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THE DETERMINATION OF PHOSPHATES IN SEA WATER

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The concentration of phosphates in sea water varies from 0.000 to about 2.00 microgram atoms ($\mu\text{g. atoms}$) phosphate-phosphorus per liter at 20°C . Occasionally a maximum of 3.00 $\mu\text{g. atoms}$ may be found in some deep arms of the sea.

For the determination of the concentration of phosphate-phosphorus in sea water, the method of Denigès, as modified for the purpose, is recommended. The method itself is based on the principle that when a sulfuric acid solution of ammonium molybdate is added to a soluble phosphate solution a complex phosphomolybdic acid is formed. Upon treatment with a reducing agent, such as stannous chloride, the complex acid is reduced to a blue-colored substance believed to have the composition $\text{H}_3\text{PO}_4 \cdot (4\text{MoO}_3 \cdot \text{MoO}_2)_2 \cdot 4\text{H}_2\text{O}$. The color developed is proportional to the concentration of the phosphate.

Arsenates give similar reactions with the ammonium molybdate and stannous chloride solutions. Arsenates, if present in sea water, occur in extremely small concentrations, probably less than 0.05 $\mu\text{g. atoms}$ of arsenate-arsenic per liter of sea water. The procedure here given determines the sum of the arsenates and phosphates. For sea water containing more than 1 $\mu\text{g. atom}$ of phosphate-phosphorus, the arsenates may be ignored, as the experimental error of the method will be greater than the actual amount of arsenic present.

The phosphorus and arsenic contained in organic compounds dissolved in the sea water, and in inorganic compounds other than phosphates and arsenates, have no influence upon the intensity of the blue color developed by the reagents.

Chemical Substances Required

1. Concentrated Sulfuric Acid (36 Normal)
2. Concentrated Hydrochloric Acid (12 Normal)
3. Ammonium Molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$
4. Stannous Chloride, $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$
5. Metallic Tin, Mossy
6. Sodium Chloride, NaCl .
7. Anhydrous Potassium Dihydrogen Phosphate, KH_2PO_4
8. Chloroform, CHCl_3
9. Distilled Water

The above chemicals should all be pure substances and, with the exception of the potassium dihydrogen phosphate, free of phosphates and arsenates. Special emphasis must be given to the sulfuric acid, as this substance, used for most chemical purposes, often contains compounds of arsenic as impurities.

REGENTS

Distilled Water. If the distilled water shows a trace of phosphate, it should be redistilled after making alkaline with sodium or barium hydroxide.

Dilute Sulfuric Acid. 150 ml. 36 N sulfuric acid are slowly added to 150 ml. of distilled water contained in a beaker. After cooling, the solution is diluted to 300 ml.

Sulfuric Acid-Ammonium Molybdate solution. 10 g. of ammonium molybdate are dissolved in 100 ml. distilled water. This solution is added to 300 ml. of the 18 N sulfuric acid. The reagent must be kept in dark amber glass-stoppered bottles and protected from light to prevent photochemical reactions which will reduce the reagent. If this is done the solution should be stable for weeks.

Stannous Chloride Solutions

Solution I. 2.15 g. of the dihydrate of stannous chloride are dissolved in 20 ml. 12 N hydrochloric acid and diluted to 100 ml. with cooled distilled water which has been freshly boiled to remove the dissolved oxygen. A piece of mossy tin is placed in the solution to retard the oxidizing effects of the air. The solution is stored in amber glass-stoppered bottles and is very stable over a long period of time as long as metallic tin is present.

Solution II. 5 ml. of Solution I are diluted to 25 ml. with cooled distilled water which has been freshly boiled. A small piece of mossy tin is placed in the solution. On exposure to the air for a few hours, the solution will oxidize; thus, it is advisable to prepare a fresh supply of the solution each day. The oxidation may be materially retarded by placing a Mohr seal over the solution and siphoning off the amount necessary for immediate addition to the standards and samples. (The Mohr seal is a layer of paraffin oil.)

Sodium Chloride Solutions

Primary solution. 168 g. of sodium chloride are dissolved in distilled water and diluted to 5 liters. This solution contains 20‰ of chloride ions and may be used directly for the preparation of Standard Phos-

phate Solution III (as described below) when the chlorinity of the sea water under investigation is more than 18‰.

Diluted Solutions. For the preparation of the Standard Solutions III to be used when the chlorinity of the sea water under investigation is less than 18‰, diluted solutions of sodium chloride are prepared from the primary solutions. For this purpose 1 liter of a diluted solution containing k ‰ of chloride may, with sufficient accuracy, be prepared by diluting 50 k ml. of the primary solution to 1000 ml. by means of distilled water.

Example: A sea water under investigation has a chlorinity of 15‰. Then 50 k is equal to 50 x 15, which is 750, and the desired sodium chloride solution is prepared by diluting 750 ml. of the primary sodium chloride solution to 1 liter by means of distilled water.

PREPARATION OF STANDARD PHOSPHATE SOLUTIONS

Emphasis must be given to the necessity for occasional check on all standard solutions. The statements given below are based upon the experience of many investigators. However, unknown factors may influence the stability of the solutions, and possible faulty technique of the investigator may cause alterations in the concentration of the standard solutions. Therefore, all standards must be subjected to rigorous control which may best be accomplished by preparation of duplicate standards from time to time.

Standard Solution I. 0.340 g. of pure anhydrous potassium dihydrogen phosphate, which has been previously dried to remove hygroscopic moisture, is dissolved in distilled water and transferred quantitatively to a 1 liter volumetric flask. The solution is diluted to volume, thoroughly mixed and 0.2 ml. chloroform added. 1 ml. of this solution contains 2.5 μ g. atoms of phosphate-phosphorus. The addition of chloroform retards bacterial action. The solution is generally stable for about a year.

Standard Solution IIa. 10 ml. of Standard Solution I are pipetted into a 1 liter volumetric flask, diluted to volume by means of the primary solution of sodium chloride containing 20‰ of chloride ions, and the solution then thoroughly mixed. 1 ml. of this solution contains 0.025 μ g. atom of phosphate-phosphorus. 0.2 ml. chloroform is added. The solution is generally stable for many weeks.

Standard Solution IIb. 40 ml. of Standard Solution I are diluted to 1 liter in a volumetric flask by means of the primary solution of sodium chloride containing 20‰ of chloride ions and thoroughly mixed.

1 ml. of this solution contains 0.10 $\mu\text{g.}$ atom of phosphate-phosphorus. 0.2 ml. chloroform is added. The solution is generally stable for many weeks.

Standard Solutions III. These standard solutions are prepared by diluting a certain volume (from 0.10 ml. to 2.00 ml.) of Standard Solution IIa or IIb measured with a micro buret or Mohr pipet either to exactly 50 ml. or to exactly 100 ml. by means of a sodium chloride solution. The concentration of the sodium chloride solution used must be approximately in accordance with the chlorinity of the sea water that is to be investigated. If the chlorinity of the sea water is $k \text{ ‰}$, the chloride concentration of the sodium chloride solution must be between the limits, $k \pm 1 \text{ ‰ Cl}$.

The number of standard solutions to be prepared depends upon the colorimetric method used and the phosphate-phosphorus concentration in the waters being investigated. The standard solutions given in Table I comprise those that are used when colorimetric estimations are made by means of Nessler tubes.

For certain waters with a relatively high concentration of phosphate 50 ml. Nessler tubes are utilized. For other waters with a lower concentration of phosphate 100 ml. Nessler tubes are to be preferred. The standard solutions may be prepared directly in the Nessler tubes if they are used. If Hehner cylinders or some form of a colorimeter are employed, selected standards are prepared according to the method of comparison.

The rapid formation of a yellow coloration and the gradual fading of the blue color in the saline solution necessitates the preparation of new Standard Solution III for each series of samples that are compared contemporarily with the standard solutions.

In the preparation of the Standard Solutions IIa, IIb and III, distilled water might be used instead of solutions of sodium chloride. Supposing that this is done, the color comparison of a sea water sample with a standard solution of the same concentration of phosphate shows that the color development and also the resulting color intensity are different for sea water and the standard solution. When sodium chloride solutions are used for preparation of the standard solutions these differences are in a considerable degree eliminated. Preferably the Standard Solutions IIa, IIb, and III are to be prepared by means of phosphate-free sea water of the same chlorinity as the sea water samples to be investigated. Should unforeseen conditions make it desirable to have the standard solutions prepared by means of distilled water this may be done, but the values found by a colorimetric determination of the concentration of phosphate in sea water are then to be multiplied by the factor, 1.15.

TABLE I.—PREPARATION OF STANDARD SOLUTIONS III

Ml. of Standard Solutions IIa and IIb to be Diluted:		Concentration of the Pre- pared Standard Solutions when Diluted to:	
IIa	IIb	50 ml. IIa	100 ml. IIb
(μ g. atom of Phosphate-Phosphorus per liter of solution)			
0.00	0.00	0.00	0.000
.10	—	.05	.025
.20	—	.10	.05
.40	—	.20	.10
.60	—	.30	.15
.80	—	.40	.20
1.20	—	.60	.30
1.60	—	.80	.40
2.00	—	1.00	.50
—	.60	1.20	.60
—	.70	1.40	.70
—	.80	1.60	.80
—	.90	1.80	.90
—	1.00	2.00	1.00
—	1.10	2.20	—
—	1.20	2.40	1.20
—	1.30	2.60	—
—	1.40	2.80	1.40
—	1.50	3.00	—
—	1.60	—	1.60
—	1.80	—	1.80
—	2.00	—	2.00

TESTING OF THE REAGENTS

A blank determination should always be made to insure the absence of phosphates and arsenates in the reagents and in the sodium chloride solution supposed to be free of these substances. This is accomplished by treating 50 ml. or 100 ml. of the primary sodium chloride solution with the reagents in the same manner as accorded the standards and the samples taken for analysis. If an appreciable color intensity is found in the blank determinations, new solutions are prepared successively in order that the contaminated reagent may be found and eliminated.

THE PROCEDURE FOR THE DETERMINATION OF THE CONCENTRATION OF PHOSPHATE-PHOSPHORUS IN SEA WATER

If the samples of sea water contain much plankton this should be removed by filtration or centrifugalization. However, it is only in extreme cases when such a procedure is necessary.

The samples for the phosphate determination are to be analyzed as soon as possible after they have been taken from the sea. Whether the determination is made by means of Nessler tubes or by any other method of comparison, it is recommended that 50 ml. or 100 ml. of the sea water sample be taken for the determination. The volumes of the standard solutions are determined by the volumes of the samples. The sea water samples and the standard solutions for the comparison are to be brought to the same temperature conditions so that the sea water sample and each of the standard solutions have temperatures within an interval of 2° C.

1 ml. of the sulfuric acid-ammonium molybdate reagent is added and mixed with each of the standard solutions and sea water samples. About 0.20 ml. of Stannous Chloride Solution II is likewise added and thoroughly mixed with each sample and standard solution. Maximum color intensity develops in about seven minutes and comparisons are then made with the standards. About twelve samples can be conveniently handled at a time. It is recommended that samples should not be stored for more than a few hours before the analyses are made and that they should not be exposed to direct sunlight while awaiting temperature equilibrium with the standard solutions.

Should conditions necessitate comparison with standards made from distilled water, then the concentration values for phosphate-phosphorus shown in Table I must be multiplied by the factor, 1.15.

Nessler tubes used for phosphate determinations soon become coated with a blue reduction compound of molybdenum that hinders the matching of the colors being produced in the tubes. Thus, it is important to clean the tubes occasionally with aqua regia. If other forms of apparatus are used a similar cleaning is essential.

Caution must be observed that glassware is not used alternately for phosphate and for silicate determinations without thoroughly cleaning with aqua regia between each type of determination.

Accuracy of Results. The accuracy of the determination is about 5 to 10 per cent. Because of the very small concentrations of phosphate in sea water, correction is not necessary for the slight deviation in volume of sea water due to the deviation in temperature from 20° C. This statement also applies to the standard solutions.

APPARATUS REQUIRED

In accordance with the above description, the following solutions and apparatus are recommended for use aboard ship. It is assumed that ample provision will be made for taking duplicate solutions and apparatus in case of accident or breakage. Duplicate materials and solutions should always be packed in separate containers.

1. Apparatus for sampling the sea water.
2. Amber glass-stoppered bottles 250–500 ml. capacity with sulfuric acid-ammonium molybdate solution.
3. Amber glass-stoppered bottles 50–100 ml. capacity with Stannous Chloride Solution I.
4. Amber glass-stoppered bottles 50–100 ml. capacity with Stannous Chloride Solution II.
5. Bottles or demijohns of sufficient capacity with primary solution sodium chloride.
6. Bottles or demijohns of sufficient capacity with distilled water.
7. Amber glass-stoppered bottles of capacity 100–250 ml. with Standard Solution I.
8. Amber glass-stoppered bottles of capacity 100–250 ml. with
 - (a) Standard Solution IIa, and
 - (b) Standard Solution IIb.
9. Apparatus for preparation of standard solutions:
 - (a) 10 ml. pipet, calibrated;
 - (b) 1000 ml. volumetric flask, calibrated;
 - (c) Pipet of capacity 0.2 ml.;
 - (d) 5 ml. microburet, graduated to 0.05 ml.;
 - (e) Buret stand;
 - (f) A suitable number of Erlenmeyer flasks, 125 ml. capacity, for preparation of standard solutions for comparison with sea water according to the method used for the color comparison; the standard solutions may be prepared directly in the Nessler tubes, thus making the Erlenmeyer flasks unnecessary.
10. Filtering or centrifuging apparatus for removing plankton from the sea water if necessary.
11. Apparatus for applying the reagents to the standard and sea water samples:
 - (a) Measuring flasks or cylinders of capacity 100 ml., 50 ml., and 25 ml. If Nessler tubes are used and if these are marked to the volumes 100 ml. and 50 ml., respectively, the measuring flasks may be unnecessary.

- (b) Pipet marked to deliver 1 ml. of the sulfuric acid-ammonium molybdate reagent.
 - (c) Pipet marked to deliver 0.2 ml. of the solution of Stannous Chloride Solution II.
12. Apparatus for colorimetric measurements:
- (a) Nessler tubes with *graduation marks at the same height* (diameter of 20 mm. and 16 mm. for tubes containing 100 ml. and 50 ml., respectively) may be convenient, or
 - (b) Hehner cylinders, or
 - (c) Colorimeter, visual or photoelectric, or
 - (d) Photometer.
13. Thermometer graduated to 1° C.
14. Bottles with distilled water for cleaning the measuring apparatus when necessary.

NOTES ON THE DETERMINATION

1. Organic phosphorus and the lower valence compounds of phosphorus are not determined by this method unless special treatment is given the sample. Arsenates, if present, yield a blue reduction color by this method.
2. With low concentration of phosphate there is a proportionately greater reduction color than would be expected.
3. If the hydrogen-ion concentration in the molybdate reagent is insufficient, the stannous chloride reduces the reagent itself as well as the complex phosphomolybdic acid. If the hydrogen-ion concentration is too great the sensitivity of the reagent is reduced materially.
4. By dissolving the solid stannous chloride dihydrate in concentrated hydrochloric acid and then diluting, hydrolysis is prevented.
5. Upon prolonged standing the concentration of the dilute standard phosphate solutions is often materially reduced because of bacterial action. This may be prevented by the introduction of a small amount of chloroform.
6. The samples should be analyzed for phosphate as soon as possible. If this is not done, errors may result. First, the phosphate concentration may be appreciably reduced through phytoplanktonic action, and second, the phosphate concentration may increase through bacterial or enzymatic action on the organic phosphorus which exists in solution, or suspension.
7. Temperature affects the equilibrium established in the reaction. The standards and samples should thus be near the same temperature during color development and comparison. For example, if the phosphate is determined in two solutions of the same concentra-

- tion but the temperature of one in 15° C. and the other 30° C., the intensity of the reduction color in the warmer is noticeably greater.
8. Three drops of stannous chloride solution are often recommended in this determination, but as mentioned previously a yellow color is formed in sea water when this is done. Reducing the quantity to one drop still leaves a twelve-fold excess above the theoretical amount probably required and at the same time reduces the intensity of the yellow coloration so that it does not interfere materially. The intensity of the blue color is reduced about ten per cent at the same time.

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