perchloric acid and the organic phosphorus is converted to phosphate. Oxidation in a perchloric acid medium is superior to sulfuric acid: (a) The perchlorate salts formed upon digestion of sea water are readily water-soluble in contrast to certain sulfates; (b) A small blank correction for impurities must be applied. Interference by arsenic is prevented by volatilization of the arsenic chloride formed by heating the samples with concentrated hydrochloric acid. Total phosphorus is determined colorimetrically, and the organic phosphorus is calculated by deducting the inorganic phosphorus. Preliminary digestion with nitric acid is desirable before samples of high organic content are digested with perchloric acid.

## INTRODUCTION

The occurrence and distribution of phosphorus in its various forms in the sea are of interest in connection with the productivity of the sea. Phosphorus occurs in the sea as inorganic phosphate and as organic phosphorus in soluble organic forms and particulate matter. Inorganic phosphate is conveniently determined by a colorimetric method (Robinson and Thompson, 1948; Wooster and Rakestraw, 1951), and a considerable fund of information has been collected regarding its distribution. On the other hand, little information is available on the occurrence and distribution of organic phosphorus because of the difficulty experienced in its determination.

Organic phosphorus as particulate matter has been determined by separation of the particulate matter, oxidation in acid solution, and estimation by the colorimetric method for inorganic phosphate (Redfield, Smith and Ketchum, 1937). What is retained as particulate matter depends on the method of separation and of washing. Knowledge of the particulate organic phosphorus content gives incomplete information regarding the organic phosphorus content of the sea unless the soluble organic phosphorus content is also known.

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Most frequently the total organic phosphorus content, which includes both soluble and particulate organic phosphorus, is obtained by determining total phosphorus and subtracting inorganic phosphate. Total phosphorus is determined by colorimetric estimation after acid oxidation of organic phosphorus to phosphate. A number of procedures have been reported for the oxidation of organic phosphorus in sulfuric acid solution. Kreps and Osadich (1933) oxidized with 3% hydrogen peroxide, Kalle (1933) with potassium persulfate, and Cooper (1934) with 30% hydrogen peroxide. But these methods failed to account for the oxidation of arsenite to arsenate and its subsequent estimation along with phosphate by the colorimetric method. Failure to correct for arsenate introduces considerable error, since arsenic occurs in the sea to the extent of 0.2 to 0.5  $\mu\text{g-at/L}$ , mostly as arsenite (Gorgy, Rakestraw and Fox, 1948), while the organic phosphorus content is only about 0.5 to 0.6  $\mu\text{g-at/L}$ . Kalle (1935) attempted to prevent the interference of arsenate by reducing it to arsenite with thiourea. However, Cooper (1937) was unable to reduce arsenate in sea water with Kalle's method. Redfield, Smith and Ketchum (1937) digested sea water samples with hydrogen peroxide in sulfuric acid solution and reduced the arsenate by heating with sodium bisulfite for a period of eight hours. Harvey (1948) described another modification in which the samples were heated in the autoclave at 130° C in sulfuric acid solution with sodium sulfite to reduce arsenate.

Extensive investigations of various procedures for the determination of total phosphorus by Robinson and co-workers (Jesseph, 1932; Selbo, 1940) have indicated several inherent disadvantages in methods that employ sulfuric acid for the digestion of samples. It is difficult to obtain sulfuric acid that is sufficiently free of interfering impurities. Anhydrous sulfate salts of sodium, calcium, etc. separate on digestion and redissolve in distilled water with difficulty. Small undissolved salt nuclei often remain and cause excessive color development of the colloidal phosphomolybdate blue reduction complex.

Preliminary work by Selbo and Robinson (Selbo, 1940) has indicated that oxidation of organic matter in sea water could be effected by perchloric acid, the use of which results in a number of advantages. (a) The interfering impurities present in perchloric acid are of small magnitude. (b) The organic matter is quickly oxidized by the perchloric acid. (c) Salts, very soluble in water, are obtained upon digestion of the sample. A more complete examination of the method, made by Hansen and Robinson (Hansen, 1950), is reported in this paper. Recently, application of the perchloric acid method for lake waters (Robinson, 1941) has been made to sea water analysis by Rochford (1951).

## THE PROPOSED METHOD

## REAGENTS AND STANDARD SOLUTIONS

All chemicals used in this investigation were of analytical grade.

*Sulfuric Acid*: 36 Normal, arsenic-free acid.

*Perchloric Acid*: 60 or 72% acid.

*Hydrochloric Acid*: 12 Normal acid.

*Sodium Hydroxide*: 1 Normal sodium hydroxide solution.

*Ammonium Hydroxide*: 15 Normal ammonium hydroxide.

*Ammonium Molybdate-Sulfuric Acid Reagent*: Dissolve 10 g of ammonium molybdate,  $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ , in 100 ml of distilled water. Add this solution to 300 ml of 18 N sulfuric acid. Keep reagent in an amber glass-stoppered bottle and protect from light to prevent photochemical reactions from occurring.

*Stannous Chloride Solution I*. Dissolve 2.15 g of stannous chloride,  $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ , in 20 ml of 12 N hydrochloric acid and dilute to 100 ml with freshly boiled distilled water. Store the solution in an amber glass-stoppered bottle and add a piece of mossy tin to counteract air oxidation. The solution was stable for about a month.

*Stannous Chloride Solution II*. Dilute 5 ml of Solution I to 25 ml with freshly boiled distilled water. Add a small piece of mossy tin to stabilize the solution. Prepare a fresh solution each day.

*Standard Phosphate Solution I*. Dissolve 0.340 g of anhydrous potassium dihydrogen phosphate,  $\text{KH}_2\text{PO}_4$ , in distilled water and make up to 1 liter. Add 0.2 ml of chloroform to retard possible bacterial action. One ml of this solution contained 2.50  $\mu\text{g}$ -at phosphate-phosphorus.

*Standard Phosphate Solution IIa for Inorganic Phosphate*. Dilute 10 ml of Standard Solution I to 1 liter with a sodium chloride solution having a chlorinity similar to that of the sea water being tested. Add 0.2 ml of chloroform to retard bacterial action. One ml of this solution contained 0.0250  $\mu\text{g}$ -at phosphate-phosphorus.

*Standard Phosphate Solution IIb for Inorganic Phosphate*. Dilute 40 ml of Standard Solution I to 1 liter by means of the sodium chloride solution and add 0.2 ml of chloroform. One ml of this solution contained 0.100  $\mu\text{g}$ -at phosphate-phosphorus.

*Standard Phosphate Solutions IIa and IIb for Organic Phosphorus*. Because no salt-effect correction is necessary for samples digested with perchloric acid, these standards were prepared in the same way as Standard Phosphate Solutions IIa and IIb for inorganic phosphate, except that they were diluted with distilled water rather than with sodium chloride. The phosphate-phosphorus concentrations of the standards remained the same in both cases.

## APPARATUS

The colorimetric estimations were made for the most part with a Model AC Fisher Electrophotometer, using filter A, of 625 m $\mu$  average wavelength, and glass cells with optical paths of 5 cm. Some of the estimations were made with the Electric Eye Photometer developed by Ford (1950).

## ANALYTICAL PROCEDURES

*Inorganic Phosphate.* Mix thoroughly 0.5 ml of ammonium molybdate reagent and 0.20 ml of Stannous Solution II with a 50 ml sea water sample as soon after collection as possible. Allow seven minutes for color development, then measure the resulting color in the Electrophotometer, using distilled water as the reference solution. Obtain the phosphate value from a calibration curve prepared with standard phosphate solutions.

*Total Phosphorus.* Treat 50 ml samples of sea water with 3 ml of 60% perchloric acid in a 125 ml Erlenmeyer flask. This volume of perchloric acid is equivalent to the salts of a typical sea water sample with a salinity of 30‰ plus an excess of 0.2 ml for oxidation purposes. After evaporation on a hot plate to fumes of perchloric acid, place a cover glass on the flask and heat the sample for about 5 minutes just below the fuming temperature of perchloric acid. In order to drive off the arsenic present, add 3 ml of 12 N hydrochloric acid and fume off rapidly. Let cool and add 30 ml of distilled water to dissolve the salts. Neutralize the excess acid with a normal sodium hydroxide solution, using phenolphthalein as the indicator. Add dilute perchloric acid drop by drop until the solution is slightly acidic. Dilute the solution to 50 ml and mix thoroughly with 0.50 ml of ammonium molybdate solution and 0.30 ml of Stannous Chloride Solution II. After maximum color development, estimate the color in the Electrophotometer, using distilled water as the reference solution. Obtain the phosphate value from a calibration curve prepared with standard phosphate solutions.

Prepare blank solutions by treating a sodium chloride solution of the same chlorinity as sea water with perchloric acid in the manner just described.

*Notes on the Procedures.* Frequently it has been noted (Lundell and Hoffman, 1945) that under certain conditions perchloric acid reacts violently with organic material. This hazard is eliminated by the initial oxidation of the more easily oxidizable material by using nitric acid before perchloric acid is added. The amount of organic material in a typical sea water sample is relatively small, hence there is no hazard when it is oxidized with perchloric acid, but if samples of ab-

normally high organic content such as plankton or mud are being analyzed, it is advisable to digest with a small amount of nitric acid first. Water samples from East Sound, San Juan Islands, which contained as much as 2  $\mu\text{g-at}$  organic phosphorus per liter, were digested directly with perchloric acid without trouble. However, as a precaution, plankton samples obtained by filtration were treated with small portions of nitric acid before oxidation with perchloric acid, and all such experiments proceeded without violence. *It should be emphasized that, before using perchloric acid, one should be familiar with its hazards and with the precautions to be followed to insure its safe use (Kuney, 1947).*

## INVESTIGATION OF THE METHOD OF ANALYSIS

### TESTING THE METHOD OF ANALYSIS

The method just described was tried on known amounts of sodium glycerophosphate in a sodium chloride solution, Cl = 17‰. A satisfactory recovery of the phosphorus was obtained.

The organic phosphorus content of a sea water sample was determined by the perchloric procedure and by the sulfuric acid-hydrogen peroxide procedure. The excess perchloric acid was neutralized with sodium hydroxide and the excess sulfuric acid with ammonium hydroxide, as in the procedure described by Redfield, et al. (1937). The results are recorded in Table I. The total phosphorus concentrations in each case were corrected for a blank.

TABLE I. DETERMINATION OF ORGANIC PHOSPHORUS IN SEA WATER

<i>Sulfuric Acid Procedure</i>		<i>Phosphate Concentrations (<math>\mu\text{g-at/L}</math>)</i>	
		<i>Perchloric Acid Procedure</i>	
<i>Total P*</i>	<i>Organic P</i>	<i>Total P**</i>	<i>Organic P</i>
2.11	0.53	2.36	0.78
1.89	0.31	2.49	0.91
2.08	0.50	2.47	0.88
2.48	0.90	2.54	0.96
2.36	0.78	2.36	0.78
2.32	0.74	2.53	0.95
<hr/>		<hr/>	
2.21 av.	0.63 av.	2.46 av.	0.88 av.

Inorganic phosphate concentration 1.58  $\mu\text{g-at/L}$ .

\* Corrected for a blank of 1.09  $\mu\text{g-at/L}$ .

\*\* Corrected for a blank of 0.36  $\mu\text{g-at/L}$ .

The blank for the sulfuric acid procedure was considerably greater than that for the perchloric acid procedure. Thus the more than 3 ml of concentrated sulfuric acid and 8 ml of ammonium hydroxide

gave a blank of 1.09  $\mu\text{g-at}$  phosphorus per liter, the 3 ml of perchloric acid and several drops of sodium hydroxide solution a blank of 0.36  $\mu\text{g-at/L}$ . Various attempts to purify the sulfuric acid and ammonium hydroxide did not reduce the magnitude of the blank.

As shown in Table I, the results for total phosphorus (and also for organic phosphorus) by the perchloric acid method were somewhat larger than those obtained by the sulfuric acid procedure. It is not known whether this is due to greater efficiency of oxidation by the perchloric acid or to error arising from the establishment and application of the quite large blank for the sulfuric acid method. However, in general it may be said that results obtained by a method that utilizes a large blank are apt to suffer in accuracy.

The organic phosphorus was calculated by deducting the inorganic phosphate, which amounted to 1.58  $\mu\text{g-at/L}$ , from the total phosphorus value. An organic phosphorus content of 0.63  $\mu\text{g-at/L}$  was obtained by the sulfuric acid method, 0.88  $\mu\text{g-at/L}$  by the perchloric acid procedure. Provision for reduction of arsenate was not included in either method, hence the final concentrations found represented the sum of phosphate, arsenate and oxidized arsenite.

#### TIME OF COLOR DEVELOPMENT AND STABILITY OF COLOR

It was determined that, after the addition of reagents, maximum color intensity developed in 3 minutes in distilled water or in sea water digested with perchloric acid; this color was stable for about 8 minutes before fading began. On the other hand, in undigested sea water samples full color developed in about 6 minutes and was stable for 27 minutes before fading began.

#### EFFECT OF PERCHLORATE UPON COLOR INTENSITY

For some time it has been known that, for a given amount of phosphate, less color is developed in the estimation of phosphate in an undigested sea water sample than in distilled water. This is known as the salt effect. When sea water is digested with sulfuric acid or perchloric acid, hydrogen chloride is evolved and salts of sulfate or perchlorate remain. However, no salt effect was noted (Table II) when phosphate was estimated in a sulfate or perchlorate medium and its color compared with that developed in the distilled water medium. This is in accordance with Cooper's (1938) explanation that the salt effect is due to the formation of certain yellow molybdenyl halides.

#### ARSENITE INTERFERENCE

It is generally thought that arsenic is present in sea water mainly as arsenite with inappreciable amounts of arsenate. However Armstrong and Harvey (1950), as a result of experiments which they per-

TABLE II. EFFECT OF VARIOUS SALTS ON COLOR DEVELOPMENT

Medium g/50 ml	Phosphate Concentration ( $\mu\text{g-at/L}$ )	
	Added	Found
2 g NaCl	1.20	1.00
2 g Na <sub>2</sub> SO <sub>4</sub>	1.20	1.20
	2.20	2.20
2 g NaNO <sub>3</sub>	1.60	1.50
2 g NaClO <sub>4</sub>	1.60	1.60

formed, suggested that arsenate may be the predominating form. According to Zinzadze (1935), arsenate but not arsenite forms a blue reduction product with the reagents for the phosphate determination, and thus it would be estimated along with phosphate. However, if arsenite is oxidized to arsenate in the oxidization of organic phosphorus, there would be interference in the estimation of total phosphorus. Experimentally it was demonstrated by the authors that this is the case (Table III). Sea water samples with added arsenite were digested

TABLE III. OXIDATION OF ARSENITE WITH PERCHLORIC ACID IN A SEA WATER MEDIUM

Added	Arsenite Concentration ( $\mu\text{g-at/L}$ )	
	Found as Arsenate	
	Fumed 5 min.	Fumed 1 hr.
2.00	0.22	0.96
	0.10	1.08
	0.10	2.03
	0.19	2.00
	0.17	1.62

with perchloric acid and estimated colorimetrically. From these values was subtracted the total phosphorus of the sea water, which contained no added arsenite. The total phosphorus value also included arsenic which was present in the sea water and which had been oxidized to arsenate as well as phosphorus. The results indicated that the added arsenite had been oxidized, but it was not determined quantitatively.

It was also noted that arsenite causes a blue color with the phosphate reagents even though it is not thought to form a complex. Fig. 1 shows the extent of the color development by arsenite in different media. Almost as much color is developed by arsenite in distilled water or in a medium containing the digested sea water salts as is developed by an equivalent amount of phosphate, while in a chloride medium considerably less color is formed. Thus arsenite in sea water would cause an error of comparatively small magnitude.



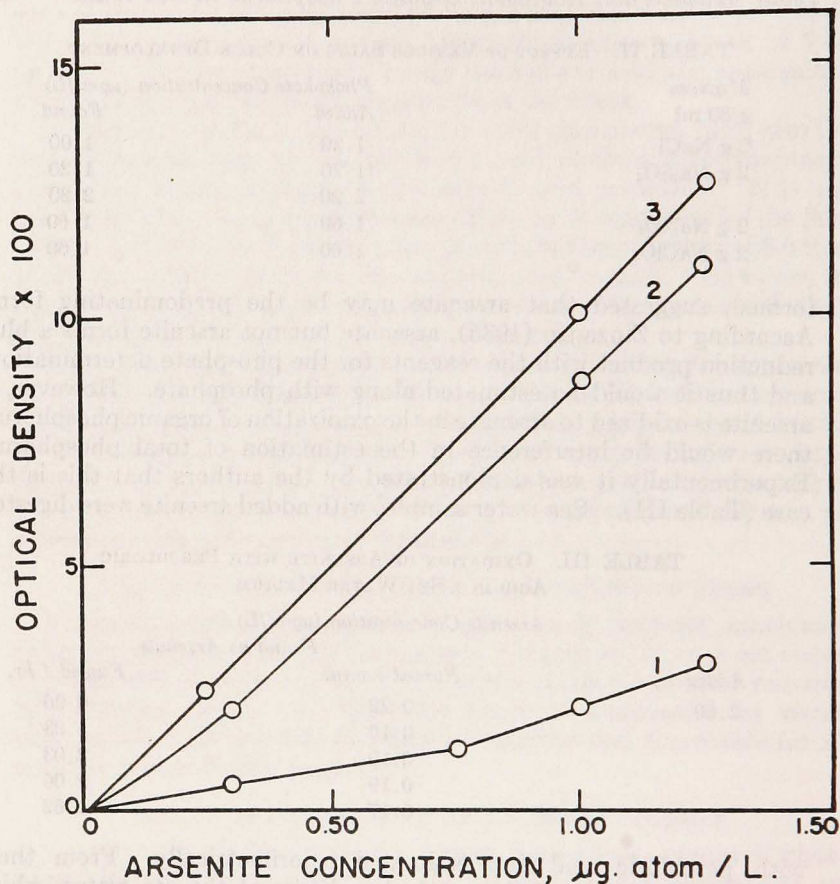


Figure 1. Arsenite interference in various media at  $Cl^- = 17 \text{ ‰}$ . 1. Arsenite in sodium chloride solution. 2. Arsenite in distilled water or in digested sea water. 3. Calibration curve for phosphate in sodium chloride solution.

It was found that the color developed by arsenite depends upon the chloride concentration. This is demonstrated by the results shown in Fig. 2. An arsenite concentration of  $12 \text{ } \mu\text{g-at/L}$ , which is 20–50 times its probable concentration in sea water, was used to accentuate the effect at various chloride concentrations. Considerably less color was developed by arsenite at the higher chlorinities. Thus, in undigested sea water arsenite interference can ordinarily be neglected, but it should be considered when samples without chloride are being analyzed.

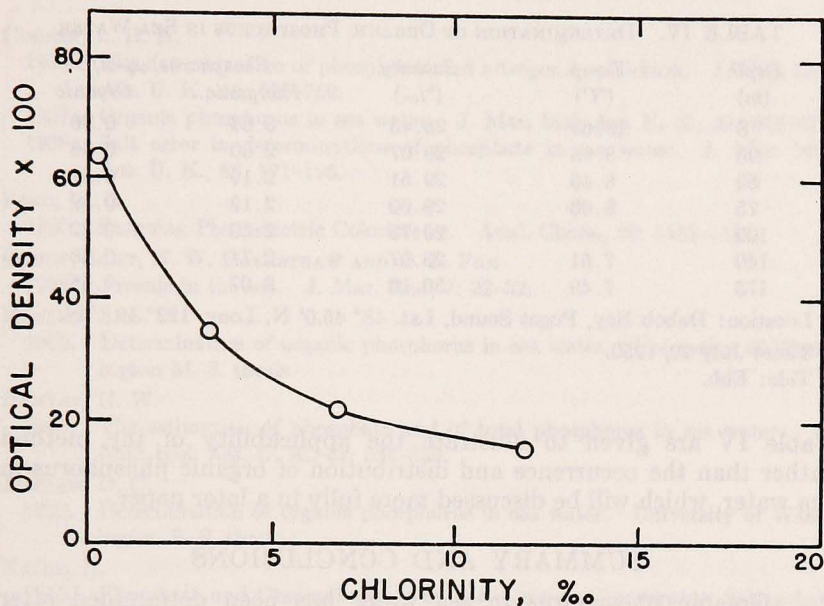


Figure 2. Arsenite color development with variation in chlorine concentration.

#### ELIMINATION OF ARSENIC

In an attempt to eliminate interference by arsenic, a reduction procedure with sulfite was tried. It was found that, in a sea water sample digested with perchloric acid, arsenate was apparently reduced to arsenite after heating for one hour at 90° C in the presence of 0.4 g of sodium bisulfite. The method was not satisfactory, however, as the results were erratic.

In order to completely eliminate all forms of arsenic, a volatilization procedure was developed. After the sea water samples were digested for 5 minutes with perchloric acid, 3 ml of concentrated hydrochloric acid were added and rapidly fumed off. This procedure was effective when tried with samples to which known amounts of arsenite had been added. The arsenic was probably driven off in the form of arsenic trichloride.

#### ANALYSIS OF SEA WATER SAMPLES

The values given in Table IV were obtained when sea water samples collected in Dabob Bay were analyzed by the recommended procedure. The inorganic phosphate concentration increased with depth, as is usually the case with stratified waters. The organic phosphorus values showed no definite correlation with depth. The results in

TABLE IV. DETERMINATION OF ORGANIC PHOSPHORUS IN SEA WATER

Depth (m)	Temp. (°C)	Salinity (‰)	Phosphorus, $\mu\text{g-at/L}$	
			Inorganic	Organic
S	18.63	25.46	0.66	0.36
20	8.96	29.07	2.00	0.36
50	8.46	29.51	2.19	0.57
75	8.06	29.60	2.19	0.59
100	—	29.72	2.50	0.32
140	7.51	29.97	2.74	0.48
175	7.49	30.10	3.07	0.45

Location: Dabob Bay, Puget Sound, Lat. 48° 45.0' N, Long. 122° 49.7' N.

Time: July 20, 1950.

Tide: Ebb.

Table IV are given to illustrate the applicability of the method rather than the occurrence and distribution of organic phosphorus in sea water, which will be discussed more fully in a later paper.

### SUMMARY AND CONCLUSIONS

1. Organic phosphorus in sea water has been determined after oxidation of the organic material with perchloric acid. With samples of high organic content, preliminary digestion with nitric acid should precede the perchloric acid digestion.

2. Perchloric acid is superior to sulfuric acid as a digestion medium; its salts are more readily water soluble and its blank smaller.

3. Neither perchlorate nor sulfate give a salt effect in the colorimetric determination of phosphate.

4. In the colorimetric determination of phosphate the speed of color development in a medium containing perchlorate is the same as that in distilled water, both of which have faster rates of development than a medium containing chloride.

5. Arsenite in a chloride medium yields but a slight color intensity with the phosphate reagents; in a distilled water medium its color intensity is almost equal that of an equivalent amount of phosphate.

6. Arsenic can be prevented from interfering in the determination of organic phosphorus by treatment of the samples with concentrated hydrochloric acid and volatilization of the arsenic.

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