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DETERMINATION OF INORGANIC PHOSPHATE
IN SEA WATER BY A BUTANOL
EXTRACTION PROCEDURE

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Charles M. Proctor

Research Conducted through the
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COLLEGE STATION, TEXAS

Appendix I
Project 40
Final Report.

THE AGRICULTURAL AND MECHANICAL COLLEGE OF TEXAS
Department of Oceanography
College Station, Texas

Research Conducted for the
Texas A. & M. Research Foundation
Projects 24 and 40

DETERMINATION OF INORGANIC PHOSPHATE IN SEA WATER

BY A BUTANOL EXTRACTION PROCEDURE

Technical Report

This is a preliminary report of work that was begun on Project 40 and is being continued on Project 24. Part of this material was presented at the 8th Southwest Regional Meeting of the American Chemical Society at Little Rock, Arkansas, December 5, 1952. Presentation of material in this report does not constitute final publication.

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Report Prepared June 22, 1953

by
Charles M. Proctor

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I. Introduction

Phosphate is intimately involved in the fixation of energy by plants (3) and in the assimilation, storage, and utilization of food, and hence of energy, both animals and plants (10). Phosphorus in the form of phosphate--no other form has yet been demonstrated in protoplasm--thus ranks as one of the trace elements essential to life. The maximum concentration of inorganic phosphate observed in the open sea is 3 μM (micro molar) with 2 μM being the highest concentration generally encountered (17). Values of 0.00 μM are often encountered, even in areas of great biological activity. This value is beyond the limit of sensitivity of the Denigès (4) method for phosphate, as usually employed (6, 7, 8, 13, 15, and 17). Greater sensitivity is required in studies of phosphate turnover and of the limiting effect of phosphate concentration on plant and animal growth.

The molybdenum-blue method for determination of phosphate was introduced by Osmond in 1887 (11) and is the basis of nearly all modern micro-analytical methods for phosphorus. In Osmond's method the sample is treated with molybdic acid. The resulting phospho-molybdate complex is then reduced with stannous chloride, or other suitable reagent, to give the phospho-molybdenum blue color. For determination of phosphate in sea water, reference is usually made to the procedure of Denigès (4). Although visual color comparison methods are still often used in field work and on shipboard (13) the method is usually adapted to the photo-electric colorimeter with modifications to suit the material being analysed (6, 7, 15, and 17). Increased sensitivity may be obtained by using a colorimeter absorption cell with a long light path (7), or by taking a large sample and concentrating it for analysis with standard absorption cells. The latter course is complicated in sea water since concentration of the sample gives a very high salt content which interferes with formation of the phosphomolybdate complex. The present method was suggested by Dr. Donald W. Hood of this laboratory and is based on the observation of Denigès (4) that the phosphomolybdate complex is soluble in organic solvents. The blue color may then be developed by reduction in the organic phase. The phosphomolybdenum blue is thus concentrated without increasing the salt interference and greater sensitivity becomes practical with standard colorimetric equipment. Interference from citrate, nitrite, fluoride, oxalate (1) and other unspecified ions (16) are also reduced or eliminated.

II. Procedure

A. Reagents

Analytical Reagent grade (ACS) used throughout.

SULFURIC ACID, 18N and 1.2N.

WASH ACID, 1.2N H_2SO_4 saturated with iso-butanol at room temperature (about 10% i-BuOH).

AMMONIUM MOLYBDATE, 10% solution stored in polyethylene bottle.

ISO-BUTANOL.

ISO-PROPANOL (or ethanol).

STANNOUS CHLORIDE SOLUTION, 2% in 2.4N HCl. Dissolve 2.3g $\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$ in 20 ml of 12N HCl. Dilute to 100 ml and store in a dark bottle with a few pieces of mossy tin. Stable for several months.

REDUCING SOLUTION. 2 ml of stannous chloride solution to 100 ml of wash acid. Mix as needed. Stable for about half a day.

PHOSPHATE STOCK STANDARD, 3 mM 0.408g of KH_2PO_4 to one liter of distilled water. One ml of CCl_4 was added to prevent bacterial and mould growth.

PHOSPHATE WORKING STANDARDS were made as needed by diluting the stock standard with artificial sea water, or with distilled water when required. CCl_4 was used as preservative.

ARTIFICIAL SEA WATER, Lyman and Fleming (9). For 20 liters use: NaCl, 470 g.; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 213 g.; Na_2SO_4 , 78.3g.; KCl, 13.2g.; NaHCO_3 3.84g.; KBr, 1.92g.; NaF, 0.060g. Dissolve in about fifteen liters of distilled water. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 29.4g.; SrCl_2 , 0.48g. Dissolve separately in about 2 liters. H_2BO_3 , 0.52g. Dissolve in about 100 ml of hot water. Add the alkaline earth solution slowly to the solution of salts with vigorous mixing then add the boric acid. Dilute to 20 liters and mix well. A 20 liter carboy with siphon tube was used for storage. Final mixing was accomplished by aspirating air through the siphon tube. About 10 ml of CCl_4 were added as preservative.

B. Instrument

The Beckman Model B Spectrophotometer with 1 cm Corex cells was used for absorbancy readings. The sensitivity control on the instrument permits reasonably accurate readings over most of the range from 0 to 2 absorbancy units as shown in Figure 1.

C. Method

75.0 ml samples of sea water 0.02 to 10 μM in phosphate (0.04 to 20 μg of P) are run into 200 ml separatory funnels. The samples should be at room temperature. Add 5.0 ml of 18N H_2SO_4 to each, then add 5.0 ml of 10% ammonium molybdate. The samples should be swirled in the flasks while adding these reagents, so that local concentrations do not build up. Add 15.0 ml of β -BuOH to each funnel and shake for one minute to extract the phospho-molybdate complex. (Note 1) Discard the aqueous layer. Back extract the organic layer twice with

25 ml portions of wash acid (Notes 2 and 3) then add 10 ml of reducing solution and shake for one minute. Discard the aqueous layer and wash once with 25 ml of wash acid. Mix 3.00 ml of the i-BuOH layer with 0.30 ml of i-PrOH (Note 4) and read the absorbancy at 730 m μ in a 1 cm Corex cell.

Note 1. The solubility of i-BuOH is temperature dependent so that samples to be compared must be run at the same temperature. One minute extraction seems to be sufficient.

Note 2. The wash acid and reducing solution are saturated with i-BuOH so that concentration of phosphomolybdate in the organic phase should remain constant after extraction. Slight losses of the i-BuOH phase are thus unimportant.

Note 3. Broken tip pipettes of 10 and 25 ml capacity were cut off just below the bulb. These made convenient finger pipettes for addition of reducing solution and wash acid respectively. These volumes are not critical.

Note 4. The i-PrOH, or EtOH, prevents separation of water droplets from the i-BuOH solution which might "fog" the absorption cell windows.

III. Experimental

A. Spectrum

The absorbancy spectrum of phosphomolybdenum blue in the iso-butanol layer is shown by Figure 2. Water saturated iso-butanol, with iso-propanol added, was used to set the instrument to zero. A spectrum of water saturated butanol, with iso-propanol, was determined against butanol and is shown at the bottom of Figure 1. No great absorption due to the water and iso-propanol is noticed below 900 m μ . The iso-butanol spectrum was not checked, but due to structural similarities, no significant difference would be expected at wave lengths below 800 or 900 m μ . Based on these data, samples are read at 730 m μ against pure solvent, iso-butanol.

B. Color Stability

Samples of artificial sea water with 3.0 μ M added phosphate, distilled water with 3.3 μ M, and Port Aransas channel water were tested and absorbancies compared over four hours. Table I gives these results. The artificial sea water sample showed considerable fading. To check fading in sea water, tests were made with various concentrations of added phosphate in the channel water. Table II indicates that fading is inconsequential in the first 75 minutes.

C. Sensitivity

The data of Table II show a spread typical of small groups of samples. They have been plotted in Figures 3 and 4. The mean

Table I

Color Stability. Absorbancy change with time.

Time after reduction (min.)	5	10	20	60	120	240
Distilled water blank	0.003	0.003	0.004	0.005	0.004	0.002
H ₂ O + 3.3 μ M PO ₄	0.658	0.661	0.664	0.667	0.663	0.665
Pt. Aransas Channel	0.093	0.094	0.095	0.095	0.096	0.096
Artificial S. W. + 3.0 μ M PO ₄	0.563	0.567	0.571	0.563	0.554	0.538

Table II

Color Stability of Channel Water With Added Phosphate

added PO ₄	30 min.	75 min.
0.004M	0.091	0.092
0.10	0.112	0.114
0.20	0.127	0.128
0.30	0.148	0.148
3.00	0.604	0.600

absorbancy coefficient was found to be 0.19 using the equation

$$A = \log \frac{I_0}{I_t} = E l c$$

where

A = absorbancy (optical density)

I₀ = incident light intensity

I_t = transmitted light intensity

E = absorbancy coefficient, micromolar

l = length of light path through cell in cm

c = concentration in micromoles

Many phosphate methods in the current literature have similar, or almost identical sensitivities which are about one tenth the sensitivity of the present method. The differences in procedure are dictated by the type of sample being analysed, but many current methods have micromolar absorbancy coefficients of about 0.022 (2 and 6). The method of Wooster and Rakestraw (17) gave a micromolar absorbancy of 0.021 when tested in this laboratory.

D. Interferences

1. Chloride

Of the substances that interfere with the formation of phosphomolybdenum blue (2), the only ones that are present in sea water in sufficient concentration to cause trouble are: chloride, silicate and arsenate. Chloride in high concentration probably replaces part of the molybdenum in the phospho-molybdate complex. Arsenate and silicate form molybdenum complexes similar to that formed by phosphate. The chloride effect is shown in Figure 4. It will be noted that the tests made at 12 and 18 %¹ chloride content are almost indistinguishable. It is thus possible to circumvent chloride effects in sea water by using phosphate standards made with artificial sea water or with filtered low phosphate sea water.

2. Silicate

The usual range of silicate in sea water is from 0.0 to about 200 µM (14). In the first trials of this extraction method, the amount of acid (5 ml of 10 NH₂SO₄) and ammonium molybdate (5 ml of 5%) were essentially those used by Berenblum and Chain (1) for phosphate in biological materials. Some color production

¹ % = per mille, parts per thousand

by silicate was found under these conditions. By increasing the acid concentration to 18N (final strength in the reaction mixture 1.2N) and molybdate to 10% (final, 0.6%) silicate interference was eliminated as shown in Figure 1. The high acidity, and consequently high molybdate concentration (7), are in marked contrast to the conditions of the Wooster and Rakestraw method for phosphate in sea water (17). Wooster and Rakestraw gave no data on silicate interference but this writer has found that silicate additions amounting to 40, 80, and 133 μM do not interfere in the Wooster-Rakestraw phosphate determination.

3. Arsenate and Arsenite

Total arsenic in sea water usually varies between 0.2 and 0.5 μM (5). Of this, only part is present as arsenate. Tests were made to determine the absorbancy due to added arsenate. 13 μM arsenate gave an absorbance increment less than that due to 1 μM of added phosphate. On this basis, the maximum error in phosphate determination if all the arsenic in sea water were in the form of arsenate would be only about 0.03 μM . This is just at the significance level of the phosphate determination. Arsenite, the predominant form, in sea water causes a relatively small error (8).

E. Partition of Acid Between Artificial Sea Water and iso-Butanol

To determine the possible change in acidity due to extraction, a single, roughly quantitative test was run at 25°. The volume of the artificial sea water layer, acidified with H_2SO_4 , was 33 ml; the volume of the organic layer, 19 ml. Titration with 1/20 N NaOH using methyl orange indicator gave acid normalities of 1.03 and 0.105 in the water and the alcohol respectively. The partition ratio of titratable acids of the kinds present in H_2SO_4 acidified artificial sea water is thus 10:1. The amount of acid thus lost from the aqueous phase is negligible (7).

F. Interference by Stopcock Lubricant

Two portions of acidified sea water, with no molybdate, were extracted with i-BuOH in 125 ml pyrex bottles. The bottom of the ground glass stopper of one of these bottles was smeared with Lubri-Seal brand stopcock lubricant (distributed by A. H. Thomas Company, Philadelphia)¹. The absorbance of the two i-BuOH layers was compared over the spectral range from 600 to 1020 $\text{m}\mu$. The difference in absorbance was zero at each wave length.

¹ This is a report of a test run on one sample and does not necessarily constitute an endorsement of the product or a statement of its uniformity.

IV. Discussion.

The method of phosphate analysis presented here is an order of magnitude more sensitive than methods currently in use and has about the same relative accuracy. It is also subject to fewer interferences (C.f., Ref. 1). It will serve for study of low concentrations of phosphate and of changes in phosphate concentration that are too near the limit of resolution of existing methods.

Day to day reproducibility of the method is not as good as could be desired. This is not a serious drawback, however, since it is necessary to run frequent controls with any colorimetric procedure. No difficulty in getting check results within each group of analyses need be expected if each step in the procedure is made part of a routine. Temperature control was not used in these studies. The laboratory temperature usually did not change during the course of an analysis and no difficulty from this source was experienced. Samples should be allowed to come to room temperature before extraction.

The concentration of the iso-butanol layer should not change after extraction. By having the wash acid and the reducing solution at the same temperature as the extracts and just saturated with iso-butanol the concentration of the extracts should not change due to iso-butanol dissolving in the wash solutions or by addition of excess iso-butanol.

Investigation of certain aspects of the method are still in progress. Among these are extension of the method to different sample volumes, use of other solvents, and possible utilization of more effective reductants.

V. Summary

1. A method is described for determination of inorganic phosphate in sea water by extraction of the phospho-molybdate complex into iso-butanol and reduction in the organic phase with stannous chloride.
2. The method is an order of magnitude more sensitive than methods currently in use and has the same relative accuracy.
3. Arsenic and silicate, in the amounts ordinarily encountered in sea water, do not interfere.
4. Certain aspects of the method are still under investigation.

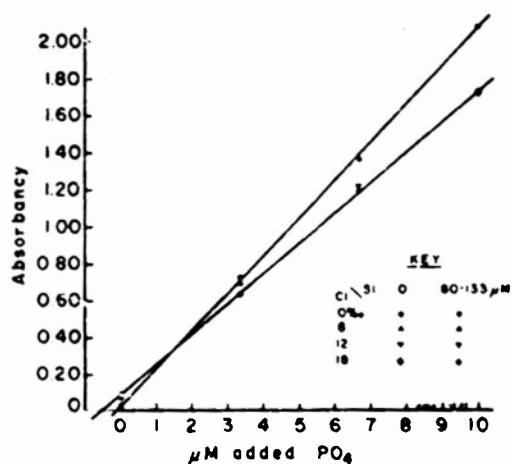


Fig. 1 Salt and silicate effects. Top line 0% chlorinity. Bottom line 12 and 18%. Little difference between 12 and 18%. 6% not graphed. Silicate has negligible effect. Graphs are linear from 0 to 2 absorbancy units.

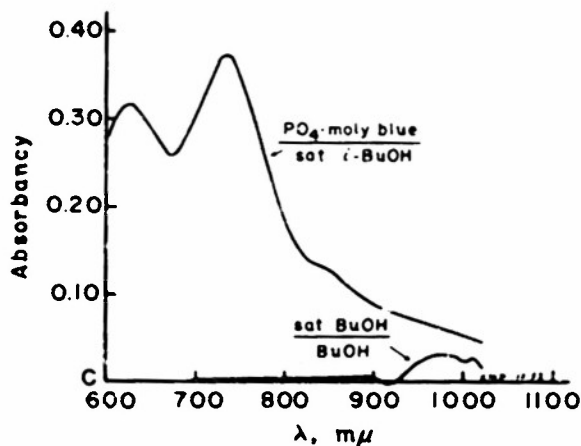


Fig. 2 Spectrum of phosphomolybdenum blue against solvent. Absorption maximum at about 733 mμ. Spectrum of water saturated butanol against butanol shows absorption only in the red.

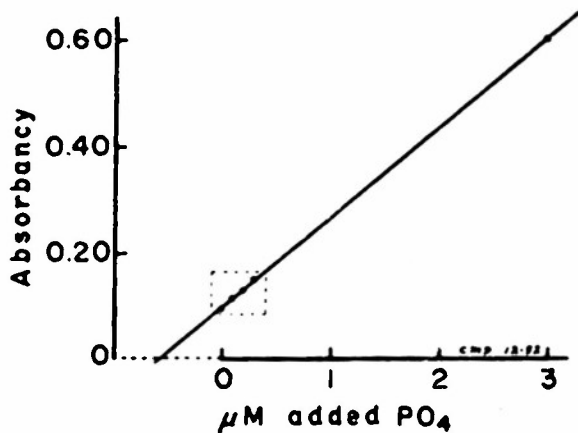


Fig. 3 Absorbancy vs. phosphate added to channel water. The lower part of the line is shown expanded in Figure 4.

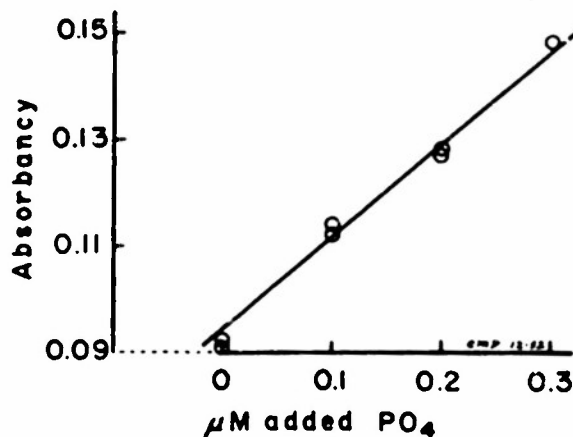


Fig. 4 Absorbancy vs. added phosphate. The increase in absorbancy per μM of added phosphate is nearly 10 times that found with current methods.

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