THE ESTIMATION OF PHOSPHATE AND OF TOTAL PHOSPHORUS IN SEA WATERS

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THE ESTIMATION OF PHOSPHATE

Description of an Absorption Meter

During an investigation of the total phosphorus (present as dissolved phosphate, dissolved organic phosphorus compounds, microplankton and suspended detritus) in samples of sea water, it became necessary to estimate phosphate in solution by an objective method. Estimation by eye of the molybdenum blue formed in the Atkins-Denigès method of analysis appeared to vary in precision from day to day; moreover, it was quite unsatisfactory in waters made turbid with added bacteria or other organisms.

Discussion with Dr W. R. G. Atkins, F.R.S., indicated that any method was to be avoided which measured the change in intensity of illumination falling on a photoelectric cell because very small voltage changes would have a considerable effect on the intensity of the light source. Such an instrument would not only need calibration, but this might change with time as the characteristics of the cell altered. The desiderata were defined as:

(1) The use of balanced photoelectric cells, the light falling upon them being kept constant except for fluctuations in intensity of the light source, which are not easily avoided.

(2) Fluctuations in the intensity of the light source to have no effect upon the null point.

(3) Any change with time in the characteristics of the photocells to become at once obvious and correctable.

(4) Readings in units directly proportional to the light absorbed by the molybdenum blue formed on adding stannous chloride to the liquid, and not based on a calibration curve.

It was also desired that no more than 70 c.c. of liquid should be sufficient for a measurement to be made.

A simple instrument has been developed which meets these requirements (Fig. 1). The observation tube T is filled with water and reagents; the slide, carrying lamp and photocell P_2, is then moved until no current flows through the galvanometer. The distance of lamp from P_1 is measured and the pointer on the slide set to read the *virtual* distance of L to P_2 on a logarithmic scale.

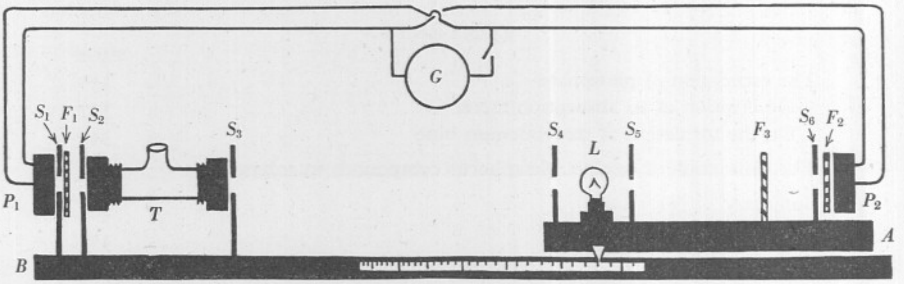

Fig. 1. Photoelectric meter. A, slide on which lamp and P_2 are mounted. B, base, 120 cm. overall length, on which P_1 and T are mounted, carrying scale showing logarithm of virtual distance of lamp from P_1. F_3, neutral filter made from two glass plates with smoked faces. G, galvanometer 483 ohms resistance, 2 sec. period, 200 mm. per μA. deflexion. L, lamp, car headlight 48 W. 12 V. with coiled filament in shape V, *circa* 4 mm. from corner to corner, held in bracket which is earthed, and operated through a dimming switch. P_1, P_2, selenium photocells, 23 mm. aperture, made by Evans Electroselenium Ltd., Bishop's Stortford. F_1, F_2, red light filters, Ilford 608 Spectrum. S_1 to S_6, light stops, diameter of aperture $\frac{1}{2}$ in. in S_1, $\frac{17}{32}$ in. in S_2, $\frac{7}{16}$ in. in S_3, $\frac{1}{2}$ in. in S_6. T, observation tube, glass $\frac{7}{8}$ in. internal diameter 15 cm. long with ground ends against which windows are held, having a thin coat of vaseline between the ground glass and window surfaces; the windows are removable for cleaning.

Since the 15 cm. observation tube is filled with liquid with refractive index *c.* 1·4, the virtual distance is some 4·3 cm. less than the actual distance. Thus if the actual distance were 80·0 cm. when no deflexion was shown on the galvanometer the virtual distance would be 75·7 cm. and the reading (or log d_0) 1·8791.

Stannous chloride is then added, the liquid replaced in the tube, and when the blue has fully developed the slide is moved towards P_1, until no current flows through the galvanometer. The virtual distance of lamp from P_1, or log d_1, is then shown by the pointer.

The increase in absorption of light passing through the 15 cm. column, due to the formation of molybdenum blue, is

$$\frac{d_0^2}{d_1^2} = 2\,(\log d_0 - \log d_1) \quad \text{or} \quad 2\Delta,$$

and the extinction coefficient of the molybdenum blue formed in the solution

$$E_{\text{cm.}} = \log_{10} \frac{\text{incident light}}{\text{transmitted light}} \text{ per cm. length of column}$$
$$= \frac{2\,(\log d_0 - \log d_1)}{15} \quad \text{or} \quad \frac{\Delta}{7{\cdot}5}.$$

The value of Δ is a direct measure, strictly proportional to the increase in blue colour in the solution, brought about by adding the reductant.

The value of the extinction coefficient needs further qualification. It changes with the wave-length of light, and therefore with both the composition of the red light transmitted by different filters and with the sensitivity of the photocell within this wave band. It is different if the same red filter is used with a different kind of photocell, or the eye, and it is different if the same photocell is used with another red filter which transmits light of different spectral composition.

Readings of $\log d_0$ and $\log d_1$ can be made to within 0·0005 on the scale; thus the instrumental error in values of Δ does not exceed ± 0·001. The colour formed in sea water due to the presence of 1 mg. phosphate-P per m.^{3} requires a movement of *c.* 0·0040 on the scale ($\Delta = 0{\cdot}0040$). Hence the instrumental error does not exceed that attributable to ± 0·25 mg. P per m.^{3}

Adjustment of the absorption meter is carried out as follows. The lamp filaments and stops S_3, S_2 and S_1 are carefully aligned with the slide in its nearest and farthest extreme positions, by observing the shadows cast by the stops with the light filters and photocells removed. The observation tube is inserted and the annularity of the light ring cast on S_1 checked by inspection.

The photocells and light filters are replaced and the slide moved until no current flows through the galvanometer. After the light has been on for several minutes the slide is readjusted to zero current (or null point) position and the light dimmed by the dimming switch. The galvanometer will show a deflexion as the light dims, coming back to zero as the light fades out. This appears to be caused by the two photocells differing in either their 'change in sensitivity with illumination', or in the speed with which they attain constant potential. When due to the former it can be remedied.

The sensitivity of a selenium cell changes if the illuminated area on its surface is moved. The 'change in sensitivity with illumination' likewise changes, but to a lesser extent.

To remedy the change in null point with change in intensity of light source, it was sufficient to move the stop S_6 in the vertical plane. This allowed the two photocells to be matched with respect to this characteristic. This having been done, slowly dimming the light source had no effect on the null-point position. Once matched the photocells appear to remain matched. In another similar instrument, an alternative method was used. A resistance was interposed between the galvanometer and one of the photocells; this also necessitated altering the neutral filter F_3.

Two further properties of selenium photocells require consideration. If one cell is illuminated much more brightly than the other for more than a few seconds, it will fatigue and require a short time to return to equilibrium. There is no need to let this happen, because after the tube has been refilled with another sample, the lamp is turned on slowly through the dimming switch while the slide is being moved towards the new null-point position.

After the photocells have been in the dark for some time (as when the apparatus has not been in use), they require a short period to attain a full response after the light is switched on. In consequence the null point changes during the first 1 or 2 min. However, if the cells are illuminated (equally) for 10 min. and the light is then switched off for a short period, the response is more rapid when the light is switched on again. The null point steadied in 30–40 sec. and remained steady for several hours in a dust-free room.

Owing to this property of the cells the following procedure was adopted. After use, the observation cell is filled with water, replaced, and the slide set at null point, the lamp being then switched off. When next required the lamp is switched on about 10 min. before starting a series of measurements and only switched off for the short periods required for changing the contents of the observation tube.

After the initial alignment and matching of the photocells, the strict linearity of the readings was checked. This is necessary to make sure that (*a*) alignment is perfect at all positions of the lamp and (*b*) there is either no reflexion from the walls of the tube, or that the very small amount of reflected light follows the inverse square law.

The tube was filled with water and reagents and log d_0 noted. Several neutral glass filters were inserted between F_1 and S_2, one at a time, and the d_1 values noted. Then two or more were inserted, and Δ for the combination was found to equal the sum of the Δ's observed for each separately.

A further check was made to be sure the simple theory involved in calculating the virtual distance was obeyed.

The tube was withdrawn and the slide moved until no current flowed through the galvanometer. The *actual* distance between lamp and photocell (d_a) was measured in cm. A neutral filter having an optical density of about 0·4 was then inserted between F_1 and S_2, the slide moved to zero current position, and the actual distance between lamp and photocell (d_b) measured.

The optical density of this neutral filter for the particular wave band of red light and photocell used equals

$$2\,(\log d_a - \log d_b).$$

The filled tube was then replaced, the slide moved until no current flowed, and log d_c, being the log of the *virtual* distance of lamp from photocell, read. The neutral filter was again inserted, the slide moved to the new zero deflexion position, and log d_e noted

$$2\,(\log d_a - \log d_b) \text{ equalled } 2\,(\log d_c - \log d_e).$$

This evidence indicated that the instrument obeyed the simple theory involved. It also satisfied the other initial desiderata.

On the Formation of Molybdenum Blue

When stannous chloride is added to sea water containing ammonium molybdate and sulphuric acid, either no colour is formed, colour is formed only when phosphate is present, or colour is formed when no phosphate is present.

The conditions under which these eventualities take place are shown in Fig. 2.

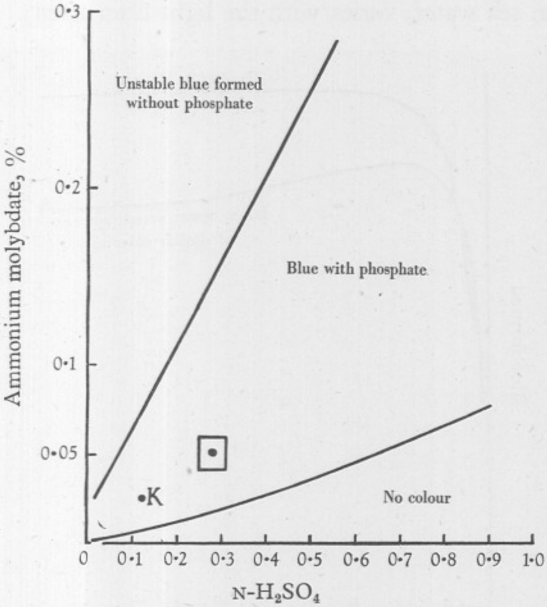

Fig. 2. Diagram showing the effect of varying concentrations of sulphuric acid and molybdate on the formation of blue in sea water, from data by Gripenberg (1929) and Kalle (1934). The point within the square represents the conditions investigated in this communication, the point marked *K* the conditions investigated by Kalle.

The following observations relate to the formation of molybdenum blue in sea water and in very dilute solutions of phosphate to which had been added:

(1) Sulphuric acid, making the solution *c.* 0·28 N with a pH of 0·82.

(2) 0·05% ammonium molybdate,

and had been reduced by the addition of at least 0·00045% $Sn^{\cdot\cdot}$.

This corresponds to the addition of 2 c.c. of Atkins-Denigès reagent to 100 c.c. of sea water.

On the addition of stannous chloride, the colour develops rapidly (Fig. 3) and is followed by a relatively slow fading, which becomes still slower as fading progresses.

With phosphate in distilled water colour development is less rapid, and the depth of colour, due to the presence of the same amount of phosphate, is greater than in sea water or salt solution.

In distilled water and in sea water, both the rate of colour development and the final depth of colour produced are greater with increasing temperature, and are greater if more stannous chloride is used, up to but not beyond a limiting quantity.

The blue substance produced in distilled water has a different light-absorption band to that produced in sea water (Buch, 1929; see also Cooper, 1938, who finds that the 'salt error', or ratio of colour produced in distilled water to that produced in sea water, varies with the light filter used).

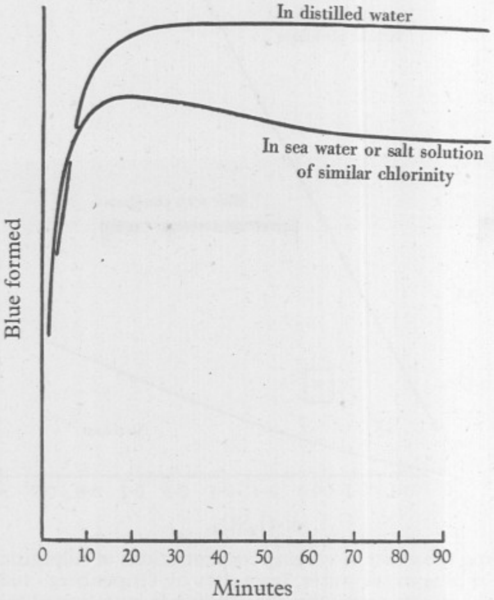

Fig. 3. Diagram showing rate of formation and subsequent fading of blue, due to the same concentration of phosphate in distilled and in sea water, at 10° C.

These blue substances have colloidal properties. The molybdenum blue formed by mixing trivalent molybdenum with molybdate has been seen to consist of aggregates or micelles of very different sizes (Schirmer, Andrieth, Gross, McClellan & Seppi, 1942) when viewed under an electron microscope. It seems reasonable to assume that the subsequent fading of the blues produced in acid water or acid salt solutions is due to aggregation of colloidal particles or micelles.

A study of these variables which affect the production of the blue substance in sea water containing phosphate was made in order to decide upon a reasoned procedure in carrying out analyses.

Variation in acidity. The effect of relatively small variations in acidity and in molybdate concentration upon the quantity of blue formed in sea water containing phosphate are shown in Fig. 4. It is seen that a 1% increase in acid decreases the colour by about 0·5%.

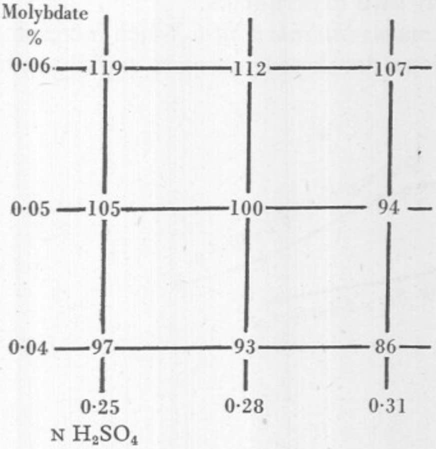

Fig. 4.

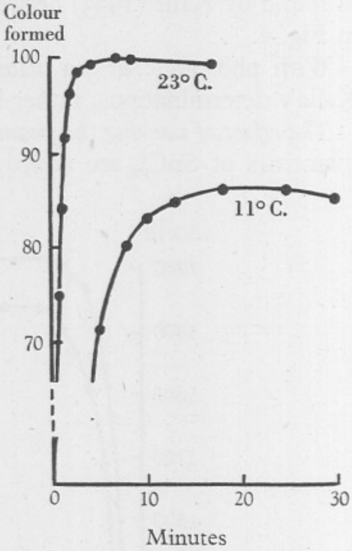

Fig. 5.

Fig. 4. Effect of varying concentrations of sulphuric acid and of molybdate on the blue formed in sea water containing phosphate. The colour produced with 0·05% ammonium molybdate and 0·28 N-acid is shown as '100'.

Fig. 5. Diagram showing the rate of colour formation and the amount of blue developed in the same sea water at 11 and 23° C., after addition of the same quantity of stannous chloride.

Time taken for ionic equilibrium to be attained after adding the acid molybdate to sea water. The following experimental data show that a period of some 3 min. is taken for equilibrium to be attained:

Stannous chloride added	Intensity of blue formed
At once	>161
After 1 min.	124
3	100
6	99·4
26	100·5
60	100
3 hr.	100
24	95·6

The effect of temperature. Increasing the temperature increases the velocity of the reactions involved. The maximum colour is developed sooner and the interval of time during which the colour is within ±0·5% of its maximum value becomes shorter. (This interval is also shorter if the quantity of stannous chloride is reduced.) Increasing the temperature also increases the amount of blue colour developed (Fig. 5).

With phosphate in distilled water the increase in colour was found to be 0·96% per rise of 1° C. within the range of 8–20° C. This is the same value as found by Kalle (1933) between 5 and 30° C. under the condition indicated in Fig. 2.

With phosphate in sea water the increase was 1·2% per rise of 1° C. in Kalle's determinations, rather less in my own experiments.

The effect of varying the quantity of stannous chloride added. When increasing quantities of $SnCl_2$ are added, the colour developed increases rapidly until about 12 times more Sn than the phosphate-P present has been reached (Gripenberg, 1929). Thereafter there is a slow increase in colour developed. With considerable additions the development of blue in phosphate solution becomes disproportionately greater than in sea water.

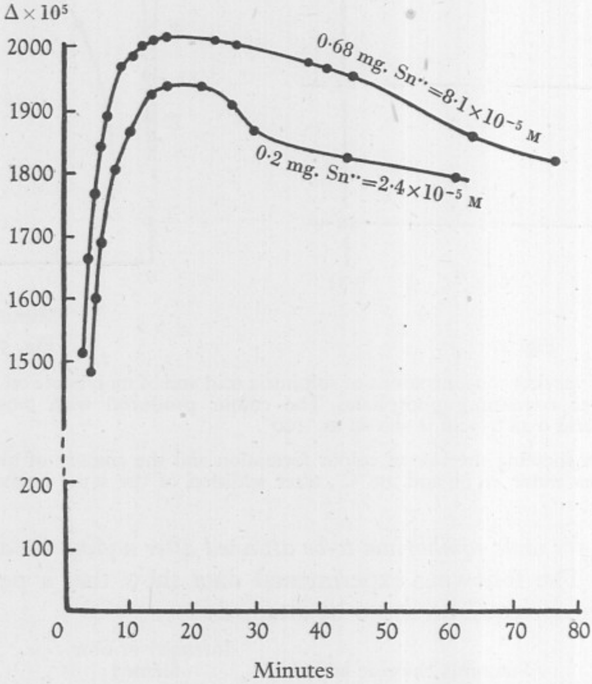

Fig. 6. The formation and fading of blue developed in a sea water, enriched with 40 mg. phosphate-P per m.^{3} at 10·5° C. Upper curve shows the course with 0·68 mg. Sn˙˙ added to 70 c.c. of liquid, lower curve with 0·2 mg.

The present investigation has been confined to additions varying between 0·2 and 0·8 mg. Sn˙˙ to 70 c.c. of liquid—initial concentrations lying between $2·4$ and $9·6 \times 10^{-5}$ molar Sn˙˙ and not exceeding $0·15 \times 10^{-5}$ molar phosphate.

Within this range the increase in colour due to each additional 0·1 mg. Sn˙˙ added amounts to approximately 1%.

Increasing the stannous chloride also increases the velocity of colour formation, and, in addition, delays the onset of fading (Fig. 6).

On mixing with sea water to which acid and molybdate have been added, the stannous ions are rather soon oxidized. By adding N/100 iodine after intervals and back-titrating with thiosulphate, it was possible to follow the fall in concentration of stannous ions (Fig. 7).

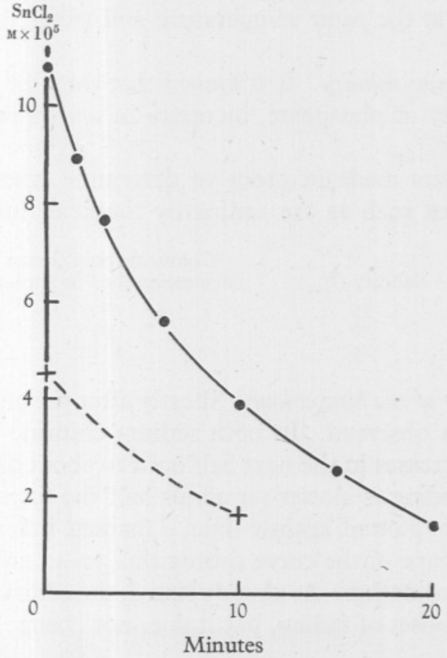

Fig. 7. Diagram showing the rate of oxidation of stannous chloride added to sea water containing 2 % of Atkins-Denigès reagent.

The effect of particulate matter in suspension and of the products of organic decay. The presence of either bacteria or of kaolin in sufficient quantity to make the water just visibly cloudy does not interfere with reasonably exact measurement of the blue formed on adding stannous chloride.

Series of estimations have been made with a sea water in which bacteria had grown after the addition of asparagin in sufficient quantity to raise its absorption coefficient in red light by $E_{cm.}$ 0·0036. The effect of this particulate matter was to reduce the blue colour formed on adding stannous chloride by 4%.

A further series of estimations have been made with a natural sea water just visibly cloudy with kaolin, which had entered the sea after heavy rains. There was sufficient in suspension to increase the absorption coefficient of red light of the water by $E_{cm.}$ 0·009. This had the effect of reducing the blue colour formed by 5%.

In another experiment a natural sea water was incubated for several days with a considerable quantity of added plankton organisms until they had decayed. After passing through filter paper the water showed no marked cloudiness. Determinations of the increment in blue colour developed due to the addition of 20 mg. phosphate-P per m.^{3} were made in order to find out whether organic decay products affected the production of blue colour. The increment found ($\Delta = 0{\cdot}0832$) at 15° was the same as that in unpolluted sea water ($\Delta = 0{\cdot}0830$) at the same temperature and using the same quantity of stannous chloride.

The effect of varying salinity. It is known that the blue colour formed, due to the same quantity of phosphate, increases in sea waters when the salinity falls below about 25 °/₀₀.

An experiment was made in order to determine whether there was any variation at salinities such as are ordinarily found in inshore and open sea waters:

Salinity (°/₀₀)	Colour formed (mean of determinations in triplicate)
35	100
30	99·5
25	101·8

The rate of fading of the blue colour. Shortly after the colour has reached its maximum, fading is observed. In both sodium chloride solution and in sea water the colour decreases in the next half hour to about 94 % of its maximum value. Thereafter fading is slower (at about half the former rate). When the red light absorbed is plotted against time, a marked inflexion in the curve is apparent, and the shape of the curve during this 30–40 min. differs somewhat from one sea water to another. As already stated, the addition of more stannous chloride delays the onset of fading, but it does not change the general form of the curve.

These observations led to the following hypothesis. Immediately after formation, the molecules aggregate to form micelles and these continue to aggregate—growing larger and fewer. The rate of aggregation, quick at first, slows later. As the molecules aggregate and gradually grow larger, the blue colour fades. The light absorbed by the solution not only becomes less but moves from the red towards shorter wave-lengths. On inspection the fading blue solutions are seen to become both less intense and to acquire a blue-green hue. Addition of 0·05 % of gum arabic, acting as a protective colloid, smooths out the inflexion in the curve as the blue fades while greater additions delay the inception of fading.

That aggregation would cause fading seems certain on considering the situation in reverse, for if particles of a slowly dissolving dye are suspended in water the colour increases as the particles dissolve.

This hypothesis provides an explanation of observed characteristics in the rate at which the blue colour develops.

On the rate at which blue develops. As a first approximation, the light absorption (Δ_t) was taken as proportional to the quantity of phosphate which had reacted at time t during the reaction, since the maximum colour developed (Δ), when the rate of development and fading balance each other, has been found proportional to the phosphate present at the commencement.

Then $\Delta - \Delta_t$ is a measure of the unreacted phosphate concentration at time t; if the logarithm of this is plotted against time an almost linear relation is found. It is almost but not quite linear with phosphate in distilled water, salt solution or sea water.

This indicates that of the several ions taking part in the reaction all but one—phosphate—are in excess. The apparent velocity approaches that of a monomolecular reaction controlled by the concentration of unchanged phosphate ($\Delta - \Delta_t$) present at any moment.

The velocity will tend to depart somewhat from the course of a monomolecular reaction because (*a*) the concentration of stannous chloride is falling throughout the reaction (Fig. 7) and also because (*b*) fading of the blue, due to aggregation of molecules and then further aggregation of the micelles so formed, is not likely to follow the course of a monomolecular opposed reaction.

With regard to (*a*), it is seen from Fig. 6 that increasing the initial concentration of reductant increases the reaction velocity. It would be expected that if the oxidation of stannous chloride by atmospheric oxygen throughout the period of blue formation could be retarded, this would have the same effect. Experiment confirmed this. The addition of sulphite (0·01 M) increased the rate of blue formation. Hence the quantity of stannous chloride present is not in sufficiently gross excess throughout to allow the monomolecular reaction to proceed entirely unaffected.

With regard to (*b*), if fading is most rapid immediately after the formation of blue and does not continue at a rate proportional to the intensity of blue colour during the early part of the reaction (which it does not do, *vide supra*), Δ_t is more than proportional to the phosphate which has taken part in the reaction at time t. In consequence the apparent velocity calculated from this first approximation (Δ_t = phosphate which has reacted at time t) should (i) be greater than the mean velocity early in the reaction while the major part of the blue is being formed, (ii) dwindle for a short period after the initial rapid development of most of the blue while this is most rapidly aggregating, and (iii) indicate a slight rise in apparent velocity towards the end of the reaction after the greater part of the blue has been formed and has had time to undergo its early aggregation. Experiment shows that this is so.

The velocity constant for a monomolecular reaction was calculated for successive intervals of time during the formation of blue:

$$k\,(\text{apparent}) = \frac{2{\cdot}3}{t_2 - t_1} \log_{10}\left(\frac{\Delta - \Delta t_1}{\Delta - \Delta t_2}\right).$$

The following is illustrative.

Time (min.)	Δ_t or light absorbed	Interval (min.)	*k* (apparent)
0	0	—	—
0·935	0·0900	0·933	0·769
1·45	0·1130	0·517	0·604
1·935	0·1285	0·483	0·586
2·47	0·1410	0·533	0·575
3·25	0·1530	0·783	0·540
3·915	0·1595	0·667	0·504
6·0	0·1710	2·083	0·587
8·0	0·1742	2·0	[0·55]
10·0	0·1752	—	—
13	$0{\cdot}1758=\Delta$	—	—

A number of such experiments have been made and the value of the 'apparent velocity constant' plotted as in Fig. 8. A fall in the value followed by a slight rise was always found, before the value finally fell to zero at the point where maximum colour is attained. These phases during the course of the formation of blue are typical of the reaction in sea water, in salt solution and in distilled water.

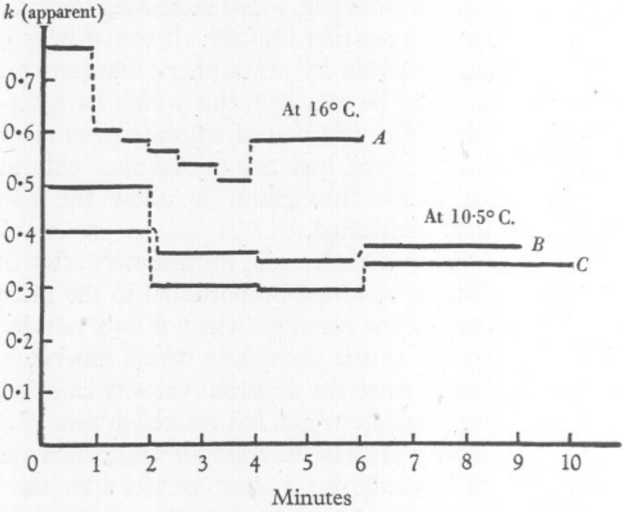

Fig. 8. The value of the apparent reaction velocity during intervals while blue was being formed in phosphate-enriched sea water. *A* at 16° C. and $6\cdot3\times10^{-5}$ M-Sn$^{\cdot\cdot}$; *B* at 10·5° C. with $8\cdot1\times10^{-5}$ M-Sn$^{\cdot\cdot}$; C at 10·5° C. with $2\cdot4\times10^{-5}$ M-Sn$^{\cdot\cdot}$.

The addition of a protective colloid hinders the aggregation of the electronegative blue micelles, thereby increasing the rate of colour formation. Gelatin, electropositive at this acidity, proved more potent than arabic. Both substances have a secondary effect, reducing the total colour developed possibly because they form complexes with the molybdic acid. Citric, oxalic

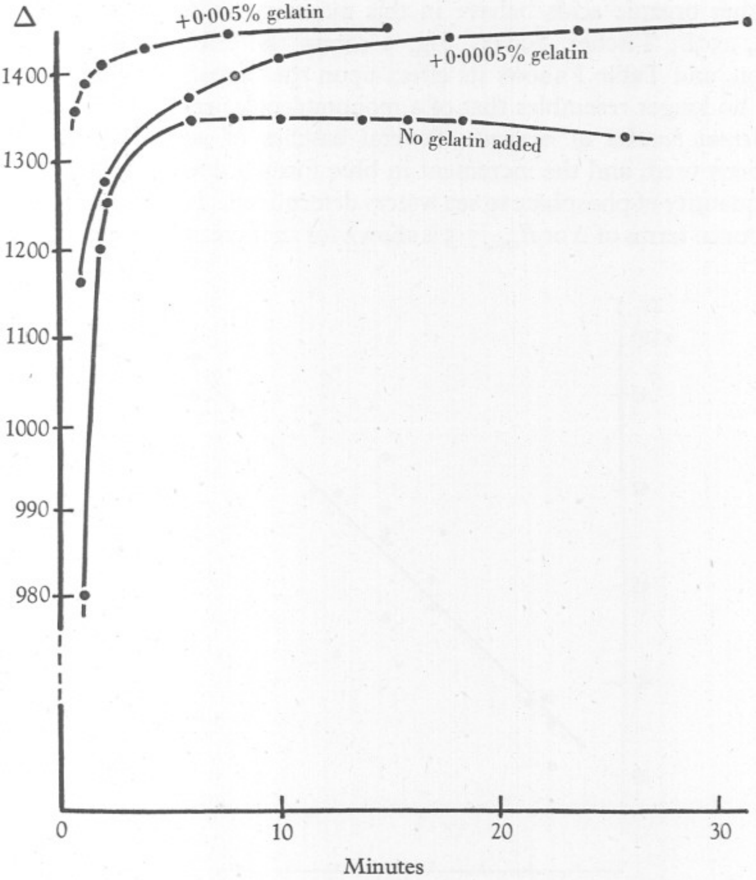

Fig. 9. The effect of a protective colloid on the formation of blue in a phosphate-enriched sea water.

TABLE I. INFLUENCE OF GELATIN ON THE APPARENT VELOCITY CONSTANT IN SEA WATER AT 16° C.

No addition		With addition of gelatin	
Interval (min.)	*k* (apparent)	Interval (min.)	*k* (apparent)
0 –0·92	1·4	0 –0·90	1·75
0·92–1·28	1·2	—	—
1·28–1·75	0·9	0·90– 2	0·334
1·75–2·15	0·44	—	—
2·15–4	1·0	2 – 4	0·260
4 –6	0·6	4 – 6	0·194
		6 – 8	0·174
		8 –12	0·10
		12 –24	0·11

Maximum colour reached in 10 min.

Maximum colour reached in 30 min

and other organic acids behave in this manner (Kalle, 1935; Berenblum & Chain, 1938; Tischer, 1934). Fig. 9 shows the effect of gelatin upon the reaction, and Table I shows its effect upon the 'apparent velocity constant', which no longer resembles that of a monomolecular reaction.

Different batches of reagent. Several batches of acid molybdate reagent have been used, and the increment in blue formed due to the addition of the same quantity of phosphate to sea waters determined. In Fig. 10 the increment in colour in terms of Δ or $E_{cm.}/7{\cdot}5$ is shown for an increase of 1 mg. phosphate-P per cm.^{3} as found on a number of occasions. This *increment value* appears to be almost constant, when the same quantity of stannous chloride has been used, and provided that the effect of temperature is taken into consideration—an increase of 1·2% per rise of 1° C. The line in the diagram is drawn to show this temperature relation.

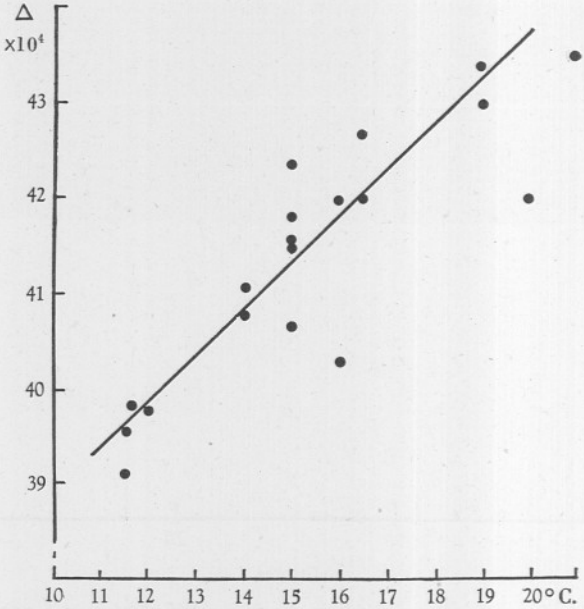

Fig. 10. The points show the increment in Δ due to the addition of 1 mg. phosphate-P per m.^{3}, obtained with different batches of reagent and different sea waters on different dates. The line shows the temperature relation as found by Kalle, being an increase of 1·2% per degree rise in temperature. The quantity of stannous chloride added varied between 0·35 and 0·44 mg. $Sn^{\cdot\cdot}$ to 67 c.c. of sea water plus 3 c.c. of reagents.

Since it has been noticed that the rate of colour formation and fading, at the same temperature, etc., differs slightly between one sea water and another, and since particles in suspension affect the colour formation, a precise agreement would not be expected. The majority of the values lie within about ±2% of the expected, which is greater than the probable experimental error.

Hence there appears some reason to suppose that sea waters may be met with which contain a substance which slightly inhibits the formation of blue.

Linear relation between phosphate present and colour formed. A number of experiments have been made which show proportionality. The following series of observations is illustrative:

	Δ observed	mg. P per m.^{3} calculated*	Increase
With distilled water	0·0032		
	0·0032		
	0·0024	Reagent-blank = 0·003Δ	
With filtered sea water	0·0120	2·20	
	0·0115	2·09	
Do. + 5 mg. phosphate P per m.^{3}	0·0320	7·1	5·1
	0·0335	7·5	5·4
Do. + 10 mg. phosphate P per m.^{3}	0·0535	12·4	10·3
	0·0533	12·3	10·2
Do. + 15 mg. phosphate P per m.^{3}	0·0735	17·2	15·1
	0·0735	17·2	15·1
Do. + 20 mg. phosphate P per m.^{3}	0·0910	21·6	19·5
	0·0900	21·5	19·3
	0·0905	21·4	19·3

* Being Δ observed less the reagent-blank, divided by the increment value for the temperature of the experiment, 14° C., this increment value being 0·00408Δ per mg. P per m.^{3}.

Successive reactions. If, after adding the stannous chloride and allowing the colour to develop fully, phosphate (in 0·28 N-H_2SO_4) is added within the next few minutes there is a second development of blue. The amount formed in this second reaction is slightly less than the increment due to adding the same quantity of phosphate initially. This lesser formation of colour appears due to some of the stannous chloride having been oxidized during the first reaction.

However, if, at the same time as adding the phosphate, a second addition of stannous chloride is made (about two-thirds of the quantity originally added) the amount of blue which develops is nearly equal to the increment when the same amount of phosphate is added initially. For instance:

Using filtered sea water at 18° C., the following readings were obtained on the photoelectric meter.

	log cm. virtual distance lamp to photocell	
	Sample A	Sample B
67 c.c. sea water + acid molybdate + Sn	1·8465	1·8470
After adding 20 mg. phosphate-P per m.^{3} and more Sn	1·7590	1·7600
Increment due to addition	0·0875	0·0870
After adding a further 20 mg. phosphate-P per m.^{3} and more Sn	1·6740	1·6760
Increment due to addition	0·0850	0·0840
67 c.c. sea water + 20 mg. phosphate-P per m.^{3} + acid molybdate and Sn	1·7620	1·7610
Increment over mean value of 1·8467 for water without added phosphate	0·0847	0·0857

Procedure adopted. (1) To 67 c.c. samples of sea water in 100 c.c. flasks were added 3 c.c. of an acid molybdate solution made by dissolving 6·5 g. of ammonium molybdate in 500 c.c. of water and 110 c.c. of concentrated sulphuric acid. This acid molybdate solution attains ionic equilibrium within 48 hr. and thereafter undergoes little or no change when stored in hard glass, but changes in contact with ground glass. It is equivalent to the Atkins-Denigès reagent diluted with an equal quantity of water. The addition is made most conveniently by means of a simple automatic pipette (Fig. 11) while swirling the sea water, in order to avoid the possible formation of chlormolybdate (*vide infra*).

(2) The sea-water samples plus reagent are each transferred to the observation tube of the photoelectric meter, the d_0 readings obtained, and the liquids returned to the flasks.

(3) Three drops of a solution of stannous chloride in dilute hydrochloric acid, containing between 0·3 and 0·45 mg. $Sn^{\cdot\cdot}$, are then added while swirling each sample of the sea water plus reagent. Each sample is returned to the observation tube and the d_1 reading taken when the colour formation reaches a maximum. The time interval, after adding the reductant, during which 99·5 % of the maximum colour is present, varies with temperature at

11° C.	14–23 min.
14° C.	12–18 min.
16° C.	11–15 min.
23° C.	5–14 min.

The difference between d_1 and d_0 or Δ is thus obtained for each sample, and the temperature noted. Before calculating the phosphate content it is necessary to subtract the value for the reagent-blank.

(4) The observation tube is very thoroughly washed to rid it of any trace of stannous ions, and the operations repeated using the same reagent and stannous chloride solution with distilled water. With distilled water the time interval after adding reductant during which 99·5 % of the maximum colour is present is longer than with sea water at

9° C.	45–145 min.
15° C.	15– 45 min.
20° C.	10– 30 min.

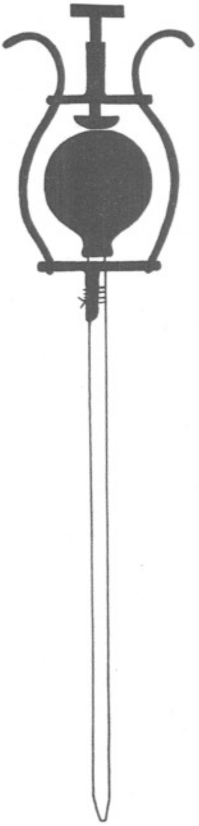

Fig. 11. Simple automatic pipette used for adding reagent while shaking contents of flask, show- ing rubber ball on glas pipette and plunger wit limited travel adjusted t suck in and deliver tl required quantity.

The value found for Δ for each sample of sea water minus the value of Δ for distilled water (the reagent-blank) is then directly proportional the phosphate content of the sea-water sample.

(5) The increment value for unit addition of phosphate-P per m.^{3} may be fo by enriching samples with 20 mg. phosphate-P per m.^{3}. This is conveniently don adding 0·3 c.c. of a solution containing 4·5 mg. phosphate-P per l. of 0·28 N-H to 67 c.c. samples of filtered sea water by means of a Krogh syringe pipette.

Alternatively, the increment may be determined by adding phosphate additional reductant after the d_1 reading has been obtained (p. 351).

The values of phosphate content obtained by the phosphomolybdate method of estimation include any *arsenate* which may be present in the water. Rakestraw & Lutz (1933) found between 7 and 24 mg. As per m.^{3} in various samples of sea water, and if all this were present as arsenate it would cause the formation of blue colour equivalent to 3–10 mg. phosphate-P per m.^{3}. That at least some of the arsenic present in natural sea waters is in the form of arsenate seems probable, because if small quantities of sodium arsenite (20–40 mg. As per m.^{3}) are added to sea water it oxidizes to arsenate, the rate of oxidation varying between one sea water and another.

The values of phosphate found also include any particles of inorganic phosphate suspended in the water which dissolve in the 0·28 N-acid. Turbid inshore waters may contain considerable quantities. Thus a water collected off the Suffolk coast after filtration and addition of the acid reagent was found to contain 3·7 mg. phosphate-P per m.^{3}; but, when the acid reagent was added before filtering, 10·5 mg. per m.^{3} were found. In the clear transparent waters of the English Channel, Cooper (1948) finds evidence of particulate phosphate in suspension varying hand in hand with the quantity of particulate iron present.

In consequence, it is to be expected that subsamples from the same sea water may vary in their phosphate content. In clear waters from the English Channel variations exceeding ± 1 mg. phosphate-P per m.^{3} appear to be rare.

With subsamples of a filtered water, on the other hand, the variation appears to lie within or almost within the instrumental error, ± 0·25 mg. P per m.^{3}.

THE ESTIMATION OF ORGANIC PHOSPHORUS COMPOUNDS IN SOLUTION

Several attempts to estimate the total phosphorus in sea-water samples culminated in the successful methods devised by Kalle (1935) and by Redfield, Smith & Ketchum (1937).

The present investigation aimed at modifying this latter method to obtain a quicker and simpler technique. For survey purposes it had the disadvantage that the estimation involves several processes taking time; it also involved subtracting rather high and variable reagent blank-values.

Preliminary experiments, using visual colorimetry, showed that autoclaving at 135–140° C. for 5–6 hr. in 0·28 N-H_2SO_4 completely hydrolysed such compounds as nucleic acid, phospho- or glyceroproteins dissolved in sea water. It also completely hydrolysed the phosphoric esters in diatoms.

In control experiments using distilled water instead of sea water, the reagent-blank values were high and variable. It became apparent that phosphate leached out of the glass flasks into the 0·28 N-acid during autoclaving at 140° C., even after the flasks had previously been cleansed by heating in concentrated sulphuric acid. Finally, 100 c.c. silica flasks were used. These give consistent reagent-blank values equal to the values obtained without autoclaving.

During the cooking any arsenite was oxidized to arsenate; by adding a small quantity of sodium sulphite before heating, this was not only prevented but added arsenate was almost completely reduced to arsenite. These latter observations were confirmed after the method of phosphate estimation had been more fully developed.

Experiment showing the effect of adding arsenate to an aged and filtered sea water. Samples of the water, with and without the addition of 44 mg. arsenate-As per m.³, were acidified to 0·28 N with sulphuric and 0·15 % $Na_2SO_3.7H_2O$ added. After autoclaving for 5 hr. at 140° C. and cooling, the volumes were made up to the original volumes and an acid molybdate solution added to give 0·05 % ammonium molybdate.

	Phosphate found mg. P per m.³
Water only	7·6, 6·7, 6·7
Water and arsenate	7·0, 6·9

The photoelectric estimation of molybdenum blue allowed determinations to be made in water turbid with bacteria. This, in turn, allowed the question whether hydrolysis of the organic phosphorus was complete to be attacked by another and more exact method.

When sea water is enriched with asparagine (20 mg. per l.) and the bacteria allowed to proliferate, the inorganic phosphate is used up by the bacteria, which are singularly rich in phosphorus, having a C/P ratio of about 22. When phosphate was added to such a slightly turbid water and then estimated, the increase in phosphate due to this addition found by experiment was 4 % less than when the same quantity of phosphate was added to a control in which bacteria had not developed. Following this preliminary experiment a sea water containing 14½ mg. inorganic phosphate per m.³ was enriched with asparagine. Ten silica flasks were charged with this, five being incubated until all the dissolved phosphate had been used by bacteria while the other five were acidified and stored in the cold. Then, after hydrolysis at 140° C., the resulting phosphate (including the equivalent of any arsenic present) was estimated (Table II).

TABLE II. PHOSPHATE, IN MG. P PER M.³, FOUND IN FLASKS

After storage without development of bacteria	After incubation and utilization of 14·5 mg. P per m.³ of dissolved phosphate by bacteria
22·6	21·6
22·75	21·7
22·2	21·0
22·4	21·2
22·6	21·6
Mean 22·51 ± 0·149	Mean 21·42 ± 0·165 + 4 % = 22·28 ± 0·172

Hence at least 93 % of the phosphorus in the bacterial tissue had been reconverted to phosphate in solution. Assuming that the bacterial cells, after cooking, reduced the colour formation by 4 % as they did in the previous experiment, virtually all the phosphorus in the bacterial tissue was reconverted to phosphate.

In the earlier experiments it was found that the use of ammonium molybdate solutions in distilled water led to high reagent-blank values; furthermore some anomalous results were obtained. This necessitated a study of the inter-ionic changes which take place in solutions of ammonium molybdate during storage and on their mixing with acidified sea water. The following observations were made.

Formation of silicomolybdate. On storing aqueous solutions of ammonium molybdate in glass bottles, their use resulted in high reagent-blank values, less so if the molybdate was dissolved in dilute sulphuric acid, and not at all if dissolved in 3 N or 6 N-acid.

No such progressive increase in the reagent-blank values resulted from the use of a 1·65 % solution of ammonium molybdate in 0·56 N-H_2SO_4 stored in waxed bottles.

Presumably silicomolybdate ions were formed during storage in glass and these were reduced to silicomolybdate blue by stannous chloride. The reduction takes place more rapidly than that of phosphomolybdate; another difference is that the same amount of blue is formed in sea water as in distilled water. In consequence the 'reagent-blank' resulting from the presence of silicomolybdate is not subject to salt error.

Experiment. A 1·65 % solution of ammonium molybdate in 0·56 N-H_2SO_4 was used which had been stored in a glass bottle for 2 months, its reagent-blank having meanwhile risen to a high value while the liquid had acquired a faint yellow tint.

With filtered sea water the blue formed after adding stannous chloride was the same, whether the reductant was added a few minutes or 18 hr. after the molybdate solution. This showed that the silicomolybdate ions were stable in sea water containing acid and molybdate at the customary concentration and acidity. The silicomolybdate blue developed more rapidly (80 % within 50 sec. at 10° C.) than the phosphomolybdate blue develops from the reduction of phosphomolybdic acid at the same temperature (80 % in *c.* 4·5 min.) and its subsequent rate of decay was less.

A further experiment was made with the same stored reagent in order to find whether the presence of a large quantity of silicomolybdate ions would interfere with the estimation of phosphate.

A filtered sea water was found to contain 7·0 mg. phosphate-P per m.^{3}, using a reagent with very low reagent-blank. The following estimation was then made using the glass-stored acid molybdate solution.

	Colour developed	
With distilled water (duplicate)	118·1 117·9	reagent-blank
With filtered sea water (duplicate)	145·2 144·8	
With same + 20 mg. phosphate-P per m.^{3}	222 222	

Increase in colour due to addition of 20 mg. P/m.^{3} = 77·0
Colour developed in filtered sea water (145) less reagent-blank (118) = 27·0
Phosphate content of filtered sea water $= \frac{27\cdot 0 \times 20}{77\cdot 0}$ or 6·8 mg. phosphate-P per m.^{3}

Silicomolybdate ions are formed on addition of a silicate to a relatively strong (1·65%) solution of ammonium molybdate in water or dilute sulphuric acid, but they are not formed if small quantities of silicate are added to 0·05% molybdate in 0·28 N-H_2SO_4. Thus they are not formed at room temperature *subsequent* to the addition of the molybdate reagent in phosphate determinations.

Some evidence was obtained of the formation of silicomolybdate while a 5% solution of ammonium molybdate mixes slowly with the acidified sea water, but not if a 1·65% solution in dilute sulphuric is used.

Formation of chlormolybdate. Evidence was obtained suggesting that within a limited range of acid and of molybdate concentration chloromolybdic ions may be formed in the presence of chloride. The supposed chloromolybdate is reduced by stannous chloride to a blue; the chlormolybdate is unstable at greater dilution in sea water made 0·28 N with sulphuric acid, slowly reverting to molybdate. The above conclusions rest on the following observation.

(*a*) When sulphuric acid was added to sea water without shaking, so that the upper levels in the flask were less acid, and molybdate then added, the flask being later shaken before adding reductant, over twice as much blue colour developed than when the water and acid had been thoroughly mixed prior to the addition of the molybdate.

(*b*) A solution was made containing 1·67% ammonium molybdate and 3½% sodium chloride in 0·28 N-sulphuric acid. After it had stood for 4 days, its addition to acidified sea water in the customary proportions followed 15 min. later by stannous chloride led to the formation of a strong blue colour. If the reductant was added 30 min. after the acid-salt-molybdate solution, the blue colour formed was 25% less. The experiment suggested that chlormolybdate ions had formed in the acid-salt-molybdate solution, and on adding this to sea water made 0·28 N with sulphuric acid, these ions slowly reverted.

The foregoing observations led to the adoption of the following procedure in estimating the total phosphorus in a water sample—phosphorus present as phosphate in solution, as organic phosphoric esters in solution, in microplankton and in organic detritus, and as phosphate present as inorganic phosphate particles.

Procedure adopted for estimation of total phosphorus in water samples. To 67 c.c. of sea water in 100 c.c. silica flasks are added (i) exactly 1 c.c. of concentrated sulphuric acid which has been diluted with an equal volume of water, (ii) 4 drops of a saturated solution of sodium sulphite. The flasks are then covered and heated for 5–6 hr. in a pressure cooker at between 30 and 40 lb. per sq.in. After cooling the volume of each is made up to 68 c.c. with distilled water, the contents being thoroughly mixed. Then, while the contents are being swirled in the flask, 2 c.c. of a solution containing 6·6 g. of ammonium molybdate in 400 c.c. of water plus 6 c.c. of concentrated sulphuric acid are added. This addition is conveniently made by means of an automatic pipette (Fig. 11); the use of a burette or any contact with ground glass is to be avoided. The molybdate solution is stored in a waxed bottle, and appears to attain interionic equilibrium within 48 hr. after being made.

The d_0 and d readings are taken. From the Δ values so obtained the phosphate contents are calculated after subtracting the reagent-blank.

These values are subject to a small, almost insignificant, correction because the acidity is reduced by about 4% as the sulphur dioxide formed is driven off during cooking. This reduction in acidity causes 2% more colour to be formed: therefore the calculated values of phosphate content are subject to a correction of −2%.

The estimations of phosphate by this (or Redfield's) method will not include the equivalent of any arsenate present in the water.

Variable quantities of microplankton organisms and of particulate phosphate are likely to occur in subsamples of the same sea water. Some data showing the variability encountered in offshore English Channel waters are given in the following.

(i) Sea water collected near the Eddystone Lighthouse, 30 September 1946, at a depth of 2 m., contained an appreciable quantity of particulate matter. Estimation of phosphate in seven subsamples showed the presence of 12·0, 12·6, 12·6, 12·6, 12·7, 12·8, 12·8 mg. phosphate-P per m.^{3}.

Estimation of total phosphorus in seven subsamples showed the presence of 16·1, 16·1, 16·1, 16·5, 16·5, 17·0, 17·0 mg. P per m.^{3}.

(ii) Subsamples of raw sea water collected 10 miles south of the Eddystone at a depth of 2 m. on 5 December 1946 were found to contain 12·9, 12·9, 13·2, 13·4, 13·6, 14·0 mg. phosphate-P per m.^{3}, and 15·7, 17·3, 17·65 mg. total P per m.^{3}.

Storage of sea-water samples. Early in this investigation it was found that when sea water is stored in glass bottles, the quantity of total phosphorus in solution and suspended particles decreases. This might be expected, since there is a rapid development of bacteria firmly attached to the glass surface during storage in small vessels and since bacteria are singularly rich in phosphorus. However, when sea water, collected during the summer when the organic phosphorus in solution is at a maximum, was saturated with chloroform, which hinders but does not entirely stop bacterial growth, or was acidified (0·28 N) and stored for a few days, there was also a reduction in total phosphorus. A clean glass surface adsorbs organic matter when in contact with sea water (Harvey, 1941; ZoBell & Grant, 1943), and presumably some organic phosphorus is lost by adsorption during storage. I am indebted to Dr J. F. Danielli for the information that a glass surface may adsorb a layer, some 50 Å. thick, of certain proteins or nucleic acids. This suggests that storage in small glass vessels could reduce the concentration of organic matter in solution materially.

It was therefore essential to find some method of storing samples without loss of organic phosphorus by attachment to the storage bottle. Experiment indicated, as far as the methods of estimation permitted, that this can be done by means of a simple device. Before filling with a measured quantity of sea water, the storage bottles were 'baited' with a small quantity of recently precipitated aluminium hydroxide, in order that the adsorbable organic matter and any epiphytic bacteria might attach to the large surface exposed by this finely divided and reactive solid rather than to the glass, and a few drops of chloroform were also added to hinder the proliferation of bacteria. After storage the sulphuric acid was added to the storage bottle; this quickly dissolved the alumina, unseating the adsorbed organic matter and attached bacteria. The whole contents of the bottle were then poured into the silica reaction flask.

Later a suspension of thorium carbonate was substituted for aluminium hydroxide as bait, because the latter becomes less rapidly soluble in sulphuric acid after more than a week or 10 days' storage.

Water collected 22 July 1946 near Eddystone Lighthouse, was found to contain:
Filled direct into flasks: 14·0 mg. P per m.^{3} (mean of 4 estimations).
After storage in glass bottles: 10·5 mg. P per m.^{3} (mean of 4 estimations).
After storage in 'baited' bottles: 14·7 mg. P per m.^{3} (mean of 4 estimations).

Water collected 17 August 1946 near Eddystone Lighthouse:
Filled direct into flasks: 16·3 mg. P per m.^{3} (mean of 6 estimations).
After storage in baited bottles: 16·9 mg. P per m.^{3} (mean of 8 estimations).

Water collected off Eddystone Lighthouse 17 September 1947:
Filled direct into silica flasks: 17·0, 16·7, 16·4, 15·7, 15·4, 15·0, 14·8, 14·7, 14·2 mg. P/m.^{3} (mean 15·54).
Stored 4 days in bottles baited with thorium carbonate: 17·8, 16·1, 15·6, 15·6, 14·8 mg. P/m.^{3} (mean 16·0).
Stored 4 days in glass bottles in dark: 16·4, 13·9, 13·6, 13·6, 12·7 mg. P/m.^{3} (mean 14·0).

Results obtained by using this technique, with the object of investigating the cycle of phosphorus in the sea, its changes with time, depth and place, will be the subject of a further communication. Yet it seems desirable to include a series of data, typical of several such, from clear offshore water of moderate depth in summer (Table III).

TABLE III. PHOSPHORUS IN OFFSHORE WATER

Position, 3 miles south of Eddystone Lighthouse. Depth, 67 m. 23 July 1947.

	mg. P per m.^{3}		
Depth in m.	Inorganic*	Total	Organic†
5	2·3	10·2	7·9
10	2·2	11·6	9·4
20	5·0	12·3	7·3
30	6·5	12·6	6·1
40	7·4	11·8	4·4
60	6·8	13·1	4·3
65	6·8	14·2	7·4
35 cm. above sea floor	8·4	14·7	6·3
6 cm. above sea floor	8·8	24·0	16·2
6 cm. above sea floor (200 yd. distant)	7·9	18·0	10·1

* Including the equivalent of any arsenate present. † By difference.

I would wish to acknowledge many most helpful conversations with Dr W. R. G. Atkins, F.R.S., and Dr L. H. N. Cooper, F.R.I.C., who also carried out a number of check estimations by means of a Pulfrich photometer during the early part of the investigation.

SUMMARY

A simple photoelectric meter is described which allows the molybdenum blue formed in sea water, due to the presence of phosphate, to be estimated within that due to ±0·25 mg. phosphate-P per m.^{3}.

The effects of concentration of acid, molybdate, reductant, temperature and suspended particles on the rate of formation, fading and amount of molybdenum blue, formed in sea waters containing phosphate are detailed.

Intramolecular changes taking place during storage of molybdate solutions, and while being mixed with acidified sea water, have been investigated.

The hydrolysis of organic phosphorus compounds in acidified sea water at 140° C., and the prevention of arsenate formation, are described.

Procedures, resulting from these investigations, for the estimation of phosphate, and of total phosphorus, are described.

The growth of bacteria and the physical adsorption of organic phosphorus compounds in solution on the walls of glass vessels used for storage of sea water have been investigated, and a method of prevention evolved.

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