THE ESTIMATION OF DISSOLVED PHOSPHATE IN SEA WATER[1,2]

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ABSTRACT

The molybdenum-blue method of determining dissolved inorganic phosphorus in sea water was modified for use with a photoelectric colorimeter. The effect of reagent concentration, time, and temperature on the rate and extent of color development was studied. Practical suggestions are given for carrying out routine analyses at sea.

INTRODUCTION

The molybdenum-blue method for determination of dissolved inorganic phosphorus in sea water has been in use for many years. Modifications have been made in the conditions of analysis (size of sample, reagent concentration, time of standing, and temperature) and in the methods of making the colorimetric comparison of standard and unknown samples.

In recent years attempts have been made to adapt the method for use with photoelectric colorimeters. Harvey (1948) has made a careful study of the method with a photoelectric colorimeter of his own design.

In 1948 it became evident that work of the Marine Life Research program of the Scripps Institution of Oceanography would require that large numbers of routine phosphate determinations be carried out at sea by semiskilled personnel. A preliminary study showed that further modification of the method and a better understanding of the effect of various factors on the reaction would be required in order that data obtained by various people on different ships be consistent and reliable. This paper presents the results of this work on the phosphate method, carried out during the last two years.

Absorptimetric terms and symbols used are those adopted by the National Bureau of Standards and described in Letter Circular LC-857 of the National Bureau of Standards (1947).

APPARATUS

All spectrophotometric measurements were made in a Beckman Model DU Quartz Spectrophotometer with 10-cm absorption cells having COREX windows. Certain photometric estimations of phos-

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[2] Contribution New Series No. 517 from the Scripps Institution of Oceanography.

phate were also made in a photoelectric colorimeter, the Automatic Servo-operated Photometer (ASOP) to be described elsewhere. In this instrument monochromatic light was obtained with interference-type filters peaked at a wavelength of 700 mμ;[3] 10-cm absorption cells were used, the instrument usually being balanced against an air light-path rather than against an absorption cell filled with the solvent. A correction was applied for absorbancy of the cell and solvent.

Reagents were added either from micro-burets or from specially designed reagent dispensers to be described elsewhere.

FORMATION OF MOLYBDENUM-BLUE

Addition of a small quantity of a sulfuric acid solution of ammonium molybdate to a sample of water containing phosphate ions results in formation of a phosphomolybdate complex. Subsequent reduction with an acid solution of stannous chloride produces a blue color, the intensity of which varies with the concentration of phosphate ions initially present. The rate at which color forms and then fades and the intensity of the color formed depend also on the concentration of reagents and on the temperature and salinity of the sample.

Robinson and Wirth (1935), using either Atkins or Truog-Meyer reagents, found that the maximum intensity of color occurred seven minutes after addition of reagents, but they observed no fading until after an hour. More recently, Robinson and Thompson (1948) found that the maximum color intensity developed in about seven minutes, but they made no statement about duration of this maximum intensity.

In this laboratory, the formation of molybdenum-blue was studied by adding reagents to a sample and by transferring the sample to an absorption cell which was immediately placed in the spectrophotometer. Readings of transmittancy were made at frequent intervals; during intervals when readings were not being made, the absorption cell was out of the light path and hence in the dark. In a study of the reagents used by Wattenberg (1937), essentially the same as those employed by Robinson and Thompson (1948), it was found that in sea water color developed within 5 minutes to within 2% of maximum absorbancy, remained within 2% for about 10 minutes, then faded.

It was desirable to extend the flat part of the curve so that large numbers of samples could be analyzed efficiently at sea. Harvey (1948) showed that adding increased quantities of stannous chloride increased the rapidity of color development, delayed the onset of

[3] Although Harvey (1948) states that the blue substance produced in distilled water has a different light absorption band from that produced in sea water, the absorption spectrum studied with the spectrophotometer showed peak absorbancy at 700 mμ in distilled water, in 3.3% NaCl solution, and in sea water.

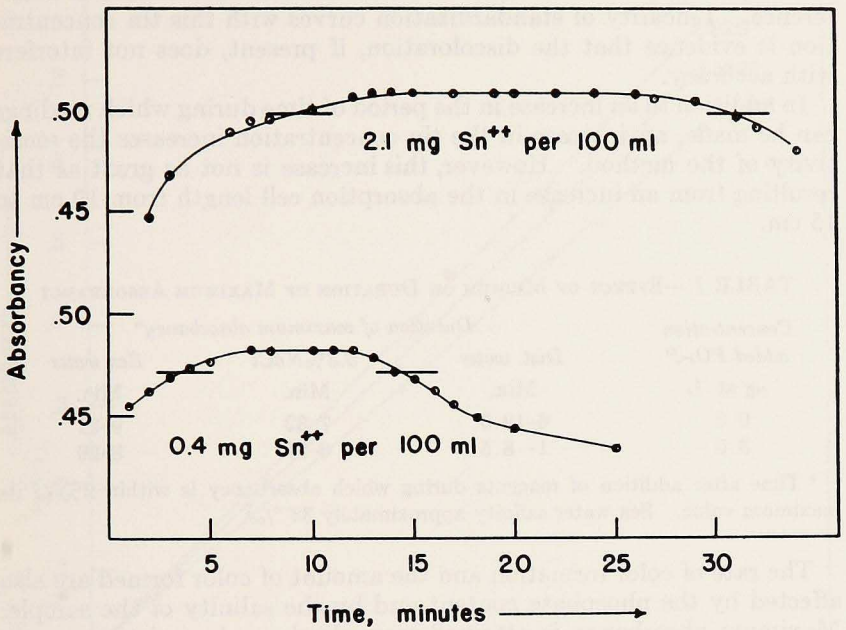

Figure 1. Effect of concentration of stannous tin on stability of molybdenum-blue. Samples are sea water with 1.5 μg at/L of added PO_4-P.

fading, and increased the amount of color formed with a given quantity of phosphate. He used concentrations of 0.43 to 0.64 mg Sn^{++} per 100 ml of sample; most workers have used concentrations approximating these.

The maximum practicable tin concentration seems to be about 2.1 mg Sn^{++} per 100 ml sample; upon further increase, reagent blanks develop a colloidal precipitate of tin oxides and are unreadable. With this concentration of tin, color develops within 10 minutes to within 2% of maximum absorbancy and remains within 2% for 20 minutes (Fig. 1). With such a long period of time during which the absorbancy remains constant, it is possible to make large numbers of determinations rapidly, scheduling additions of reagents and readings of absorbancy without waste of time.

Objection has been raised to increased tin concentration on the grounds that a yellow coloration appears and interferes with readings. This color is evident particularly with low phosphate concentrations and with the long absorption paths customarily employed in visual colorimetry. When measurements are made at the peak absorbancy

(700 mμ) in a photoelectric colorimeter there is no evidence of interference. Linearity of standardization curves with this tin concentration is evidence that the discoloration, if present, does not interfere with accuracy.

In addition to an increase in the period of time during which readings can be made, an increase in the tin concentration increases the sensitivity of the method. However, this increase is not as great as that resulting from an increase in the absorption cell length from 10 cm to 15 cm.

TABLE I.—Effect of Medium on Duration of Maximum Absorbancy

Concentration added PO_4-P	*Duration of maximum absorbancy**		
	Dist. water	*3.3%NaCl*	*Sea water*
μg at L	Min.	Min.	Min.
0.6	6–19.5	7–32	9–31
3.0	1– 8.5	6–17	8–26

* Time after addition of reagents during which absorbancy is within 2% of its maximum value. Sea water salinity approximately 33 °/₀₀.

The rate of color formation and the amount of color formed are also affected by the phosphate content and by the salinity of the sample. Maximum absorbancy is attained more slowly and persists longer in samples with low phosphate content. However, if color is considered constant while it is within 2% of maximum absorbancy, it is found that, in sea water, color is constant at all but the highest concentrations of phosphate (greater than 2.4 μg at/L) from 10 minutes until 30 minutes after reagents are added. At higher phosphate concentrations, color is constant only until 25 minutes after reagents are added.

When reagents are added to either distilled water or to a 3.3% sodium chloride solution containing added phosphate, the rate of color formation is much more sensitive to phosphate content than is that of a sea water dilution (see Table I). The molybdenum-blue color is more stable in sea water than in either salt solution or distilled water.

The rate of color development and the amount of color formed are also affected by the temperature of the sample. At a lower temperature, maximum absorbancy is not attained so rapidly and is not so great as that at a higher temperature. Thus, as temperature increases, the slope of the standardization curve, hence the sensitivity of the method, increases.

If a series of standardization curves is run at different temperatures and if the absorbancy index of each curve is plotted as a function of temperature, it is found that the absorbancy index varies linearly with

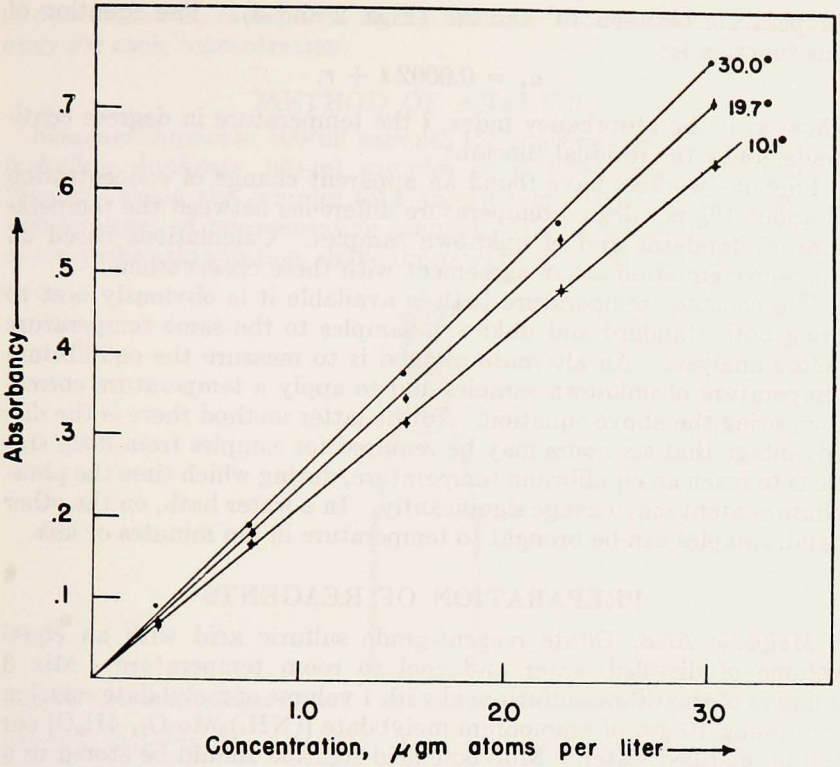

Figure 2. Phosphate standard curves at various temperatures, using sea water with added phosphate. These curves have been passed through the origin to emphasize the change in slope.

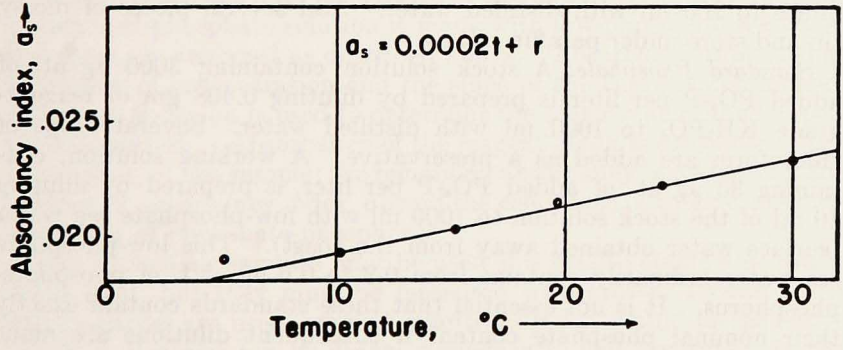

Figure 3. Absorbancy index as a function of temperature.

temperature between 10° and 30° (Figs. 2 and 3). The equation of this function is:

$$a_s = 0.0002\, t + r,$$

where a_s is the absorbancy index, t the temperature in degrees centigrade, and r the residual constant.

Previous workers have found an apparent change of concentration of about 1% per degree temperature difference between the temperature of standards and of unknown samples. Calculations based on the above equation are in agreement with these observations.

If a constant temperature bath is available it is obviously best to bring both standard and unknown samples to the same temperature before analysis. An alternate method is to measure the equilibrium temperature of unknown samples and to apply a temperature correction, using the above equation. In the latter method there is the disadvantage that six hours may be required for samples from deep stations to reach an equilibrium temperature, during which time the phosphate content may change significantly. In a water bath, on the other hand, samples can be brought to temperature in ten minutes or less.

PREPARATION OF REAGENTS

Molybdic Acid: Dilute reagent-grade sulfuric acid with an equal volume of distilled water and cool to room temperature. Mix 3 volumes of this dilute sulfuric acid with 1 volume of molybdate solution containing 10 gm of ammonium molybdate [$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$] per 100 ml distilled water. Molybdic acid solution should be stored in a dark bottle and should not be allowed to come in contact with rubber.

Stannous Chloride: Place 2.1 gm of granular tin metal (reagent grade 20 or 30 mesh) in a small beaker and add 22 ml of reagent-grade hydrochloric acid. After solution is complete, usually in 3–4 hours, dilute to 200 ml with distilled water. Add several pieces of mossy tin and store under paraffin oil.

Standard Phosphate: A stock solution containing 3000 μg at. of added PO_4-P per liter is prepared by diluting 0.408 gm of reagent-grade KH_2PO_4 to 1000 ml with distilled water. Several drops of chloroform are added as a preservative. A working solution, containing 30 μg at. of added PO_4-P per liter, is prepared by diluting 10 ml of the stock solution to 1000 ml with low-phosphate sea water (surface water obtained away from the coast). This low-phosphate sea water ordinarily contains from 0.3 to 0.6 μg at/L of phosphate-phosphorus. It is not essential that these standards contain exactly their nominal phosphate content if subsequent dilutions are made accurately, since, in standardization, the *slope* of the curve, absorbancy

vs concentration, is required rather than the *absolute value* of absorbancy for each concentration.

METHOD OF ANALYSIS

Measure duplicate 100-ml samples for analysis (see *Notes 1 and 2*), including duplicate 100-ml samples of distilled water for reagent blanks. Bring the samples to a known temperature or measure the temperatures of representative samples. To each sample add 1.0 ml of molybdic acid solution, shake thoroughly, and add 0.2 ml of stannous

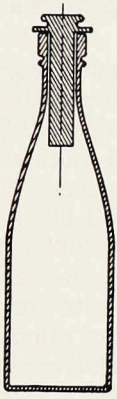

Figure 4. Phosphate sampling bottle. Dimensions of plug depend on volume of bottle used

chloride solution (see *Note 3*). After shaking thoroughly, record time of reagent addition. At least 10 minutes, but not more than 25 minutes, after addition of reagents, measure absorbancy of samples and reagent blanks at 700 mμ in a 10-cm absorption cell (see *Note 4*).

In order to relate absorbancy to concentration, 3 or 4 standard curves are determined for each batch of reagents. A series of dilutions of standard phosphate solution is made with low-phosphate sea water and these are analyzed as described above. Absorbancy is plotted as a function of concentration and the slope of the curve is determined. The given equation is used to determine the magnitude of the temperature correction. Absorbancy of the reagent blank is subtracted from absorbancy of the sample; the remaining absorbancy is divided by the product of absorbancy index, a_s, and the cell length, l, to give the concentration of phosphate-phosphorus.

Note 1: Smaller samples may be used if a small-volume absorption cell is used, but they must be measured with greater accuracy. If 50-ml samples are used, reagent quantities given should be halved.

Note 2: Samples can be drawn conveniently and accurately in the sampling bottle shown in Fig. 4. The bottle, although it contains a

volume somewhat greater than 100 ml, is closed by a loose-fitting brass plug with dimensions such that it will displace sufficient water so that the bottle will deliver 100 ml (precision of about ± 0.5 ml) when the plug is removed.

Note 3: Molybdic acid solution should not be pipetted from a stock reagent bottle because of the danger of contamination. If a buret is used, it must be remembered that molybdic acid solution turns blue on prolonged exposure to light or in contact with rubber. Similarly, stannous chloride solution is readily oxidized when exposed to air. Specially designed reagent dispensers are convenient for precise addition of reagents at sea.

Note 4: Absorption cell windows are gradually stained upon repeated exposure to solutions containing molybdenum-blue. This stain can be removed by filling the cell with a 10% solution of hydrofluoric acid and allowing it to stand for 15 minutes. The extent of this staining and its effect on the absorbancy of samples can be determined by measuring the absorbancy of the cell filled with distilled water, after first balancing the photometer for 100% transmittancy through an air light-path. If such cell checks are made before and after each group of determinations, it is possible to correct for the absorbancy of the cell itself. Then absorbancies of samples can be measured relative to an air light-path rather than relative to a cell filled with water.

RANGE AND ACCURACY OF METHOD

Ayres (1949) showed the value of plotting "absorbtancy" (100 minus transmittancy) against log concentration in studying range and accuracy of a photometric analytical method. He showed, on theoretical grounds, that the straight line portion of this curve reveals the optimum range of the method and that the slope of this line is a measure of the relative analytical error. The optimum range is a function of the wave length used, and the relative error is a function of the concentration of substance being studied.

Such a study of the method described above shows that, when photometric readings are made at 700 mμ in 10-cm absorption cells, the optimum range is from 1.0 to 3.5 μg at. P/L. Although Beers Law seems to obtain down to the very low concentrations, the relative error is greater below 1.0 μg at/L. In a typical curve, shown in Fig. 5, the relative analytical error in the optimum range is 2.8% per 1% photometric error. Between 0.4 and 0.8 μg at/L, however, it is 4.7%. Since the photometric reading error may be reduced to about 0.2%, the relative analytical error becomes only a fifth of these values, or less than 1% in both cases.

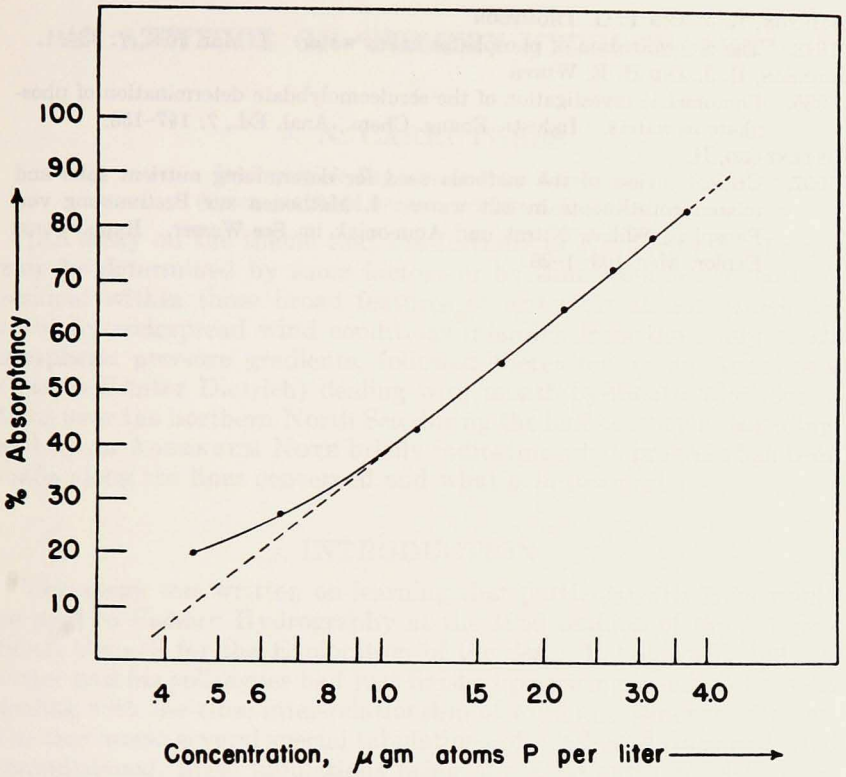

Figure 5. Curve for estimating optimum range and accuracy, according to method of Ayres (1949). Sea water at 10.1°, measured on 10-cm absorption cells at a wave-length of 700 mμ.

There are other important sources of error not treated by Ayres' method. These include sampling errors, error in determining the relation between absorbancy and concentration, and error in determining reagent blank. The presence of certain of these errors, in addition to those inherent in visual colorimetry, makes one question the usual estimates of 5 to 10% accuracy for the traditional method. Although the precision of measurements made by the method described above is usually better than 2% of concentration, the accuracy at the present time cannot be said to exceed 5%.

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